

***Forsythia suspensa* extract enhances performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function, and gut morphology in broilers**

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ABSTRACT This experiment aims to determine the effects of *Forsythia suspensa* extract (FSE) as an antibiotic substitute on performance, antioxidant status, anti-inflammatory function, intestinal morphology, and meat fatty acid deposition in broilers. 192 male Arbor Acre broilers (1-day-old, weighing 45.6 ± 1.3 g) were randomly allocated to 3 treatments, 8 replicate pens per treatment, 8 broilers per pen. The treatments contain a control diet (corn-soybean meal basal diet, CTL), an antibiotic diet (basal diet + 75 mg/kg chlortetracycline, CTC), and an FSE diet (basal diet + 100 mg/kg FSE; FSE). The experiment includes phase 1 (day 1 to 21) and 2 (day 22 to 42). Compared with CTL and CTC, broilers supplemented with FSE showed higher ($P < 0.05$) ADG and ADFI in phase 2 and overall (day 1 to 42). On day 21, serum catalase and total antioxidant capacity contents were enhanced ($P < 0.05$) in broilers fed FSE compared with CTL. On day 42, broilers fed FSE showed increased ($P < 0.05$) serum superoxide dismutase and glutathione peroxidase

contents, and enhanced ($P < 0.05$) apparent total tract digestibility of dry matter, organic matter, gross energy, total carbohydrates, and phosphorus, as well as reduced ($P < 0.05$) nitrogen and phosphorus excretion in feces compared with CTL. These broilers also showed decreased ($P < 0.05$) n-6/n-3 polyunsaturated fatty acid ratio in thigh meat, and tumor necrotic factor- α , interleukin-1 β and interleukin-6 contents in the liver on day 42 compared with CTL. The villus height was increased ($P < 0.05$) in the duodenum, jejunum, and ileum of broilers fed FSE compared with CTL. In conclusion, dietary *F. suspensa* extract supplementation as a chlortetracycline substitute under non-challenge conditions enhanced performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function, and intestinal morphology in broilers. Moreover, *F. suspensa* extract may also benefit environment by reducing nitrogen and phosphorus excretion and benefit human health via modulating meat fatty acid profiles in broilers.

Key words: anti-inflammatory, antioxidant status, broilers, *Forsythia suspensa* extract

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INTRODUCTION

Modern intensive raising methods for broilers severely cause various stresses, decrease the immunity, and compromise antioxidant ability of broilers, which leads to increased mortality and reduced quality of chicken meat (Mitchell and Kettlewell, 1998; Pan et al., 2018a). In-feed antibiotics promote growth and reduce incidence of mortality of broilers under intensive conditions (Cervantes et al., 2015). Despite the observed

improvement in broiler performance, use of antibiotics in feed has been criticized. Negative effects of in-feed antibiotics use related to animal husbandry and human health include the presence of residues in animal products and occurrence of antimicrobial resistance in animals and humans (Van den Bogaard et al., 2002; Singer and Hofacre, 2006). At present, the European Union has banned the use of antimicrobial growth promoters in animal feed (Barug et al., 2006) and so have the United States, South Korea, and China. Consequently, the need to develop natural and environment-friendly alternative substances to promote general health and performance in animal production has come to the forefront of research (Cervantes et al., 2015). Potential replacements for in-feed antibiotics in previous studies conducted in our laboratory include probiotics (Pan et al., 2017a), plant extracts (Long et al., 2019),

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compound enzymes (Shang et al., 2018), organic acids (Long et al., 2018a), essential oils (Xu et al., 2018), and trace minerals (Xu et al., 2017).

Natural Chinese herbal extracts are potential alternatives to antibiotics. Among them, *Forsythia suspensa* is widely used in animal production. Previous *in vitro* and *in vivo* studies in our laboratory have revealed the mechanism of action and specific application of *F. suspensa* extracts (FSE) are related to the antioxidant, anti-inflammatory, antiallergic, and antibacterial effects (Wang et al., 2008; Lu et al., 2010; Zhang et al., 2013; Zhao et al., 2017a). *In vivo* studies showed that FSE might improve immune function, antioxidant capacity, and gut morphology in broilers (Wang et al., 2008; Han et al., 2012; Zhang et al., 2013). *F. suspensa* extracts might also be beneficial to alleviate growth inhibition and immune damage of broilers under heat stress and corticosterone stimulation (Zeng et al., 2014; Zhao et al., 2017b). Previous studies in our laboratory also reported FSE could reduce transport stress, slow down muscle protein degradation and growth inhibition, and improve muscle antioxidant capacity and meat quality in broilers (Pan et al., 2018a, b). However, to the best of our knowledge, few studies focus on the possible role for FSE in replacing antibiotics under nonchallenge conditions and the possible mechanism on improvement of performance in broilers. Therefore, the present study aims to investigate the effects of FSE as an antibiotic substitute under nonchallenge conditions on performance, meat quality, meat fatty acid deposition, antioxidant status, anti-inflammatory function, and intestinal morphology in broilers.

MATERIALS AND METHODS

All the experimental procedures were agreed by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China; No. AW09089104-1). The experiment was carried out at FengNing Research Unit of China Agricultural University (Academician Workstation in Chengdejiuyun Agricultural and Livestock Co., Ltd., Hebei, China).

