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Effect of ferulic acid on growth, digestibility, digestive enzyme activity, immunity and antioxidant status of broilers

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Abstract

This study was conducted to evaluate the effects of ferulic acid (FA) on the growth, nutrient digestibility, digestive enzyme activities, immunity and antioxidant status of broilers. Ninety-six one-day-old male Arbor Acres broilers were randomly divided into two groups with six replicates of eight birds each, including control group (CON) and FA group (80 mg/kg diet). The experiment included starter (days 1–21) and finisher (days 22–42) phases. Compared with CON, FA group had higher average daily weight gain (ADWG) and lower feed to gain ratio (F:G) in the starter phase, and higher average daily feed intake (ADFI) in the finisher phase. The digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were higher in FA-fed broilers in days 19–21, but DM, OM and EE digestibility were lower in days 40–42. Ferulic acid treatment increased duodenal trypsin and jejunal amylase activity on day 21, but decreased duodenal trypsin, chymotrypsin activities and jejunal lipase activity on day 42. FA supplementation increased serum immunoglobulin M (IgM) and tumour necrosis factor-α (TNF-α) concentrations on day 21. Ferulic acid-fed broilers had greater hepatic glutathione peroxidase (GSH-Px) activity and lower serum malondialdehyde (MDA) level on day 21. On day 42, the serum superoxide dismutase (SOD) activity, hepatic SOD and GSH-Px activities was decreased in FA group. In conclusion, FA as an exogenous antioxidant at dosage of 80 mg/kg diet enhanced growth performance via improvement of digestive enzyme activities, immunity and antioxidant status of broilers in starter phase.

Key words: feed supplement; immunologic function; natural antioxidant; phenolic acid
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Introduction

Population growth in China and changes in lifestyle and food preferences have led to increasing demand for animal protein. As one of the most important meat sources, broiler chicken production has increased tremendously in the past decades (Yuan *et al.*, 2018). However, modern large-scale broiler production provokes stressful situations for the animals, such as fast growth, overcrowding, heat, handling, disease and infection (Farahat *et al.*, 2016). These stressors elevate the production of free radicals and break the balance between the oxidation and antioxidant defence systems of chickens, resulting in oxidative stress (Espinosa-Diez *et al.*, 2015), which causes damage to DNA, proteins and lipids, leading to immune inhibition, inflammation and growth restriction (Jia *et al.*, 2014). Therefore, attempts have been made to conquer oxidative stress and enhance immunity and performance of broilers with antioxidants.

Many studies have reported on supplementation with natural antioxidants that originated from herbs, spices and propolis, which enhanced antioxidant capacity, immunity and growth performance in broilers (Rizzo *et al.*, 2008; Saeed *et al.*, 2017). These effects are possibly related to the antioxidative potential of secondary metabolites, especially phenolic compounds (Duskaev *et al.*, 2020). Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a natural phenolic acid, which is widely distributed in cell walls of plants, cereal grains and fruits (Kumar & Pruthi, 2014). In recent years, FA and its derivatives were found to possess

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various biological and pharmacological properties, among which the best documented were its antioxidant properties (Srinivasan *et al.*, 2007). Supplementation of FA has been shown to increase the content of GSH, enhance antioxidant activity, and decrease the level of MDA in the liver of rats (Kim *et al.*, 2011; Balasubashini *et al.*, 2004). Inclusion of FA in diets to improve antioxidant capacity was promoted for pigs (Li *et al.*, 2015; Wang *et al.*, 2020). These experiments confirmed the potential effects of FA to influence performance and health of rats and pigs.

Although FA has been studied in rats and pigs, its effects on poultry are less known Galal *et al.* (2008) reported that the use of propolis (rich in FA) in the diet of laying hens reflected increased feed intake, egg mass and lymphocytes counts. Similarly, dietary propolis supplementation improved weight gain, lymphocyte and monocyte counts of Muscovy broiler ducks (Abdel-Rahman & Mosaad, 2013).The results of these experiments led the authors to hypothesize that feeding FA would improve the growth performance, antioxidant capacity and immunity of broilers. Furthermore, the responses of the broilers to FA supplementation might differ during two feeding periods.

Thus, the present study was conducted to evaluate the effects of FA supplementation on growth performance, nutrient digestibility, digestive enzyme activities, antioxidant status, and immune response of broiler chickens.

