

Effect of chestnut wood extract on performance, meat quality, antioxidant status, immune function, and cholesterol metabolism in broilers

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ABSTRACT Plant extracts have been proved as natural antioxidants resources as well as alternative feed additives in livestock and poultry species. Chestnut wood extract (**CWE**) as a source of hydrolysable tannic acid was used to evaluate the growth performance, nutrient retention, meat quality, antioxidant status, and immune function of broilers. A total of 168, day-old Arbor Acre male broilers (weight 46.59 ± 0.44 g) were randomly divided to 3 treatments, 7 replicate pens per treatment, 8 broilers per pen. The treatments contain a control diet, **CON** (corn-soybean meal basal diet); an antibiotic diet, **CTC** (basal diet + 75 mg/kg chlortetracycline); and chestnut wood extract diet, **CWE** (basal diet + 1,000 mg/kg chestnut tannins). At the finisher phase, final body weight was higher ($P < 0.05$) in CWE supplemented diet than in CON. Average daily body weight gain was higher ($P < 0.05$) and feed gain ratio was lower ($P < 0.05$) in broilers fed CWE than in those fed CON at the finisher phase. Crude protein digestibility was higher ($P < 0.05$) in broilers offered CWE than that in broilers fed CON and CTC diets. Breast muscle pH

value at 24 h (pH_{24 h}) was higher ($P < 0.05$) in broilers fed CWE than that in those fed CON and CTC diets. The bursa weight was higher ($P < 0.05$) in broilers offered CWE than that in those fed CON and CTC. Total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) values were higher ($P < 0.05$) in both breast muscle and thigh muscle of broilers offered CWE supplemented diet than those in broilers fed CON and CTC diets. Similarly, broilers offered with CWE diets showed higher ($P < 0.05$) T-AOC, GSH-PX, and SOD value in serum than those fed CON and CTC diets. Serum concentration of IgG was higher ($P < 0.05$) in broilers offered with CWE diets than that in those fed CON and CTC diets. Total cholesterol, low-density lipoprotein cholesterol, and urea-N concentration were lower ($P < 0.05$) in broilers offered CWE diet than those in broilers fed CON and CTC diets. It was recommended to supply CWE at the 1,000 mg/kg level for improving antioxidant status, cholesterol metabolism, and growth performance without affecting normal meat quality in broilers.

Key words: antioxidant status, broiler, cholesterol metabolism, meat quality, chestnut wood extract

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INTRODUCTION

Plant extracts have been studied as alternative feed additives and were reported as good source of natural antioxidant to improve animal production and health status (Hu et al., 2017). However, plant extract contains higher amount of polyunsaturated fatty acids that may lead to oxidative stress in chickens (Gao et al., 2010). Tannins are secondary plant metabolites that are commonly classified under 2 groups, condensed tannins

and hydrolysable tannins. Condensed tannins hold the antinutritional factors and could decrease protein digestibility because of its protein precipitation nature, and thus, the long-term uses of condensed tannins could decrease the growth performance and nutrient digestibility in monogastric animals (Smulikowska et al., 2001; Liu et al., 2020). On the other hand, it is believed that hydrolysable tannins have a positive role in growth performance and health status in chickens (Schiavone et al., 2008). Hydrolysable tannic acid is made from wood extract that has higher polyphenolic compounds (Kumar and Vaithyanathan, 1990; Liu et al., 2018). Supplementation of tannins could improve the body weight gain in rabbits (Liu et al., 2012) and broilers (Dong et al., 2015; Xiong et al., 2016). However, Jamroz et al. (2009) found that supplementation of 250 or 500 mg/kg chestnut tannins had no significant

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effects on body weight gain and feed-to-gain ratio, whereas 1,000 mg/kg diets reduced the final body weight in broilers. Furthermore, chestnut tannins could improve intestinal morphology, cytokine expression, and antioxidant activities in broilers (Liu et al., 2018). Dietary supplementation of chestnut wood extract (CWE) as hydrolysable tannins could reduce carcass fat deposition and water-holding capacity of meat and increase polyunsaturated fatty acids in fat tissues of pigs (Rezar et al., 2017). Moreover, hydrolysable tannic acid is commonly used in monogastric animals as a natural source of antioxidants (Biagi et al., 2010; Starčević et al., 2015). Dietary supplementation of antioxidant can play a great role in improving the health status in poultry. Oxidative stress is defined as the rise in reactive oxygen species level, which could reduce the live weight gain via altering normal metabolism and cause poor meat quality by increasing plasma corticosterone level which is associated with paler breast meat in broilers (Kannan et al., 1997). In addition, oxidative stress induces biological damage to DNA, proteins, and lipids and is responsible for several health problems that affect growth performance and productivity of livestock (Xing et al., 2019). In a recent study, Ebrahim et al. (2015) reported that hydrolysable tannic acid is effective in attenuating oxidative stress in broilers. In addition, Schiavone et al. (2008) noted that feed ingredients rich in tannins can be used as a substitute of antibiotics in poultry. Voljč et al. (2013) also reported that sweet CWE could reduce DNA damage and plasma malondialdehyde (MDA) in broilers with a combination of α -tocopherol.