Experimental Samples

The FSE is derived from a climbing plant widely distributed in China. The dried fruits of *F. suspensa* were purchased from Tong Ren Tang Company (Beijing, China). In brief, dried fruits of *F. suspensa* were ground to powder (100 g), extracted with 500 mL of 80% methanol, sonicated for 3 h, filtered, and extracted twice (500 mL each time). The filtrates were combined and dried by rotary vaporization (Rotavapor R-124, Büchi, Flawil, Switzerland) (Wang et al., 2008). In this experiment, this procedure could be repeated for several times to get enough FSE, and the major active antioxidant constituents isolated from FSE have been identified as forsythoside A (33.0 mg/kg), forythialan A (82.6 mg/kg), phillygenin (33.4 mg/kg) and phillyrin (163.4 mg/

kg) in our laboratory (Lu et al., 2010). We had previously proved forsythoside A, forythialan A, and phillygenin were the most active antioxidant compounds via *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging experiment and an *in vivo* diquat-induced male Sprague Dawley rats oxidative stress experiment carried out by Lu et al. (2010). The antibiotic (chlortetracycline, CTC) was provided by Beijing Tongli Xingke Agricultural Technology Co., Ltd. (Beijing, China), which was proved beneficial for the performance of broilers by Dong et al. (2011).

Experimental Broilers and Management

A total of 192 one-day-old male Arbor Acres broilers (weighing 45.6 ± 1.3 g) were provided by Arbor Acres Poultry Breeding Company (Beijing, China). All broilers were raised on wire-floored cages in an environmentally controlled room with continuous light (10 to 20 lux) and had *ad libitum* access to feed and water. Incandescent lighting was continuous in the room, and the light bulbs had plastic filters designed to block ultraviolet radiation under conditions of the experiment. The ambient temperature was maintained at 33°C at the beginning and decreased as the birds progressed in age to ensure a final temperature of 24°C until the end of the 42-D experiment. All broilers were inoculated with inactivated infectious bursa disease vaccine on day 14 and 21 and Newcastle disease vaccine on day 7 and 28. The experiment was conducted in 2 phases, consisting of phase 1 from day 1 to 21 and phase 2 from day 22 to 42.

Experimental Design, Diets and Processing Procedure

The treatments contained a control diet (corn-soybean meal basal diet, CTL), an antibiotic diet (basal diet + 75 mg/kg CTC, CTC), and an FSE diet (basal diet + 100 mg/kg FSE; FSE). There were 8 replicate pens per treatment with 8 broilers per pen. Table 1 shows the composition and nutrient levels in basal diet. All nutrient levels in basal diet (in mash form) meet or exceed the requirements of NRC (1994).

On day 21 and 42, broilers were fasted for 12 h and then weighed to determine ADG, ADFI, and feed conversion ratio. From day 40 to 42, the fecal samples were collected and cleaned (without feather and diet in feces) in each pen every day, and mixed fecal samples were collected from 3 D, dried in an oven (65°C) for 72 h, and ground to pass through a 1-mm sieve. Diets and fecal samples were analyzed for DM, ash, CP, ether extract (EE), calcium (Ca), phosphorus, neutral detergent fiber, and acid detergent fiber in accordance with the methods of AOAC (2012). Gross energy (GE) was determined by an automatic isoperibolic oxygen bomb calorimeter (Parr 1281, Automatic Energy Analyzer; Moline, IL). Organic matter (OM) was calculated using the following formula: $OM = 1 - \text{ash content (DM-base)}$. Total carbohydrates were calculated using the following formula:

Table 1. Composition and nutrient levels of basal diets (% , as-fed basis).

Ingredients	Phase 1 days 1 to 21	Phase 2 days 22 to 42
Corn	58.17	64.26
Soybean meal	30.44	24.05
Corn gluten meal	2.00	2.50
Fish meal	2.00	2.00
Soy oil	3.38	3.60
Dicalcium phosphate	1.50	1.04
Limestone	1.30	1.35
Salt	0.30	0.30
L-Lysine	0.01	0.08
Methionine	0.14	0.04
Threonine	0.01	0.03
Chromic oxide	0.25	0.25
Vitamin-mineral premix ¹	0.50	0.50
Nutrient levels ²		
Metabolizable energy (Kcal/kg)	3,050	3,150
Crude protein	21.04	19.06
Calcium	1.04	0.90
Digestible phosphorus	0.45	0.35
Lysine	1.10	1.00
Methionine	0.50	0.38
Threonine	0.80	0.74
Tryptophan	0.28	0.24

¹Premix supplied the following per kg diet: vitamin A, 11,000 IU; vitamin D, 3,025 IU; vitamin E, 22 mg; vitamin K₃, 2.2 mg; thiamine, 1.65 mg; riboflavin, 6.6 mg; pyridoxine, 3.3 mg; cobalamin, 17.6 µg; nicotinic acid, 22 mg; pantothenic acid, 13.2 mg; folic acid, 0.33 mg; biotin, 88 µg; choline chloride, 500 mg; iron, 48 mg; zinc, 96.6 mg; manganese, 101.76 mg; copper, 10 mg; selenium, 0.05 mg; iodine, 0.96 mg; cobalt, 0.3 mg.

²Nutrient levels were analyzed values except for metabolizable energy and digestible phosphorus.

Total carbohydrates = DM - (CP + EE + ash) (Gerritsen et al., 2010). Chromium content was analyzed using an atomic absorption spectrophotometer (Z-5000 Automatic Absorption Spectrophotometer; Hitachi, Tokyo, Japan) according to Williams et al. (1962). Phosphorus content was analyzed using a UV-vis spectrophotometer (U-1000; Hitachi, Tokyo, Japan). The apparent total tract digestibility (ATTD) of nutrients was calculated in accordance with the study by Long et al. (2018a). Manure nitrogen and phosphorus excretion from broilers in all treatments during 42-D period experiment was calculated as per the equation by Pan et al. (2017b).