Materials and Methods

The study was conducted according to the Institutional Guidelines for the Care and Use of Laboratory Animals in China (The State Science and Technology Commission of China, 1988) and approved by the Institutional Animal Care and Use Committee of Inner Mongolia Agricultural University (Protocol number 2020066). Ferulic acid powder (purity > 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

A total of 96 one-day-old male Arbor Acres broilers were randomly divided into two groups with six replicates of eight birds each, including the control group (CON) and FA supplementation group. The CON group was provided a corn-soybean basal diet, and the FA group was provided the basal diet plus FA (80 mg/kg diet). The experimental period lasted for 42 days. Feed intake and bodyweight (BW) were recorded by replication at days 22 and 42. The average daily weight gain (ADWG), average daily feed intake (ADFI) and feed to gain ratio (F:G) were calculated at days 1–21, 22–42 and 1–42.

The birds were kept in stainless-steel wire cages (100 × 60 × 50 cm) with eight chickens/cage and maintained in an environmentally controlled henhouse. Feed and water were available ad libitum. The inner temperature was kept at 35 °C for the first week, then gradually reduced by 2–3 °C per week to a final temperature of 25 °C. This temperature was maintained until the end of the experiment. For three days, the birds were exposed to light for 24 hours a day, and then they were exposed to a photoperiod with 16 hours of light and 8 hours of darkness during days 4–42. All birds were inoculated against Newcastle disease, infectious bronchitis and infectious bursal disease on days 7 and 21.

The level of addition of FA was selected according to the producer's recommendations and information obtained from previous experiments (Saeed *et al.*, 2019; Kim *et al.*, 2011). The basal starter rations and finisher ration were formulated based on NRC requirements (National Research Council, 1994) and met the nutrients recommendations of the Ministry of Agriculture (2004). The ingredient and chemical composition of the basal diets are presented in Table 1.

Table 1 Formula and chemical composition of basal diets for broiler chickens (as-fed basis)

Ingredients, g/kg	Starter (days 1–21)	Finisher (days 22–42)
Corn	527.00	59.00
Soybean meal	400.00	33.80
Soybean oil	30.00	3.00
Dicalcium phosphate	19.00	1.80
Limestone	10.80	1.22
Salt	3.70	0.37
L-Lysine HCl	0.50	0.03
DL-Methionine	1.90	0.07
Vitamin and minerals premix[1]	6.00	0.60
Choline	1.10	0.11
Calculated composition		
Metabolizable energy, MJ/kg	12.42	12.62
Lysine, g/kg	13.40	11.50
Methionine, g/kg	5.50	4.00
Cysteine, g/kg	4.00	3.60
Total phosphorus, g/kg	5.70	5.70
Analysed composition		
Moisture, g/kg	103.10	101.20
Crude protein, g/kg	217.70	197.40
Ether extract, g/kg	110.50	83.70
Ash, g/kg	56.70	55.30
Calcium, g/kg	10.00	10.20

[1]Provided per kilogram of diet: vitamin A: 9000 IU, vitamin D_3: 3000 IU, vitamin E: 26 mg, vitamin K_3: 1.20 mg, vitamin B_1: 3.00 mg, vitamin B_2: 8.00 mg, vitamin B_6: 4.40 mg, vitamin B_{12}: 0.012 mg, nicotinic acid: 45 mg, folic acid: 0.75, mg, biotin: 0.20, mg, choline: 1100 mg, calcium pantothenate: 15 mg. iron: 100 mg, copper: 10 mg, zinc: 108 mg, manganese: 120 mg, iodine: 1.5 mg, selenium: 0.35 mg

The apparent total tract digestibility of nutrients was determined using an acid insoluble ash (AIA) as an internal marker as described by Siriwan *et al.* (1993). For excreta collection, clean trays were placed under the cages. The excreta were collected daily during days 19–21 and 40–42. Feathers and down were removed carefully and the excreta collected per cage during three days were pooled. The representative samples of excreta were collected and dried in an oven at 65 °C for 72 hours, and then ground to pass through a 1.0 mm mill sieve. Components of feed and excreta samples in each group, including dry matter (DM) (method 934.01), crude protein (CP) (method 954.01), ash (method 930.05), ether extract (EE) (method 954.02), calcium (method 927.02), and phosphorus (method 984.27) were analysed according to AOAC (1995). Acid-insoluble ash was analysed based on the method of Keulen & Young (1977). Digestibility values were calculated as:

$$Digestibility = 100 - [\frac{\%\ AIA\ in\ feed}{\%\ AIA\ in\ excreta} \times \frac{\%\ nutrient\ in\ excreta}{\%\ nutrient\ in\ feed}]$$