On the other hand, antibiotic has been used for few years with a view to enhance growth performance and health status in broilers (Mahfuz et al., 2019). But over-application of antibiotics in food animal production has created health hazards (Mahfuz et al., 2018a). Currently, the European Union, the United States, and other developed countries have prohibited the application of in-feed antibiotics (Li et al., 2019; Long et al., 2019). Thus, searching an alternative for in-feed antibiotics in poultry is given higher attention by the poultry researchers (He et al., 2019). A great deal of research on poultry nutrition focused on the utilization of different feed grains rich in tannins, but little work has been carried out using purified tannin as feed additives till now.

Therefore, the current experiment was conducted to examine the hypotheses that dietary CWE as a source of hydrolysable tannic acid can replace in-feed antibiotic and improve the growth performance, nutrient digestibility, meat quality, antioxidant status, immune function, and lipid metabolism in broilers.

MATERIALS AND METHODS

Experimental Design and Diets

The research was conducted at a broiler experimental unit, China Agricultural University, and approved by Animal Care and Use Committee of China Agricultural University (Beijing, China; no. CAU-XSPLAB-B-2019-02).

The CWE as a source of hydrolysable tannic acid was provided by the Gruppo Mauro SilvachimicaSrl (Cuneo, Italy), which contained $\geq 75\%$ tannin, crude fiber $< 2.00\%$, ash $< 2.50\%$, and moisture $< 8.00\%$. Tannin percent was measured by gravimetric analysis of vegetable tannin agents and extracts by using the filter Freiberg hide powder method (Kuntzel, 1954). A total of 168, day-old Arbor Acre male broilers (weight 46.59 ± 0.44 g) were randomly divided to 3 treatments, 7 replicate pens per treatment, 8 broilers per pen. All broilers were kept in an environment-controlled room. Brooding house temperature was 34°C , and it was decreased by 3°C for per week until it reached 23°C and then remained constant. The treatments contain a control diet, CON (corn-soybean meal basal diet); an antibiotic diet, CTC (basal diet + 75 mg/kg chlortetracycline); and chestnut wood extract diet, CWE (basal diet + 1,000 mg/kg chestnut tannins). Two different types of diets (starter diet for 0–21 D; finisher diet for 22–42 D) were considered to meet the nutrient levels followed by National Research Council (National Research Council, 1994) specification (Table 1). CWE and antibiotic were mixed with premix first and then mixed thoroughly with each basal diet via feed millers.

Growth Performance and Nutrient Retention

On day 21 and 42, followed by fasting for 12 h, broilers were weighed to calculate average daily weight gain, feed intake, and feed conversion ratio. Fecal samples were collected from day 40 to 42 in each pen, dried in an oven (65°C) for 72 h, and used to prepare the subsample. Diets and fecal subsamples were analyzed for dry matter, organic matter, crude protein, and ether extract (EE) according to the methods of AOAC (2004). Chromium content (2 g/kg) that was used as a marker was analyzed using an atomic absorption spectrophotometer (Z-5000 Automatic Absorption Spectrophotometer; Hitachi, Tokyo, Japan) according to Williams et al. (1962). Gross energy was determined via an automatic isoperibolic oxygen bomb calorimeter (Parr 1281, Automatic Energy Analyzer; Parr Instrument Company, Moline, IL). OM was calculated using the following formula:

$$\text{OM} = 1 - \text{ash content}(\text{DM} - \text{base}).$$

Meat Quality and Viscera Weight

On the morning of day 42, one bird per replicate ($n = 7$) close to the average body weight was slaughtered by cervical dislocation; viscera (liver, spleen, thymus, and bursa), breast muscle, and thigh muscle were collected; and then viscera percentage was calculated.