On day 21 and day 42, 2 broilers per pen (close to the average body weight in each pen) were selected for fasting wing vein blood samples collection and serum separation in the early morning. Blood was collected (about 8 mL) by cardiac puncture into a 10-mL anticoagulant-free Vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria) and centrifuged at 3,000 × g for 15 min to obtain serum. Serum samples were stored at -80°C until needed for analysis, and these same broilers were first stunned, then euthanized (using CO₂) and slaughtered for sample collection. On day 21, the liver samples were collected using a sterile 10-mL cryotube and immediately frozen with liquid nitrogen after slaughtering. On day 42, the viscera (including the whole liver, spleen, thymus gland, bursa of Fabricius), intestinal (the duodenum, jejunum, and ileum, about 5-cm fragment in the middle of each section), breast, and thigh meat samples were collected after slaughtering. The intestinal samples were stored in a 50-mL sterile centrifuge tube

filled with 4% paraformaldehyde at 4°C for 24 h. Fresh breast meat samples were used for testing meat quality immediately after slaughtering. The liver, breast, and thigh meat samples were collected using sterile 10-mL cryotube, immediately frozen with liquid nitrogen, and then stored at -80°C until needed for analysis.

Determination of Viscera Percentages and Meat Quality

On day 42, the collected viscera of broilers (n = 8) were weighed to determine the viscera percentage, including the liver, spleen, thymus gland, and bursa of Fabricius percentages, as per the following formula: viscera percentage (expressed as % of body weight) = viscera weight/final body weight × 100.

On day 42, the collected breast meat of broilers (n = 8) were used to determine meat quality. Within 24 h postmortem, the values of pH at 45 min and pH at 24 h in breast meat were measured using a glass penetration pH electrode (pH-star, Matthaus, Germany), and the ΔpH was calculated as per the following formula: ΔpH = pH at 45 min - pH at 24 h. Breast meat color was measured using a Chroma Meter (CR-410, Konica Minolta, Tokyo, Japan) and expressed as lightness (L*), redness (a*), and yellowness (b*) values. The meat color and pH value were measured in triplicate at 3 different orientations (middle, medial, and lateral) for each sample. Postmortem drip loss at 24 h was measured using the plastic bag method as described by Pan et al. (2018a).

Determination of Serum Indices and Interleukin Levels in the Liver

The concentrations of total antioxidant capacity (T-AOC), superoxide dismutase, glutathione peroxidase (GSH-Px), catalase (CAT), and malondialdehyde (MDA) in serum ($n = 8$) were determined by an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY) using colorimetric methods following instructions of the manufacturer of the corresponding reagent kit (Zhongsheng Biochemical Co., Ltd., Beijing, China). After slaughter, the liver samples ($n = 8$) on days 21 and 42 were used to determine the contents of interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) by ELISA test (Shanghai Lengton Biosciences Co. Ltd., Shanghai, China) at a wavelength of 450 nm.

Determination of Fatty Acid Profile

The homogenized skinless thigh and breast meat samples (ca. 20 g) of broilers ($n = 8$) in all treatments were defrosted and lyophilized for 60 h using a freeze dryer. Fatty acid concentrations of lyophilized day 42 breast and thigh meat were determined. The fatty acid profiles of the lipid sources were determined by gas chromatography (6890 series, Agilent Technologies, Wilmington, DE) in accordance with the procedures of Sukhija and Palmquist (1989) with slight modifications. Lipid samples were converted to fatty acid methyl esters using methanolic HCl. Undecanoic acid (C11:0) was used as the internal standard. Aliquots of 1 mL were injected into a capillary column (60 m \times 250 μ m \times 250 nm, DB-23, Agilent Technologies) with cyanopropyl methyl silicone as the stationary phase. Column oven temperature was programmed with a 1:20 split. Injector and detector temperatures were maintained at 260°C and 270°C, respectively. Nitrogen was the carrier gas at a flow rate of 2 mL/min. Fatty acid compositions in breast and thigh meat were calculated in terms of g/100 g of total fatty acids in tissue (DM-basis).

The data of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), n-6 PUFA, n-3 PUFA, and PUFA/SFA ratio (P/S) were calculated; the statistical formula is as follows:

$$\begin{aligned} \text{SFA} &= \text{C12:0} + \text{C14:0} + \text{C15:0} + \text{C16:0} + \text{C17:0} + \text{C18:0} \\ &+ \text{C20:0} + \text{C21:0} + \text{C22:0} + \text{C24:0}; \text{MUFA} = \text{C14:1} + \\ &\text{C16:1} + \text{C18:1n9c} + \text{C20:1}; \text{PUFA} = \text{C18:2n-6} + \text{C18:3n-6} \\ &+ \text{C20:4n-6} + \text{C18:3n-3} + \text{C20:3n-6} + \text{C20:4n-6} + \\ &\text{C20:3n-3} + \text{C20:5n-3} + \text{C22:6n-3}; \text{P/S PUFA/SFA}; \text{N-3} \\ \text{PUFA} &= \text{C18:3n-3} + \text{C20:3n-3} + \text{C20:5n-3} + \text{C22:6n-3}; \\ \text{N-6 PUFA} &= \text{C18:2n-6} + \text{C18:3n-6} + \text{C20:3n-6} + \\ &\text{C20:4n-6}; \text{N-6/n-3 PUFA} = \text{n-6 PUFA/n-3 PUFA}. \end{aligned}$$

Determination of Intestinal Histomorphology

On day 42, the collected small intestinal samples (1 cm) of broilers ($n = 8$) were conserved in 4%

paraformaldehyde for 24 h and then embedded in paraffin blocks before hematoxylin and eosin staining. Paraffin blocks were sectioned into 5- μ m slices, installed on glass slides, and dyed with hematoxylin-eosin for microscopic examination. Approximately 20 well-orientated villi and their adjoining crypts on each slice were selected to measure and calculate the average villus height, crypt depth, and of villus height-to-crypt depth ratio via a light microscope using a calibrated 10-fold eyepiece reticle.