On day 21 and 42, one bird per replication (six per treatment) with BW similar to the mean of the full replicate cohort was selected for blood collection after 12 hours fasting. Blood samples were taken from the wing vein using Vacutainer tubes (BD Vacutainer, 5.0 mL). After standing for 45 min at room temperature, the blood samples were centrifuged at 3000 × g for 15 min, and then serum samples were separated and stored at –20 °C for analysis of antioxidant capacity and immunity. After bleeding, the bird was slaughtered by cervical dislocation, and liver tissue samples were harvested, stored at -80 °C, and then used for antioxidant capacity assays. To determine digestive enzyme activities, the jejunum and duodenum digesta were collected into plastic tubes by massaging the tract, then immediately frozen in liquid nitrogen and stored at -80 °C until analysis.

Before analysis, the frozen jejunum and duodenum digesta samples were thawed. One g of each

sample was diluted with 9 mL of ice-cold normal saline (0.9%), homogenized and centrifuged at 9000 × g for 10 min at 4 °C. The supernatants of digesta homogenates were used for digestive enzyme activities assay. The α-amylase (C016-1-1), lipase (A054-1-1), trypsin (A080-2-2) and chymotrypsin (A080-3-1) activities were analysed with commercial colorimetric diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

The concentrations of immunoglobulin A (IgA) (JYM0012Ch), immunoglobulin G (IgG) (JYM0001Ch) and immunoglobulin M (IgM) (JYM0060Ch), interleukin-2 (IL-2) (JYM0026Ch), interleukin-6 (IL-6) (JYM0028Ch), interleukin-10 (IL-10) (JYM0040Ch) and tumour necrosis factor-α (TNF-α) (JYM0033Ch) in serum were measured using chicken-specific ELISA kits (Colorful Gene Biological Technology Co., Ltd, Wuhan, China). All measurements were conducted according to the manufacturer's instructions.

The liver sample was homogenized with sterile ice-cold normal saline (0.9%) with a weight-to-volume ratio of 1:9. The supernatant was obtained after centrifuging at 9000 × g for 10 min at 4 °C. The concentrations of hepatic superoxide dismutase (SOD) (A001-2-2), glutathione peroxidase (GSH-Px) (A005-1-2), and malondialdehyde (MDA) (A003-1-2) were determined with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to Hao *et al.* (2015).

All data were analysed using t-tests to compare the treatment and control diets (SAS Institute Inc., Cary, North Carolina, USA). Significance was considered at $P < 0.05$ and tendency when $0.05 \leq P < 0.10$.

Results and Discussion

Throughout the experimental periods, supplementation of FA did not affect the BW of broilers compared with that of the CON group ($P > 0.10$). From days 1 to 21, broilers fed FA had higher ADWG ($P = 0.048$) than CON group (Figure 1).