The pH values at 45 min and 24 h postmortem of meat sample (breast muscle) were measured at 3 locations via portable pH meter (pH-star; Thermo Fisher, Matthaus,

Table 1. Nutrient composition of experimental diets.¹

Item	Start phase (day 1–21)	Finisher phase (day 21–42)
Soybean meal	30.44	24.05
Corn	58.17	64.26
Corn gluten meal	2.0	2.5
Fish meal	2.0	2.0
Soybean oil	3.38	3.6
Dicalcium phosphate	1.50	1.04
Limestone	1.30	1.35
Salt	0.30	0.30
L-Lysine, 78%	0.01	0.08
Methionine, 98%	0.14	0.04
Threonine, 98%	0.01	0.03
Chromic oxide	0.25	0.25
Premix ²	0.50	0.50
Total	100	100
Analyzed value		
Dry matter	88.70	87.91
Crude protein	21.32	19.41
Calcium	1.0	0.90
Available phosphorus	0.45	0.35
Calculated value ³		
Metabolizable energy, MJ/kg	12.79	13.18
Total phosphorus	0.70	0.59
Digestible lysine	1.1	1.0
Digestible methionine	0.50	0.48
Digestible cysteine	0.32	0.28
Digestible threonine	0.83	0.73
Digestible methionine and cysteine	0.82	0.76

¹Chestnut wood extract (1,000 mg/kg) and antibiotic (chlortetracycline, 75 mg/kg) were mixed with premix and then mixed thoroughly with each basal diet.

²The premix provided the following per kilogram of diet: zinc, 60 mg; iron, 100 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.35 mg; selenium, 0.3 mg; vitamin A, 10,000 IU; vitamin D3, 2,850 IU; vitamin E, 30 IU; vitamin K3, 2 mg; vitamin B12, 1.2 mg; riboflavin, 6 mg; nicotinic acid, 40 mg; pantothenic acid, 12 mg; pyridoxine, 3 mg; biotin, 0.2 mg; and choline chloride, 800 mg.

³These values were calculated from data provided by NRC (1994).

Germany). Duplicate measurement of sample was performed, and the average values were considered as the final values. Drip loss was measured according to Cheng et al. (2019). Briefly, the samples were trimmed and weighed and then placed in an inflated plastic bag and hung for 24 h at 4°C. After 24 h, the samples were weighed again. Drip loss was calculated as a percentage based on weight before and after hanging. The color value of breast muscle and thigh muscle was estimated in duplicates using a chromameter (CR-410; Konica Minolta, Tokyo, Japan). The test units L*, a*, and b* indicated the lightness, redness, and the yellowness of meat, respectively.

Analysis of Antioxidant Parameters

On day 42, blood samples were obtained from jugular vein of euthanized birds ($n = 7$). Serum was prepared by centrifuging at $3,000 \times g$ for 20 min at 4°C and was preserved at -80°C for analysis. Concentration of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and MDA in serum, thigh muscle, and breast muscle were determined by using an automatic biochemical analyzer (RA-1000; Bayer Corp., Tarrytown, NY) using colorimetric methods following the instructions of commercial assay

kits (Zhongsheng Biochemical Co., Ltd., Beijing, China).

Analysis of Serum Immune and Lipid Indices

The serum IgA, IgG, IgM, IL-6, IL-1 β , and tumor necrotic factor- α (TNF- α) were measured using ELISA Kits (Shanghai Junshi Biosciences Co. Ltd., Shanghai, China), and value was measured at 450 nm. The concentrations of total cholesterol (TC), triglyceride, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol in serum sample were measured using commercial kits (Zhongsheng Biochemical Co. Ltd., Beijing, China) via an automatic biochemical analyzer (RA-1000; Bayer Corp., Tarrytown, NY).

Statistical Analysis

One-way ANOVA was applied via SPSS (2006). The pen was the experimental unit. The effects of treatment were partitioned into the main effects of CTC and CWE. Duncan's multiple range test was applied to separate the statistical differences. Mean value and SEM were used to express results. $P < 0.05$ is considered significant.

RESULTS

Growth Performance of Broilers

Compared with control (CON), final body weight (finisher phase) was higher ($P < 0.05$) in CWE-supplemented diet. At the finisher phase, average daily body weight gain was higher ($P < 0.05$) and feed gain ratio was lower ($P < 0.05$) in broilers fed CWE than those in broilers fed CON. However, no differences were found when compared with antibiotic (CTC) diet. Similarly, no differences were observed for all the performance parameters among the groups at the start and in the overall period (Table 2).

Nutrient Retention

Crude protein digestibility was higher ($P < 0.05$) in broilers fed CWE than that in those fed CON and CTC diets. No significant differences were observed for dry matter, organic matter, crude protein, gross energy, and EE retention in broilers among the groups (Table 3).