Statistical Analysis

All the data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 2008). For the performance, nutrient digestibility and excretion, and the serum antioxidant status, the pen was the experimental unit. For other measurements, the individual broiler was the experimental unit. Differences among treatments were separated by Student-Neuman-Keul's multiple range tests. Results are expressed as least squares means and SEM. Significance was designated at $P \leq 0.05$, while a tendency for significance was designated at $0.05 < P \leq 0.10$.

RESULTS

Performance of Broilers

In phase 2, broilers fed FSE showed higher ($P < 0.05$) ADG and ADFI at approximately 9% than those fed CTL and CTC. Overall (day 1 to 42), broilers fed FSE had increased ($P < 0.05$) ADG and ADFI at about 7% and 8%, respectively, compared with those fed CTL and CTC (Table 2).

Meat Quality and Viscera Percentage

Broiler fed FSE had lower ($P < 0.05$) L* value in breast meat on day 42 than broilers fed CTC. However, there are no significant differences of a*, b*, and pH values and drop loss among treatments on day 42 (Table 3). Broilers fed CTC and FSE had higher ($P < 0.05$) bursa of Fabricius percentage than those fed CTL, while there were no significant differences in other viscera percentage among treatments on day 42 (Table 4).

Serum Antioxidant Status

Effects of FSE on serum antioxidant status of broilers on day 21 and 42 are shown in Table 5. On day 21, the concentrations of T-AOC and CAT were enhanced ($P < 0.05$) in broilers fed FSE compared with those fed CTL, while the content of T-AOC was increased ($P < 0.05$) in broilers supplemented with CTC in comparison with those supplemented with CTL. On day 42, broilers supplemented with FSE showed higher ($P < 0.05$) concentrations of superoxide dismutase and GSH-Px in serum than those supplemented with CTL,

whereas broilers fed CTC showed decreased ($P < 0.05$) level of MDA compared with broilers fed FSE.

Nutrient Digestibility and Excretion

Broilers fed FSE had enhanced ($P < 0.05$) ATTD of DM, OM, and total carbohydrates, while broilers supplemented with CTC and FSE showed improved ($P < 0.05$) ATTD of GE and phosphorus in comparison with those fed CTL (Table 6). As shown in Table 7, broilers fed FSE had a reduction ($P < 0.05$) of nitrogen and phosphorus excretion in phase 2 compared with those fed CTL.

Fatty Acid Profiles in Meat of Broilers

Effects of FSE on fatty acid deposition in breast and thigh meat of broilers are presented in Table 8 and Table 9, respectively. Broilers fed diet supplemented with FSE tended to have increased ($P = 0.10$) α -linolenic acid (C18:3n3; ALA) and eicosapentaenoic acid (C20:5n3; EPA) contents in breast meat and decreased ($P < 0.05$) n-6/n-3 PUFA ratio in thigh meat compared with those fed CTL.

Anti-inflammatory Function in Liver of Broilers

Effects of FSE on inflammatory cytokines in the liver of broilers are presented in Table 10. Compared with broilers fed CTL, broilers fed FSE showed decreased ($P < 0.05$) content of TNF- α in the liver on day 21 and day 42, while these broilers also had reduced ($P < 0.05$) levels of IL-1 β and IL-6 in the liver on day 42.

Intestinal Morphology of Broilers

The villus height was greater ($P < 0.05$) in the duodenum, jejunum, and ileum of broilers fed FSE than in those fed CTL. Moreover, there was a tendency ($P = 0.10$) for increased villus height-to-crypt depth

Table 2. Effects of *Forsythia suspensa* extract on growth performance of broilers.

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Day 1 body weight, g	46.92	46.32	46.70	0.33	0.47
Days 1 to 21					
ADG, g/D	27.32	26.96	28.14	0.40	0.15
ADFI, g/D	36.99	36.42	38.60	0.91	0.25
FCR	1.35	1.35	1.37	0.03	0.90
Days 21 to 42					
ADG, g/D	68.85 ^b	71.44 ^b	75.09 ^a	1.18	<0.01
ADFI, g/D	105.87 ^b	105.05 ^b	115.85 ^a	2.08	<0.01
FCR	1.54	1.47	1.55	0.03	0.23
Days 1 to 42					
ADG, g/D	48.08 ^b	49.20 ^b	51.62 ^a	0.62	0.05
ADFI, g/D	71.43 ^b	70.73 ^b	77.22 ^a	0.86	<0.01
FCR	1.49	1.44	1.50	0.02	0.07

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

Abbreviation: FCR, feed conversion ratio.

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

Table 3. Effects of *Forsythia suspensa* extract on meat quality of the breast meat in broilers.