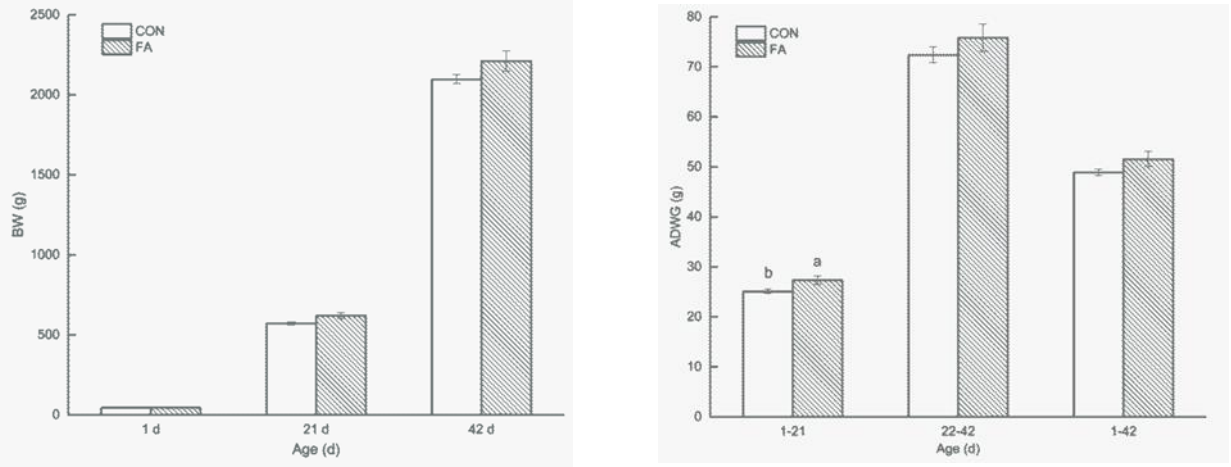

Figure 1 Effects of supplementation with ferulic acid on bodyweight and daily gain of broiler chickens

CON: control, FA: 80 mg ferulic acid/kg, BW: bodyweight, ADWG: average daily weight gain
[a,b] Means with different superscripts differ significantly ($P < 0.05$)

The ADFI of broilers was increased ($P < 0.001$) by feeding with FA in days 22–42. From days 1 to 21, broilers fed FA had lower F:G ($P = 0.002$) than CON group (Figure 2), probably as a result of their increased growth rate. However, growth performance measured as ADWG, ADFI and F:G showed no differences ($P > 0.10$) between FA and CON during the whole period.

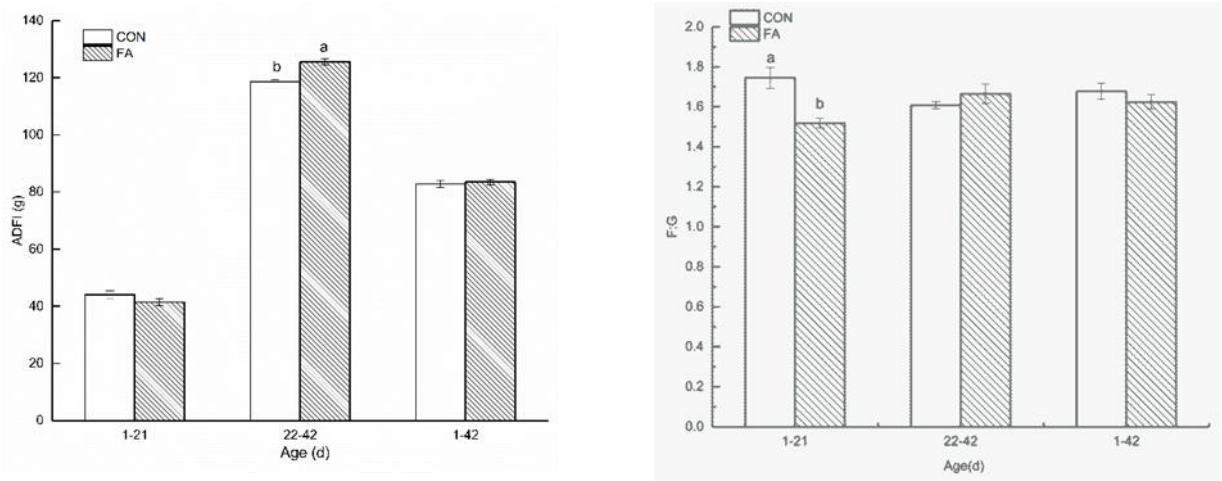

Figure 2 Effects of supplementation with ferulic acid on average daily feed intake and feed conversion ratio of broiler chickens

CON: control, FA: 80 mg ferulic acid/kg, ADFI: average daily feed intake, F:G: feed conversion ratio
[a,b] Means with different superscripts differ significantly (P <0.05)