Meat Quality and Viscera Organ Weight

The breast muscle pH value at 24 h (pH_{24 h}) postmortem was higher ($P < 0.05$) in broilers fed CWE than that in those fed CON and CTC diets, while there were no significant differences for other parameters of meat quality including drip loss percent and meat color (Table 4).

The bursa weight was higher ($P < 0.05$) in broilers offered CWE than that in those fed CON and CTC, whereas no significant differences ($P > 0.05$) were noted for other inner relative organ (liver, spleen, thymus) weight (Table 5).

Antioxidant Status in Muscle Tissue and Serum

In breast muscle, T-AOC, GSH-PX, and SOD values were higher ($P < 0.05$) in broilers offered CWE-supplemented diet than those in broilers fed CON and CTC diets (Table 6). Similarly in thigh muscle, T-AOC, GSH-PX, and SOD values were higher ($P < 0.05$) in broilers offered CWE-supplemented diet than those in broilers fed CON and CTC diets. However, no significant differences were noted for MDA value among the treatments of both samples.

Broilers offered with CWE diets showed higher ($P < 0.05$) T-AOC, GSH-PX, and SOD values in serum than those fed CON and CTC diets (Table 6). MDA value was not affected by CWE supplementation.

Immune Status in Serum

Serum concentration of IgG was higher ($P < 0.05$) in broilers offered with CWE diets than that in those fed CON and CTC diets (Table 7). No significant differences were noted for the other value of measured immune subparameters (IgA, IgM, IL-6, IL-1 β , TNF- α) among the treatments in the serum sample of experimental broilers.

Metabolic Indices in Serum

TC and LDL concentration were lower ($P < 0.05$) in broilers offered CWE diet than in those fed CON and CTC diets (Table 8). Urea-N concentration was lower ($P < 0.05$) in CWE-supplemented diets than that in CON and CTC diets. However, no significant effects were noted for triglyceride and HDL concentration among the treatments in this study.

Table 2. Effect of dietary chestnut wood extract supplementation on growth performance in broilers.¹

Item	CON	CTC	CWE	SEM	<i>P</i> value
Starter phase (day 0–day 21)					
Initial body weight (g)	46.92	46.32	46.54	0.44	0.611
Final body weight (g)	573.72	566.12	549.57	10.26	0.410
Average daily weight gain (g)	27.32	26.96	26.17	0.94	0.842
Average daily feed intake (g)	36.99	36.42	35.22	0.98	0.454
Feed-to-gain ratio (g/g)	1.35	1.35	1.34	0.02	0.941
Finisher phase (day 21–day 42)					
Initial body weight (g)	573.72	566.12	549.57	10.26	0.410
Final body weight (g)	1993.1 ^b	2067.2 ^a	2028.9 ^a	27.58	0.038
Average daily weight gain (g)	67.59 ^b	71.48 ^a	70.44 ^a	1.20	0.022
Average daily feed intake (g)	105.14	104.77	101.91	2.97	0.715
Feed-to-gain ratio (g/g)	1.56 ^a	1.47 ^b	1.45 ^b	0.04	0.032
Overall period (day 0–day 42)					
Average daily weight gain (g)	47.46	49.22	48.31	0.63	0.063
Average daily feed intake (g)	71.07	70.60	68.57	1.66	0.832
Feed-to-gain ratio (g/g)	1.49	1.43	1.41	0.03	0.264

^{a,b}Values with different superscripts within the same row indicate a significant difference at $P < 0.05$.

SEM, pooled standard error of the means. Level of significance at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract.

¹Data represented the mean value of 56 broilers per treatment.

Table 3. Effect of dietary chestnut wood extract supplementation on nutrient retention in broilers (%).¹

Item (%)	CON	CTC	CWE	SEM	P value
Dry matter	75.07	72.84	74.70	0.61	0.305
Organic matter	79.40	77.23	78.64	0.55	0.401
Crude protein	62.20 ^b	63.50 ^b	65.38 ^a	1.15	0.031
Gross energy	77.65	75.74	77.39	0.57	0.358
Ether extract	92.24	92.66	91.85	0.42	0.773

^{a,b}Values with different superscripts within the same row indicate a significant difference at $P < 0.05$.

SEM, pooled standard error of the means. Level of significant at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract.

¹Data represented the mean value of 7 sample per treatment.