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
L* ²	47.46 ^{a,b}	49.07 ^a	45.96 ^b	0.72	0.03
a* ²	2.41	3.24	2.77	0.32	0.22
b* ²	9.05	10.02	8.56	0.63	0.29
pH at 45 min	6.68	6.74	6.82	0.08	0.51
pH at 24 h	5.84	5.97	5.78	0.09	0.39
δ pH ³	0.84	0.77	1.04	0.14	0.40
Drop loss, %	2.78	2.83	3.23	0.35	0.61

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

²L*: lightness; a*: redness; b*: yellowness.

³ δ pH = pH 45 min - pH 24 h.

ratio in the duodenum and jejunum of broilers supplemented with FSE compared with those fed CTL (Table 11).

DISCUSSION

Current commercial raising of broilers has caused severe mortality and stress, which might lead to a reduction of feed intake and growth rate in broilers. The present study showed broilers fed FSE (major active antioxidant compounds include forsythoside A, forythialan A, phillygenin, and phillyrin) had increased ADG and ADFI compared with those fed CTL and CTC, and the efficacy of FSE for increased ADG and ADFI was more obvious in phase 2 than in phase 1, which might be due to the fact that FSE has accumulated effects in enhancing performance (Long et al., 2019). The main reason for the enhancement of ADG and ADFI was that phillyrin, forythialan A, phillygenin, and forsythoside A in FSE played an important role in enhancing the immune function and antioxidant capacity of broilers (Wang et al., 2008; Zhang et al., 2013). Yang et al. (2017) reported phillyrin in FSE increased immune response and inhibited the lipopolysaccharide (LPS)-induced inflammatory damage, which benefited the health status and performance of broilers. Furthermore, previous researchers in our laboratory proved that forsythialan A and B had antioxidant function of aromatic hydroxyl (Piao et al., 2008), while phillygenin and 8-hydroxy-pinorensin had antioxidant function of hydroxy substituents on the phenyl moieties (Piao et al., 2009), which also helped broilers alleviate stress and enhance performance. The ursolic acid, phillygenin,

Table 4. Effects of *Forsythia suspensa* extract on viscera percentage of broilers on day 42 (% of body weight).

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Liver	1.96	1.81	1.90	0.08	0.46
Spleen	0.12	0.12	0.14	0.01	0.40
Thymus gland	0.24	0.21	0.26	0.02	0.38
Bursa of Fabricius	0.28 ^b	0.40 ^a	0.49 ^a	0.04	0.05

^{a,b}Different superscripts within a row indicate means are different ($P < 0.05$).

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

Table 5. Effects of *Forsythia suspensa* extract on serum antioxidant status of broilers.

Item ²	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Day 21					
T-AOC, U/mL	1.00 ^b	2.50 ^a	2.64 ^a	0.34	0.01
SOD, U/mL	54.37	55.21	55.11	1.15	0.86
GSH-Px, umol/L	29.57	37.23	30.90	5.49	0.59
CAT, ng/mL	13.61 ^b	16.92 ^{a,b}	18.47 ^a	1.15	0.05
MDA, nmol/mL	7.02	7.10	6.89	0.70	0.98
Day 42					
T-AOC, U/mL	2.99	5.23	2.94	1.19	0.36
SOD, U/mL	25.78 ^b	20.49 ^b	53.24 ^a	4.21	<0.01
GSH-Px, umol/L	17.43 ^b	17.53 ^b	33.99 ^a	3.15	0.02
MDA, nmol/mL	7.04 ^{a,b}	5.96 ^b	8.32 ^a	0.37	0.01

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: Control; CTC: Chlortetracycline; FSE: *Forsythia suspensa* extract.

²T-AOC: total antioxidant capacity; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; CAT: catalase; MDA: malondialdehyde.

and rutin in FSE reduced oxidative stress-related lipid peroxidation and eliminated DPPH and ABTS [2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] *in vitro* (Kang and Wang, 2010), which might be beneficial for improving meat quality in broilers. Moreover, plenty of *in vivo* and *in vitro* studies showed the forsythoside A in FSE played an important role in improving anti-inflammatory function by inhibiting nuclear factor kappa B (NF- κ B) activation (Zhang et al., 2018), antioxidant status via activating nuclear factor erythroid-2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) signaling pathway (Wang et al., 2016; Qian et al., 2017), and decreasing apoptosis in cells (Yan et al., 2017). These benefits of forsythoside A might help modulate the health status and performance of broilers in the present study. Besides, previous studies also showed FSE reduced oxidative stress and enhanced performance under heat stress and corticosterone stimulation (Wang et al., 2008; Zeng et al., 2014) via enhancing nutrient digestibility (Xie et al., 2018), which might also be a possible reason for the enhanced performance by FSE in this study.

For broilers, a previous study in our laboratory indicated FSE enhanced ATTD of DM, OM, and GE

Table 6. Effects of *Forsythia suspensa* extract on apparent total tract digestibility of nutrients in broilers (%).

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Dry matter	71.90 ^b	74.81 ^{a,b}	75.38 ^a	0.76	0.04
Organic matter	74.79 ^b	77.42 ^{a,b}	78.01 ^a	0.67	0.03
Gross energy	74.89 ^b	77.39 ^a	77.85 ^a	0.72	0.05
Crude protein	61.44	66.40	64.15	1.85	0.24
Ether extract	89.86	91.95	89.52	0.70	0.10
Total	77.51 ^b	79.45 ^{a,b}	81.13 ^a	0.47	<0.01
carbohydrates					
Neutral detergent fiber	47.20	45.36	42.58	2.89	0.56
Acid detergent fiber	27.74	16.72	15.76	3.72	0.11
Calcium	38.81	45.61	39.82	3.13	0.32
Phosphorus	46.34 ^b	51.05 ^a	52.59 ^a	1.46	0.05

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: Control; CTC: Chlortetracycline; FSE: *Forsythia suspensa* extract.