Natural plant extracts contain a variety of phenolic compounds such as phenolic acids, flavonoids and other polyphenols, which exert health promoting effects in poultry diets (Surai, 2014). Ferulic acid is a copious and almost ubiquitous phenolic acid, present in plant cell wall components as covalent side chains (Kumar & Pruthi, 2014). Inclusion of FA in diets of other farm animals exhibited growth promoting, antioxidative and immune stimulating effects (Wang *et al.*, 2019; Wang *et al.*, 2020; Peña-Torres *et al.*, 2021). The current study assumed that FA supplementation would improve antioxidant capacity and immunity, thereby promoting growth of broilers. The current results supported the hypothesis of elevated ADWG and reduced F:G on day 21 with FA feeding. However, since there were no reports on the effects of FA on broilers, it is difficult to make direct comparisons. In Muscovy broiler ducks, Abdel-Rahman & Mosaad (2013) found the weight gain was increased and feed conversion efficiency was decreased by propolis (a supplement rich in FA). The performance-promoting effects of FA could be related to improved nutrient digestibility (Seven, 2008). It was confirmed by the increased nutrient digestibility during the starter phase in this study. Furthermore, the increases in growth performance of broilers may be associated partly with the antioxidative effects of FA, because higher antioxidant enzyme activities were observed in the current study.

The digestibility of DM, OM, CP and EE were higher (P <0.05) in broilers fed FA diet compared with CON in days 19–21 (Table 2). However, in days 40–42, decreased digestibility of DM, OM and EE (P <0.05) was observed in FA group compared with CON. Additionally, CP digestibility tended (P =0.065) to decrease in FA-fed broilers during the same period.

Table 2 Nutrient digestibility of broilers fed the control diet or a diet supplemented with ferulic acid

Items	Control diet	Diet with 80 mg ferulic acid/kg	*P*-value
Starter (days 19–21)			
Dry matter, %	65.59 ± 2.88	82.93 ± 0.61	< 0.001
Organic matter, %	68.07 ± 2.52	85.05 ± 0.53	< 0.001
Crude protein, %	62.73 ± 3.19	79.07 ± 0.70	< 0.001
Ether extract, %	61.87 ± 3.45	77.09 ± 1.28	0.002
Finisher (days 40–42)			
Dry matter, %	72.07 ± 1.79	66.86 ± 1.03	0.030
Organic matter, %	73.74 ± 1.82	68.92 ± 1.03	0.038
Crude protein, %	68.28 ± 3.24	60.66 ± 1.75	0.065
Ether extract, %	60.73 ± 1.06	50.31 ± 1.16	< 0.001

One of the most important factors for improving growth performance of animals is an increase in apparent digestibility or utilization of major nutrients. In the current study, digestibility of DM, OM, CP and EE was increased by feeding FA in the starter period. The beneficial effects of phenolic compounds on nutrient digestibility of broilers were established in many reports. For instance, Hernández *et al.* (2004) demonstrated that supplementation of plant extracts increased the apparent digestibility of DM and EE. Malekzadeh *et al.* (2018) found that thyme extract increased DM, OM and EE digestibility. Similar findings were supported by Long *et al.* (2020), who found that DM and OM digestibility was improved by *Forsythia suspensa* extract. However, limited data have been reported of FA supplementation. Seven (2008) observed that propolis enhanced the apparent digestibility of DM, OM, CP, EE of broilers under heat stress. The increase in nutrient digestibility was probably related to the activities of digestive enzymes from the pancreas and intestinal mucosa, which might be stimulated by phenolic compounds (Hashemipour *et al.*, 2013). Thus, the authors speculated that FA could enhance nutrient digestibility of broilers by increasing duodenal trypsin activity and jejunal amylase activity in their study.

No significant differences (P >0.10) were observed for FA supplementation in duodenal amylase and lipase activities on days 21 and 42 (Table 3). In comparison with the CON group, FA treatment increased (P <0.001) the duodenal trypsin activity on day 21. However, on day 42, the duodenal trypsin and chymotrypsin activities were decreased by FA supplementation (P <0.05).

Table 3 Duodenal digestive enzyme activity in broilers fed an unsupplemented diet or a diet with ferulic acid

Items	Control diet	Diet with 80 mg ferulic acid/kg	*P*-value
Day 21			
Amylase, U/mg protein	1.54 ± 0.34	1.59 ± 0.13	0.885
Lipase, U/g protein	148.51 ± 7.86	167.64 ± 25.65	0.503
Trypsin, U/mg protein	2070.92 ± 248.88	5919.28 ± 516.23	< 0.001
Chymotrypsin, U/mg protein	7.23 ± 0.26	7.27 ± 0.20	0.920
Day 42			
Amylase, U/mg protein	1.93 ± 0.32	1.79 ± 0.33	0.760
Lipase, U/g protein	118.44 ± 15.66	89.05 ± 21.76	0.332
Trypsin, U/mg protein	7243.40 ± 738.24	3761.53 ± 94.43	0.011
Chymotrypsin, U/mg protein	10.51 ± 0.69	7.48 ± 0.41	0.005