DISCUSSION

In this experiment, the CWE was used as source of hydrolysable tannic acid in broiler diets which contained more than 75% tannins. Our present study did not find any significant differences in broiler performances during the overall experimental period. Similar to the current finding, [Jamroz et al. \(2009\)](#) did not find any significant effects on body weight gain and feed-to-gain ratio in broilers fed chestnut tannins (250 or 500 mg/kg). In addition, [Brenes et al. \(2008\)](#) used grape pomace (condensed tannins about 1,500 mg/kg) at different levels (15 mg, 30 mg, and 60 mg/kg) in broiler diets and did not find any significant difference in body weight gain and feed intake compared with control, which was similar to our present finding. We hypothesized that the supplementation dosages of CWE might be smaller to change the performances parameters during the overall experimental period (day 1–42). In addition, [Čandek-Potokar et al. \(2015\)](#) reported that there were no significant effects on average daily gain and gain-to-feed ratio in pigs fed with tannin-supplemented diets. However, we have observed a higher final body weight and daily body weight gain at the finisher period, which was due to the higher crude protein retention in TA-supplemented diets of experimental broilers. [Schiaivone et al. \(2008\)](#) reported that the final body weight was higher in experimental broilers offered natural extract of chestnut wood at 0.2% level, which was also similar with our current findings. Similarly, some past studies reported that several tannin sources could improve growth performance in

Table 4. Effect of dietary chestnut wood extract supplementation on meat quality in broilers.¹

Item	CON	CTC	CWE	SEM	P value
pH-45 min	6.69	6.73	6.67	0.05	0.162
pH-24 h	5.84 ^b	6.02 ^{a,b}	6.16 ^a	0.01	0.027
Drip loss (%)	2.79	3.13	2.82	0.23	0.478
L*	47.46	46.84	47.06	0.56	0.723
a*	2.41	2.59	2.81	0.15	0.593
b*	9.06	8.20	8.86	0.51	0.391

SEM, pooled standard error of the means. Different superscript lower-case letters in the same row means significantly different at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract.

¹Data represented the mean value of 7 samples per treatment.

Table 5. Effect of dietary chestnut wood extract supplementation on viscera organ weight in broilers (% live weight).¹

Item (% live weight)	CON	CTC	CWE	SEM	P value
Liver	1.98	1.77	1.87	0.06	0.404
Spleen	0.13	0.12	0.11	0.01	0.452
Thymus	0.25	0.21	0.26	0.02	0.501
Bursa	1.9 ^b	2.2 ^b	2.5 ^a	0.02	0.036

SEM, pooled standard error of the means. Different superscript lower-case letters in the same row means significantly different at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract.

¹Data represented the mean value of 7 sample per treatment.

pigs and chickens as well ([Brus et al., 2013](#); [Starčević et al., 2015](#)).

Higher crude protein digestibility in our trail was similar to the results noted by [Brufau et al. \(1998\)](#) who used heat treatment of faba bean (*Vicia faba* L) that contains tannin in Leghorn chickens. We hypothesized that CWE as a source of hydrolysable tannins was well absorbed and enhanced digestive enzyme activities in the gastrointestinal tract of experimental broilers that may had a role in improving crude protein digestibility in this study. [Molino et al. \(2018\)](#) observed that chestnut hydrolysable tannins were easily available for microbial fermentation as it has prebiotic activity that may be beneficial for nutrient utilization. However, [Schiaivone et al. \(2008\)](#) did not find any significant differences in digestibility of dry matter, EE, and energy in broilers offered chestnut tannins which justified our present findings. Data regarding the effects of hydrolysable tannins on nutrient digestibility in broilers are still scarce. The actual mechanism to increase relative bursa weight in the present trail was not clear. However, we hypothesize that it may be associated with better immunity of birds fed CWE. The bursa is a very important central immunity organ, which is an indicator of better health status and good physiological response to body immune system

Table 6. Effect of dietary chestnut wood extract supplementation on muscle tissue and serum antioxidant status in broilers.¹

Item	CON	CTC	CWE	SEM	P value
Breast muscle					
T-AOC (U/mg)	0.90 ^b	0.80 ^b	0.96 ^a	0.03	0.014
GSH-PX (U/mg)	79.40 ^b	74.17 ^c	91.41 ^a	3.4	0.042
SOD (U/mg)	7.12 ^{a,b}	6.61 ^b	7.89 ^a	0.25	0.032
MDA (nmol/mg)	0.59	0.50	0.53	0.02	0.337
Thigh muscle					
T-AOC (U/mg)	1.01 ^{a,b}	0.86 ^b	1.11 ^a	0.03	0.022
GSH-PX (U/mg)	84.83 ^b	83.77 ^b	89.07 ^a	1.97	0.031
SOD (U/mg)	8.45 ^b	9.07 ^b	10.48 ^a	0.28	0.003
MDA (nmol/mg)	0.65	0.58	0.60	0.02	0.478
Serum					
T-AOC (U/mL)	7.79 ^b	8.55 ^{a,b}	9.28 ^a	0.22	0.011
GSH-PX (U/mL)	825.40 ^b	855.39 ^{a,b}	880.45 ^a	8.74	0.021
SOD (U/mL)	24.09 ^c	26.31 ^b	29.24 ^a	0.68	0.001
MDA (nmol/mL)	5.52	5.62	5.23	0.20	0.726