Table 7. Effects of *Forsythia suspensa* extract on nitrogen and phosphorus excretion in feces of broilers (g/kg, DM-basis).

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Days 1 to 21					
Nitrogen	400.64	388.09	396.58	16.69	0.87
Phosphorus	105.78	104.00	100.83	3.63	0.63
Days 22 to 42					
Nitrogen	170.87 ^a	148.66 ^{a,b}	138.28 ^b	7.02	0.03
Phosphorus	45.08 ^a	39.81 ^{a,b}	35.13 ^b	1.35	<0.01

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

(Zhang et al., 2013), which was also demonstrated in the present study. This finding might be possibly due to the beneficial effects of FSE in increasing digestive enzymes activities in the pancreas (such as trypsin, chymotrypsin and lipase), thus enhancing the ATTD of GE and CP (Long et al., 2019). The nutrient absorption was also related to the gut morphology development, including villus height and crypt depth. Therefore, the improved gut morphology in the small intestine could also be a possibly reason for the improved nutrient digestibility. Furthermore, broilers fed FSE also showed increased ATTD of phosphorus, which was found in few other studies. The reason for this finding might be that the main ingredients in FSE could enhance the efficiency of phytase which helped broilers utilize the

Table 8. Effects of *Forsythia suspensa* extract on fatty acid deposition in the breast meat of broilers (g/100 g total fatty acids, DM-basis).

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
C12:0	0.05	0.05	0.05	0.01	0.24
C14:0	0.46	0.45	0.45	0.01	0.23
C14:1	0.08	0.07	0.07	0.01	0.49
C15:0	0.08	0.09	0.08	0.01	0.73
C16:0	21.14	20.92	20.98	0.29	0.46
C16:1	3.03	2.84	2.94	0.41	0.66
C17:0	0.14	0.14	0.14	0.01	0.37
C18:0	8.52	8.71	8.45	0.01	0.60
C18:1n9c	29.86	29.20	29.37	0.58	0.35
C18:2n6c	28.11	28.45	28.73	0.38	0.32
C18:3n3	2.12	2.13	2.26	0.05	0.10
C20:0	0.13	0.13	0.13	0.01	0.67
C20:1	0.33	0.33	0.29	0.02	0.31
C21:0	0.49	0.57	0.58	0.08	0.45
C20:3n6	0.57	0.62	0.61	0.06	0.33
C20:4n6	2.87	3.11	2.84	0.39	0.31
C20:3n3	0.05	0.06	0.07	0.01	0.29
C20:5n3	0.30	0.31	0.31	0.01	0.10
C22:0	0.10	0.10	0.09	0.01	0.30
C24:0	0.78	0.86	0.81	0.11	0.29
C22:6n3	0.67	0.71	0.62	0.08	0.35
C24:1	0.12	0.14	0.13	0.02	0.29
SFA ²	31.90	32.02	31.75	0.69	0.77
MUFA ²	33.42	32.59	32.81	1.25	0.43
PUFA ²	34.69	35.39	35.45	0.69	0.25
n-6 PUFA	31.55	32.18	32.19	0.62	0.27
n-3 PUFA	3.14	3.21	3.26	0.08	0.15
n-6/n-3 PUFA ²	10.06	10.04	9.87	0.13	0.20
P/S ²	1.09	1.10	1.12	0.02	0.36

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

²SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; P/S = PUFA/SFA; n-6/n-3 PUFA = n-6 PUFA/n-3 PUFA.

Table 9. Effects of *Forsythia suspensa* extract on fatty acid deposition in the thigh meat of broilers (g/100 g total fatty acids, DM-basis).

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
C12:0	0.04	0.04	0.03	0.01	0.82
C14:0	0.49	0.49	0.49	0.01	0.79
C14:1	0.10	0.09	0.09	0.01	0.96
C15:0	0.08	0.08	0.08	0.01	0.79
C16:0	20.99	20.62	20.68	0.48	0.85
C16:1	3.74	3.58	3.64	0.42	0.97
C17:0	0.14	0.14	0.13	0.01	1.00
C18:0	6.91	7.18	7.00	0.40	0.93
C18:1n9c	32.86	31.79	32.08	1.13	0.67
C18:2n6c	29.06	29.50	29.49	0.91	0.85
C18:3n3	2.42	2.47	2.62	0.09	0.21
C20:0	0.11	0.11	0.11	0.01	0.71
C20:1	0.28	0.28	0.28	0.01	0.64
C21:0	0.28	0.35	0.33	0.07	0.68
C20:3n6	0.33	0.39	0.37	0.06	0.64
C20:4n6	1.21	1.62	1.43	0.43	0.81
C20:3n3	0.03	0.04	0.04	0.01	0.32
C20:5n3	0.20	0.22	0.22	0.02	0.68
C22:0	0.06	0.06	0.06	0.01	0.83
C24:0	0.35	0.48	0.43	0.13	0.74
C22:6n3	0.31	0.40	0.34	0.10	0.78
C24:1	0.04	0.06	0.06	0.02	0.68
SFA ²	29.44	29.56	29.35	0.34	0.94
MUFA ²	37.01	35.80	36.16	1.38	0.74
PUFA ²	33.55	34.64	34.50	0.02	0.72
n-6 PUFA	30.60	31.52	31.29	1.21	0.78
n-3 PUFA	2.95	3.12	3.21	0.16	0.30
n-6/n-3 PUFA ²	10.37 ^a	10.15 ^{a,b}	9.78 ^b	0.15	0.02
P/S ²	1.14	1.17	1.17	0.05	0.75