Dietary FA supplementation did not influence jejunal trypsin and chymotrypsin activities on days 21 and 42 (P >0.10) (Table 4). The jejunal amylase activity was increased (P =0.049) by FA supplementation on day 21, whereas no difference was found on day 42 (P >0.10). On the contrary, jejunal lipase activity was unaffected (P >0.10) by FA supplementation, but a decrease (P =0.015) was observed on day 42.

Table 4 Jejunal digestive enzyme activity of broilers fed an unsupplemented diet or a diet with ferulic acid

Items	Control diet	Diet with 80 mg ferulic acid/kg	*P*-value
Day 21			
Amylase, U/mg protein	1.12 ± 0.15	1.85 ± 0.26	0.049
Lipase, U/g protein	118.71 ± 10.89	152.56 ± 37.11	0.360
Trypsin, U/mg protein	3078.22 ± 386.65	2983.45 ± 701.03	0.903
Chymotrypsin, U/mg protein	7.36 ± 0.85	7.60 ± 0.54	0.840
Day 42			
Amylase, U/mg protein	1.74 ± 0.28	1.64 ± 0.30	0.811
Lipase, U/g protein	163.72 ±10.72	92.74 ± 9.52	0.015
Trypsin, U/mg protein	2622.06 ± 456.83	2112.20 ± 628.23	0.529
Chymotrypsin, U/mg protein	7.23 ± 0.52	6.13 ± 0.72	0.252

Digestive enzymes play a key role in digestion of nutrient substrates. Their activities reflect digestive capacity directly, and are closely related to growth performance of broilers (Lee *et al.*, 2003). The potential of phenolic compounds to stimulate digestive enzymes secretion was recorded in several studies (Puvača *et al.*, 2013). Hashemipour *et al.* (2013) reported that supplementation of thymol and carvacrol in broiler diet increased the intestinal and pancreatic trypsin, protease, and lipase enzymes activities. Although Seven *et al.* (2008) indicated that FA-rich propolis favoured the nutrient digestibility of broilers, there are limited reports on its impacts on digestive enzyme activities. Eyng *et al.* (2014) indicated that pancreatic enzyme activity was unaffected by feeding ethanolic extract of propolis. As expected, the current data demonstrated that the use of FA in the diet enhanced duodenal trypsin and jejunal amylase activities on day 21. A possible explanation for this may be because of the improvement of antioxidant status by dietary FA supplementation.

Relative to the CON group (Table 5), FA supplementation increased serum IgM and TNF-α concentrations (P <0.05) and tended (P =0.088) to increase serum IL-2 concentration on day 21, but these effects were not observed on day 42 (P >0.10). No significant differences were noted for the values of IgA, IgG, IL-6 and IL-10 between the treatments in serum samples of broilers on day 21 and 42 (P >0.10).

Table 5 Antibodies and cytokines in serum of broilers fed the control diet or a diet supplemented with ferulic acid

Items	Control diet	Diet with 80 mg ferulic acid/kg	*P*-value
Day 21			
Immunoglobulin A, μg/mL	63.80 ± 7.46	71.11 ± 10.99	0.585
Immunoglobulin G, μg/mL	28.18 ± 2.65	29.96 ± 1.96	0.818
Immunoglobulin M, ng/mL	320.00 ± 25.24	592.14 ± 27.00	< 0.001
Interleukin-2, pg/mL	339.01 ± 34.24	431.67 ± 33.75	0.088
Interleukin-6, pg/mL	86.38 ± 13.15	104.88 ± 13.76	0.369
Interleukin-10, pg/mL	113.92 ± 8.40	124.03 ± 14.74	0.597
Tumour necrosis factor-α, pg/mL	152.78 ± 17.99	221.57 ± 13.12	0.016
Day 42			
Immunoglobulin A, μg/mL	125.60 ± 13.43	158.56 ± 15.61	0.161
Immunoglobulin G, μg/mL	36.15 ± 3.35	30.51 ± 3.62	0.280
Immunoglobulin M, ng/mL	535.71 ± 38.70	600.60 ± 47.55	0.319
Interleukin-2, pg/mL	849.15 ± 43.31	806.45 ± 36.99	0.657
Interleukin-6, pg/mL	172.21 ± 28.58	192.52 ± 13.85	0.515
Interleukin-10, pg/mL	206.76 ± 14.96	189.56 ± 38.94	0.638
Tumour necrosis factor-α, pg/mL	323.04 ± 50.55	392.56 ± 38.30	0.277