SEM, pooled standard error of the means. Different superscript lower-case letters in the same row means significantly different at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

¹Data represented the mean value of 7 sample per treatment.

Table 7. Effect of dietary chestnut wood extract supplementation on serum immune parameters in broilers.¹

Item	CON	CTC	CWE	SEM	<i>P</i> value
IgA (g/L)	4.27	4.25	4.26	0.05	0.983
IgG (g/L)	2.28 ^b	2.29 ^b	2.38 ^a	0.03	0.033
IgM (g/L)	1.68	1.68	1.64	0.02	0.655
IL-6 (pg/mL)	125.32	133.15	128.66	1.36	0.070
IL-1 β (pg/mL)	27.97	26.92	24.80	1.51	0.163
TNF- α (pg/mL)	47.47	51.73	47.86	1.16	0.267

SEM, pooled standard error of the means. Different superscript lower-case letters in the same row means significantly different at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-6, interleukin-6; IL-1 β , interleukin1 β ; TNF- α , tumor necrotic factor- α .

¹Data represented the mean value of 7 sample per treatment.

in poultry (Mahfuz et al., 2018b). The other relative organs' weight did not differ via CWE supplementation in our study, which ensured the normal health status of broilers fed CWE during the experimental period. This is in agreement with the study of Jamroz et al. (2009) who noted that the inner organs' weight (liver, gizzard, heart, giblets) was not affected in broilers offered sweet chestnut tannins.

Manipulation of diets by different treatments can improve the meat quality in poultry (Cheng et al., 2019). The higher pH value at 24 h (pH_{24 h}) in the breast muscle of broilers fed CWE-supplemented diets might be a good indicator of meat quality because its declining rate is associated to meat tenderness (Lonergan et al., 2010; Guimarães et al., 2014). Lower pH and water-holding capacity increased the loss of soluble nutrients which ultimately led to a poor meat quality (Ozturk et al., 2011). However, this study did not find any significant differences in meat color and dressing percent, which highlighted that feeding CWE had no unpleasant effects on meat quality in broilers. We also hypothesized that dietary inclusion of CWE up to 1,000 mg/kg does not affect the overall meat quality in broilers, which may probably be the lowest level of supplementation in diets. Similar to our finding, dietary supplementation of sweet chestnut tannins had no effects on meat color in pigs (Rezar et al., 2017).

This study assumed that CWE contains hydrolysable tannic acid that may have had a role in increasing the value of T-AOC, SOD, and GSH-PX in serum, breast

muscle, and thigh muscle samples of broilers. A previous study reported that the extracts of chestnut skins had antioxidant properties, particularly against lipid peroxidation (Barreira et al., 2008). In a recent study, Liu et al. (2018) noted that inclusion of 2 g/kg chestnut tannins could increase T-AOC and SOD values in the jejunal mucosa of broilers. On the other hand, Starčević et al. (2015) found higher oxidative susceptibility of the liver and breast muscle in broilers fed tannic acid at the 5-g/kg level. Similarly, Dong et al. (2015) found that broilers fed with polyphenolic extracts as tannins had higher T-AOC, SOD, and GSH-PX values in intestinal epithelial cell line-6. Moreover, Brenes et al. (2008) noted higher antioxidant activities in serum and breast muscle of broilers offered grape pomace (tannins about 1,500 mg/kg) at 60 g/kg. With the contrast of the study by Liu et al. (2009), MDA levels in muscle were lower in rabbits fed chestnut tannins. Voljč et al. (2013) also found lower plasma MDA in broilers fed with sweet chestnut wood and α -tocopherol. The differences of MDA values may be associated with animal species, dosages, and interaction of diets in treatment. Collectively, the significant positive role on antioxidant status in serum and muscle tissue indicated that supplementation of CWE may be used as a potential natural antioxidant in broiler diets.