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

²SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; P/S = PUFA/SFA; n-6/n-3 PUFA = n-6 PUFA/n-3 PUFA.

phytate phosphorus (Long et al., 2019). The enhanced ATTD of total carbohydrates was first found in the present study, which reflected that FSE could improve the ATTD of nonstarch polysaccharide and other regular nutrients such as DM, EE, CP, and OM (Zhang et al., 2013). A study carried out by Long et al. (2019) showed that FSE could effectively replace CTC on enhancing the nutrient digestibility and reduce the excretion of nitrogen in feces of weaned piglets, which is also showed in feces of broilers in the present study. The reduced

Table 10. Effects of *Forsythia suspensa* extract on anti-inflammatory in the liver of broilers (pg/mg).

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Day 21					
IL-1 β ²	3.80	3.30	2.76	0.67	0.29
IL-6 ²	18.66	16.50	14.02	1.37	0.11
TNF- α ²	8.02 ^a	6.86 ^{a,b}	5.83 ^b	0.26	<0.01
Day 42					
IL-1 β ²	2.90 ^a	2.00 ^{a,b}	1.65 ^b	0.28	0.04
IL-6 ²	14.90 ^a	12.50 ^{a,b}	10.24 ^b	1.05	0.04
TNF- α ²	6.40 ^a	5.38 ^{a,b}	4.42 ^b	0.28	<0.01

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspensa* extract.

²IL-1 β : interleukin-1 β ; IL-6: interleukin-6; TNF- α : tumor necrosis factor-alpha.

nitrogen and phosphorus excretion in manure was because of the higher utilization of CP and phosphorus in broilers fed FSE, which could be beneficial to the environment. Moreover, this study also showed broilers fed CTC had increased ATTD of GE, total carbohydrates, and phosphorus, which is partly in agreement with the study of He et al. (2019), who reported the ATTD of DM and OM was enhanced in broilers fed CTC. These beneficial effects noted with CTC under nonchallenge conditions could also be replicated with FSE, which indicated FSE could replace CTC in enhancing nutrient digestibility.

The present study showed the villus height was greater in the duodenum, jejunum, and ileum, while there was a tendency for increased villus height-to-crypt depth ratio in the duodenum and jejunum of broilers supplemented with FSE compared with those fed CTL. These results indicated that FSE enhanced the villus development in the small intestine of broilers, which is in agreement with the finding of Han et al. (2012) and Long et al. (2019). The reason might be that the main ingredients in FSE could improve proliferation of peripheral blood lymphocytes and intestinal permeability, so as to increase the intestinal capacity to utilize nutrients (Hao et al., 2010). Previous research studies also showed similar results because herb extracts could help increase the height and width of villi and decrease duodenum crypt depth (Iser et al., 2016); improve the duodenum, jejunum villus height, and ratio of villus height to crypt depth (Wang et al., 2019); and therefore improve the gut development of broilers (Cross et al., 2007).

As noted previously, intensive raising of broilers might lead to the production of reactive oxygen species (ROS) in their body. The ROS production exceeds the scavenging capacity of the antioxidant defense system in broilers, resulting in oxidative damage of DNA, lipids, and proteins (Lu et al., 2010), as well as reduction of meat quality (Soladoye et al., 2015). The present study showed that FSE could be a functional plant substance with antioxidant properties, which could enhance antioxidant enzymes activity (e.g. CAT, GSH-Px) and non-enzyme antioxidant status and eventually reduce ROS (Falowo et al., 2014). This enhancement of antioxidant status (increased serum contents of CAT and T-AOC) was also showed in broilers fed CTC under nonchallenge conditions, which indicated the beneficial effect on antioxidant status can be repeated by FSE. The current results might be due to the main ingredients of FSE as natural antioxidants could activate the antioxidant defense system (Lee et al., 2000) and Nrf2 system (Jung and Kwak, 2010) in cell membranes of tissues. The improved antioxidant status of broilers fed FSE might also be due to the fact that the FSE could lower DPPH radical-scavenging activity and MDA content (Lu et al., 2010; Pan et al., 2018a). In addition, the results could also be due to the antioxidant effects of forsythiaside A in FSE, which could improve antioxidant capacity *in vitro* (Lu et al., 2010) and *in vivo* (Zhao et al., 2017a) via the enhancement of

Table 11. Effects of *Forsythia suspense* extract on intestinal morphology in broilers.

Item	CTL ¹	CTC ¹	FSE ¹	SEM	P-value
Duodenum					
Villus height, μm	2,092 ^b	2,234 ^{a,b}	2,395 ^a	69.33	0.05
Crypt depth, μm	237	172	229	24.13	0.20
Villus height/crypt depth	9.37	13.10	11.04	1.04	0.10
Jejunum					
Villus height, μm	1,372 ^b	1,561 ^{a,b}	1,976 ^a	107.49	0.02
Crypt depth, μm	253	195	232	29.86	0.44
Villus height/crypt depth	6.00	8.19	8.93	0.87	0.10
Ileum					
Villus height, μm	1,036 ^b	1,103 ^{a,b}	1,340 ^a	68.23	0.04
Crypt depth, μm	141	138	171	15.11	0.30
Villus height/crypt depth	7.82	8.23	8.18	0.98	0.96

^{a,b}Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹CTL: control; CTC: chlortetracycline; FSE: *Forsythia suspense* extract.

gene expression of antioxidant enzymes in cells (Cheng et al., 2014). The enhancement of oxidative stability in serum observed in the present study might reflect the improved antioxidant status of chicken meat (Pan et al., 2018a), which might be a potential way to alleviate oxidative injury of the breast muscles in broilers and to improve the meat quality for human consumption (Pan et al., 2019).