The immune status of the host plays an important role in resisting infection. Phenolic compounds in plant extracts improved host immune functions (Lee *et al.*, 2018). FA-rich propolis has been reported to possess immunomodulating activities through the activation of lymphocytes in laying hens (Galal *et al.*, 2008) and lymphocytes and monocytes in Muscovy broiler ducks (Abdel-Rahman & Mosaad 2013). Attia *et al.* (2017) indicated that the antibody titers against Avian influenza, Newcastle disease and infectious Bursal disease and IgA and IgM contents of broilers were increased with propolis supplementation. In the current experiment, dietary FA supplementation promoted the humoral immune response, leading to an increase in serum IgM concentration on day 21. Immunoglobulin A, IgM, and IgG are three major immunoglobulin involved in the maintenance of immune status in broilers. In the current study, the authors found that dietary FA supplementation increased serum TNF-α and IL-2 concentrations on day 21. As the most important T-cell growth factor, cytokine interleukin plays an important role in promoting the antibody production of the host (Choi & Lillehoj, 2000). The current results suggested dietary FA supplementation increased the TNF-α and IL-2 levels, and stimulated the production of IgM. The presence of sufficient levels of antioxidants inside the body protected immune cells from oxidative stress and thus enhanced antibody production (De La Fuente & Victor, 2000). Therefore, the immune stimulating effects of FA may be attributed to its antioxidative activity.

Ferulic acid supplementation decreased (P =0.0025) serum MDA level compared with the CON group

on day 21, whereas the serum SOD and GSH-Px activities did not vary (P >0.10) between groups (Table 6). On day 42, SOD activity was decreased (P <0.05) in FA group compared with CON.

Table 6 Serum antioxidant status of broilers fed the control diet or a diet supplemented with ferulic acid

Items	Control diet	Diet with 80 mg ferulic acid/kg	*P*-value
Day 21			
Superoxide dismutase, U/mL	4.29 ± 0.27	4.82 ± 0.44	0.337
Glutathione peroxidase, U/mL	1509.20 ± 98.04	1567.53 ± 64.36	0.650
Malondialdehyde, nmol/mL	16.87 ± 0.71	15.08 ± 0.56	0.025
Day 42			
Superoxide dismutase, U/mL	7.76 ± 0.31	5.69 ± 0.39	0.005
Glutathione peroxidase, U/mL	2051.28 ± 145.36	1803.76 ± 85.13	0.180
Malondialdehyde, nmol/mL	15.22 ± 0.38	15.82 ± 0.56	0.390

Effects of dietary FA supplementation on hepatic antioxidant status of broilers are presented in Table 7. On day 21, broilers fed FA had greater (P =0.040) GSH-Px activity in the liver than CON. On day 42, the hepatic SOD and GSH-Px activities was decreased (P <0.05) in FA group compared with CON. Moreover, an increasing trend (P =0.078) of MDA was observed in the liver

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Table 7 Hepatic antioxidant status of broilers fed the control diet or a diet supplemented with ferulic acid

Items	Control diet	Diet with 80 mg ferulic acid/kg	*P*-value
Day 21			
Superoxide dismutase, U/mg protein	360.17 ± 33.90	353.03 ± 18.23	0.838
Glutathione peroxidase, U/mg protein	85.14 ± 3.39	114.62 ± 10.75	0.040
Malondialdehyde, nmol/mg protein	2.13 ± 0.12	1.83 ± 0.19	0.215
Day 42			
Superoxide dismutase, U/mg protein	389.01 ± 35.77	259.95 ± 9.80	0.008
Glutathione peroxidase, U/mg protein	136.88 ± 11.75	73.77 ± 5.01	0.003
Malondialdehyde, nmol/mg protein	1.57 ± 0.07	2.06 ± 0.22	0.078