In the present research, serum IgG was increased in broilers fed with CWE which could be beneficial because of their fast-growing nature and susceptibility to environmental stressors. IgG is a leading antibody for mucosal immunity that can neutralize pathogens via the nonspecific defense system of body. Previous study also showed that immunity was influenced by improving the antioxidant status of broilers (Iqbal et al., 2015). However, this study did not observe any significant differences in proinflammatory cytokines. Liu et al. (2018) reported that IL-6 and TNF- α concentration of jejuna mucosa were decreased in heat-stressed broilers offered chestnut tannins. As the present experiment was conducted in normal condition, the optimum level of proinflammatory cytokines expression was expected. In this study, we have applied only one dosage of CWE; thus, this study could not conclude the firm role of CWE supplementation in immune function in broilers. Therefore, we suggest for further research to investigate the role of CWE at different levels of supplementation in immune function in broilers.

This study also ensured the positive role of CWE in cholesterol metabolism (TC and LDL) in broilers. We hypothesized that CWE may have had a role in reducing the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A in experimental broilers. Abdulkarimi et al. (2011) reported that MHG-CoA is the key regulatory enzyme for cholesterol synthesis. Furthermore, additional fiber in chestnut wood may play a role in reducing cholesterol concentration in serum of experimental broilers via improving metabolism. Similar to the current finding, Starčević et al. (2015) found a lower cholesterol concentration in serum of broilers fed tannic acid at 5 g/kg level. In addition, TC and LDL concentration

Table 8. Effect of dietary chestnut wood extract supplementation on serum metabolic indices in broilers.¹

Item	CON	CTC	CWE	SEM	<i>P</i> value
TC (mmol/L)	1.55 ^a	1.60 ^a	1.19 ^b	0.07	0.023
TG (mmol/L)	0.36	0.33	0.32	0.02	0.705
HDL (mmol/L)	1.03	1.01	0.96	0.03	0.614
LDL (mmol/L)	0.31 ^a	0.30 ^a	0.19 ^b	0.02	0.035
Urea-N (mmol/L)	1.23 ^a	1.24 ^a	1.19 ^b	0.03	0.023

SEM, pooled standard error of the means. Different superscript lower-case letters in the same row means significantly different at $P < 0.05$.

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); CWE, chestnut wood extract; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

¹Data represented the mean value of 7 sample per treatment.

were found to be lower in broilers fed tannins containing thyme extract (Abdulkarimi et al., 2011). On the other hand, hypercholesterolemia and LDL concentration in tissue were associated with endothelial dysfunction and abnormal inflammation (Barbalho et al., 2009). Our study showed that supplementation of CWE could reduce the level of uric acid in serum of broilers than non-supplemented diets. Urea-N is a microbial product and harmful for bird's health (Abd El-Hack et al., 2017). We hypothesized that serum uric acid level was closely related to higher crude protein retention in this experiment. A high concentration of urea-N in serum is a risk indicator of cardiovascular diseases, whereas the low concentration of uric acid has protective effects on oxidative stress in body (Glantzounis et al., 2005). In addition, the higher concentration of urea nitrogen in serum was reported as transport stressor in broilers (Pan et al., 2018a,b).

CONCLUSION

Broiler diets supplemented with CWE at 1,000 mg/kg level can be applied to improve antioxidant status, cholesterol metabolism, and growth performance without affecting normal meat quality. Thus, the supplementation of CWE as a potential natural antioxidant as well as an antibiotics substitute can be another strategy to improve the antioxidant status and growth performance in broilers.