As shown in the present study, dietary FSE supplementation reduced proinflammatory cytokines including TNF- α , IL-1 β , and IL-6 in the liver of broilers on days 21 and 42, which implied that FSE could alleviate inflammation of broilers. These results might be due to the beneficial effects of the forsythiaside A in FSE (Wang et al., 2016). Moreover, the phillyrin of FSE attenuated inflammation via suppression of mitogen-activated protein kinases and NF- κ B pathways (Zhong et al., 2013). Previous research studies also showed forsythiaside in FSE reduced LPS-induced acute inflammation (Zhao et al., 2017a). The mechanism by which forsythiaside in FSE exerted its anti-inflammatory effect is via inactivation of NF- κ B (Cheng et al., 2014). Furthermore, the protective mechanisms of FSE on reducing proinflammatory cytokine (IL-1 β and IL-6) response in the present study might be that the forsythiaside A in FSE could downregulate the mRNA expression of proinflammatory cytokines (Bai et al., 2018) and modulate the Nrf2-mediated antioxidant response against inflammatory injury (Zhao et al., 2017a).

The current increased antioxidant capacity by FSE has been observed in previous studies of Zeng et al. (2014) and Zhao et al. (2017b), who reported that the antioxidant properties of FSE as antioxidants could improve postslaughter meat quality in broilers subjected to stress. However, in the present study, there were no significant differences in redness, yellowness, pH at 45 min, pH at 24 h, δ pH, and drop loss among treatments, which disagreed with the results of Pan et al. (2018a, b). These differences might be due to the current study conducted under nonchallenge conditions, while other studies were carried out under dexamethasone- or corticosterone-challenge condition. The present study only found FSE could lower the L* value of breast meat compared with CTC, which might

benefit meat quality, because a previous study also showed FSE could help decrease dexamethasone-induced increase in the L* value of the breast meat in broilers (Pan et al., 2019). The increased bursa of Fabricius percentage in broilers fed FSE and CTC suggested enhanced immune function of broilers because the bursa of Fabricius was one of the major immune organs for broilers. The reason for this finding might be that the forsythiaside A in FSE could help inhibit the LPS-induced injury of the bursa of Fabricius (Sung et al., 2016) and improve the anti-inflammatory functions in cells (Wang et al., 2016).

Free radicals and ROS could reduce the capacity of the cellular intrinsic free radical-scavenging system, which might attack the cellular fatty acids and result in the onset of lipid peroxidation of membranes (Kamboh et al., 2013). A previous study in our laboratory showed FSE could reduce free radicals and desirably change the fatty acid profile in the breast muscles of poultry under stress conditions (Pan et al., 2018b). In the present study, we found broilers fed diet supplemented with FSE tended to have increased C18:3n3 and C20:5n3 contents in the breast meat, which indicated FSE might be beneficial for n-3 PUFA deposition. This finding was partly showed in studies of Pan et al. (2018b) and Pan et al. (2019), who found that dietary FSE supplementation increased PUFA and C22:6n-3 content in the breast meat of broilers under dexamethasone- or corticosterone-challenge conditions. One of the reasons for this finding might be that FSE reduced the negative effects of oxidative alterations and therefore decreased the reduction of double bonds of PUFA, accompanied with higher PUFA contents. Moreover, the potential of dietary main ingredients in FSE as antioxidants toward improvement of the n-3 PUFA (ALA and EPA) in the breast meat of broilers could also be explained by the antioxidant potential of phenolic compounds in removing and decreasing the free radical in tissue of breast meat, hence reducing lipid peroxidation (Saleh et al., 2017). Compared with breast muscles, thigh meat had higher content of fat rich in PUFAs and therefore was more susceptible to lipid oxidation (Basmacioglu et al., 2004; Juskiewicz et al., 2017). The present study found that dietary FSE supplementation

significantly decreased n-6/n-3 PUFA ratio in the thigh meat compared with CTL. The PUFA are the most sensitive fractions to oxidation processes and lipid oxidation in meat (Hashemipour et al., 2013); this finding might be due to the protective effect of FSE as a dietary natural antioxidant on acting as electron donors to provide electrons for reduction of some unsaturated fatty acids (Chikunya et al., 2004) and improving the meat quality by increasing the n-3 PUFA (ALA or EPA) levels (Ahmed et al., 2016). As noted in a previous study conducted in our laboratory, C18:3n3 and C20:5n3 as n-3 PUFA in chicken meat may be beneficial for human health (Long et al., 2018b, 2020), and lower n-6/n-3 PUFA ratio also had health benefit effects for humans, mainly in protection against cardiovascular diseases (Chikunya et al., 2004). However, the mechanisms by which FSE regulates fatty acid composition of the breast meat and thigh meat in broilers are still not fully understood, and therefore, further studies are still warranted to identify these positive functions (Pan et al., 2019).

CONCLUSION

Supplementation of dietary *F. suspensa* extract as an antibiotic substitute under nonchallenged conditions enhanced performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function, and intestinal morphology in broilers. Moreover, dietary inclusion of *F. suspensa* extract also benefited environment by reducing nitrogen and phosphorus excretion in feces, as well as modulated the fatty acid composition in meat of broilers, which may be beneficial for human consumption.

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