Excessive formation of free radicals and decreased activity of antioxidant defence systems contribute to a disturbance to the equilibrium status of prooxidant and antioxidant system in favour of pro-oxidation (Kennedy *et al.*, 2005), which gives rise to oxidative stress, growth reduction and immune suppression of broilers (Salami *et al.*, 2015). Antioxidant enzymes such as CAT, SOD and GSH-Px form part of the antioxidant defence system against oxidative stress (Wang *et al.*, 2017). Phenolic compounds are regarded as natural sources to scavenge excess free radicals (Krishnaiah *et al.*, 2011) and improve the antioxidant capacity of farm animals (Gessner *et al.*, 2017). Similarly to several other phenolic compounds, FA are able to scavenge free radicals directly via donating one hydrogen atom from its phenolic hydroxyl group (Srinivasan *et al.*, 2007). More importantly, FA could induce the activation of Nrf2-ARE pathway, which leads to an activation of antioxidant enzymes (Mahmoud *et al.*, 2020). The current results showed that dietary supplementation with FA increased hepatic GSH-Px activity of broilers on day 21. In agreement with the current findings, Li *et al.* (2015) indicated that FA increased the hepatic GSH-Px activity of finishing pigs. Wang *et al.*, (2020) confirmed that dietary FA supplementation increased hepatic CAT, T-SOD, and GSH-Px activities and upregulated the protein levels of Nrf2, HO-1 and NQO-1 in weaned piglets. The positive effects of FA-rich propolis on serum SOD and GSH-Px activities in broilers were also reported by Attia *et al.* (2017). Among the biological targets of oxidative stress, lipids are the most involved class of biomolecules. Malondialdihyde is the principal and product of lipid peroxidation. In this study, the MDA level was lower in the serum of broilers fed with FA on day 21. This decrease may be because of elevating antioxidative

enzyme activities. Based on these findings, the authors stated that FA might play an important role as an exogenous antioxidant in improving growth performance and immune status of broilers during the starter phase.

Opposite to results found during the starter phase, FA-fed broilers during the finisher phase had reduced growth performance and antioxidant status at dosage of 80 mg/kg diet. In this study, duodenal trypsin and chymotrypsin activities and jejunal lipase activity were suppressed in broilers supplemented with FA on day 42. The suppression of enzymatic activity was reflected in the decreased digestibility of DM, OM, and EE and increased ADFI. The authors have no clear explanation for these results. Ocak *et al.* (2008) found that the beneficial effects of dry peppermint and thyme on growth performance disappeared at 42 days old. In agreement with these authors, Hernández *et al.* (2004) reported the nutrients digestibility of broilers fed with plant extracts during the finisher phase was lower than during the starter phase. Zdunczyk *et al.* (2002a) reported a negative correlation between phenolic compound contents and nutrient digestibility, which was attributed to the interaction of reactive hydroxyl groups of phenolic compounds with the carbonyl group of digestive enzymes (You *et al.*, 2011). Although no evidence was presented in FA-fed broilers, a study with lambs reported that a prolonged feeding period with FA tended to decrease feedlot performance (Macías-Cruz *et al.*, 2014). Reduced serum SOD activity and hepatic SOD and GSH-Px activities and an increased hepatic MDA level were also observed in this study. Oxidized phenols (such as quinones and semiquinones) formed from FA during redox reactions could be deleterious (Truong *et al.*, 2020). The concentration-dependent antioxidant or prooxidant activities of FA were also reported in an earlier study (Maurya & Devasagayam, 2010). Therefore, the depression of growth performance and antioxidative status of broilers during the finisher phase could be because of the long-term-cumulative effects of FA.

Conclusion

Ferulic acid can serve as an exogenous antioxidant at a dosage of 80 mg/kg diet to enhance growth performance via improvement of digestive enzyme activities, immunity and antioxidant status of broilers in the starter phase.

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Authors’ Contributions

Conceptualization, XA , AG and JQ; Data curation, formal analysis and investigation, JD, RW, XJ, JZ, TG, and YH; Writing-original draft, JD; Writing-review and editing, YW; Funding acquisition, YW, AG and JQ; All authors read and approved the final manuscript.

Conflict of interest declaration

The authors certify that there is no potential conflict of interest to disclose.

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