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REFERENCES

- Abdulkarimi, R., M. Daneshyar, and A. Aghazadeh. 2011. Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. *Ital. J. Anim. Sci.* 10:101–105.
- Abd El-Hack, M. E., S. A. Mahgoub, M. Alagawany, and E. A. Ashour. 2017. Improving productive performance and mitigating harmful emissions from laying hen excreta via feeding on graded levels of corn DDGS with or without *Bacillus subtilis* probiotic. *J. Anim. Physiol. Anim. Nutr.* 101:904–913.
- Association of Official Analytical Chemists (AOAC). 2004. *Official Methods for Analysis*. 13th ed. Association of Official Analytical Chemists, Washington, DC.
- Barbalho, S. M., S. A. P. Machado, O. E. Prado, M. E. Paiva-Filho, K. A. Martuchi, L. N. Coelho, R. M. Deus, V. Sasaki, B. L. Silva, and M. Oshiiwa. 2009. Mentha piperita effect on wister rat plasma lipids. *Braz. Arch. Biol. Technol.* 52:1137–1142.
- Barreira, J. C. M., I. C. F. R. Ferreira, M. B. P. P. Oliveira, and J. A. Pereira. 2008. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chem.* 107:1106–1113.
- Biagi, G., I. Cipollini, B. R. Paulicks, and F. X. Roth. 2010. Effect of tannins on growth performance and intestinal ecosystem in weaned piglets. *Arch. Anim. Nutr.* 64:121–135.
- Brenes, A., A. Viveros, I. Gon, C. Centeno, S. G. Sáyago-Ayerdy, I. Arija, and F. Saura-Calixto. 2008. Effect grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens. *Poult. Sci.* 87:307–316.
- Brufau, J., D. Boros, and R. R. Marquardt. 1998. Influence of growing season, tannin content and autoclave treatment on the nutritive value of near-isogenic lines of faba beans (*Vicia faba L.*) when fed to Leghorn chicks. *Br. Poult. Sci.* 39:97–105.
- Brus, M., J. Dolinsek, A. CenCic, and D. Skorjanc. 2013. Effect of chestnut (*Castanea sativa Mill.*) wood tannins and organic acids on growth performance and faecal microbiota of pigs from 23 to 127 days of age. *Bulgarian J. Agric. Sci.* 19:841–847.
- Čandek-Potokar, M., M. Škrlep, N. Batorek Lukač, G. Zamaratskaia, M. Prevolnik Povše, Š. Velikonja Bolta, V. Kubale, and G. Bee. 2015. Hydrolysable tannin fed to entire male pigs affects intestinal production, tissue deposition and hepatic clearance of skatole. *Vet. J.* 204:162–167.
- Cheng, Y., Y. Chen, J. Li, H. Qu, Y. Zhao, C. Wen, and Y. Zhou. 2019. Dietary β -Sitosterol improves growth performance, meat quality, antioxidant status, and mitochondrial biogenesis of breast muscle in broilers. *Animals* 9:71.
- Dong, S., H. Li, L. Casco, Y. Xiong, K. J. Guo, and I. Zoccarato. 2015. Antioxidative activity of the polyphenols from the involucre of *Castanea mollissima blume* and their mitigating effects on heat stress. *Poult. Sci.* 94:1096–1104.
- Ebrahim, R., J. B. Liang, M. F. Jahromi, P. Shokryazdan, M. Ebrahimi, W. L. Chen, and Y. M. Goh. 2015. Effects of tannic acid on performance and fatty acid composition of breast muscle in broiler chickens under heat stress. *Ital. J. Anim. Sci.* 14:3956.
- Gao, J., H. Lin, X. J. Wang, Z. C. Song, and H. C. Jiao. 2010. Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. *Poult. Sci.* 89:318–327.
- Glantzounis, G. K., E. C. Tsimoyiannis, A. M. Kappas, and D. A. Galaris. 2005. Uric acid and oxidative stress. *Curr. Pharm. Des.* 11:4145–4151.
- Guimarães, J. B., É. C. Dos Santos, E. S. Dias, A. G. Bertechini, C. L. da Silva Ávila, and F. S. Dias. 2014. Performance and meat quality of broiler chickens that are fed diets supplemented with *Agaricus brasiliensis* mushrooms. *Trop. Anim. Health Prod.* 46:1509–1514.
- He, T. F., S. F. Long, S. Mahfuz, D. Wu, X. Wang, X. Wei, and X. S. Piao. 2019. Effects of probiotics as antibiotics substitutes on growth performance, serum biochemical parameters, intestinal morphology, and barrier function of broilers. *Animals* 9:985.
- Hu, Q., S. Li, Y. Zhang, Z. Zhuo, and J. Feng. 2017. Phytosterols on growth performance, antioxidant enzymes and intestinal morphology in weaned piglets. *J. Sci. Food Agric.* 97:4629–4634.
- Iqbal, Z., Z. Kamran, J. Sultan, A. Ali, S. Ahmad, M. Shahzad, U. Ahsan, S. Ashraf, and M. Sohail. 2015. Replacement effect of vitamin E with grape polyphenols on antioxidant status, immune, and organs histopathological responses in broilers from 1- to 35-d age. *J. Appl. Poult. Res.* 24:127–134.
- Jamroz, D., A. Wiliczekiewicz, J. Skorupińska, J. Orda, J. Kuryzsko, and H. Tschirch. 2009. Effect of sweet chestnut tannin (SCT) on the performance, microbial status of intestine and histological characteristics of intestine wall in chickens. *Br. Poult. Sci.* 50:687–699.
- Kannan, G., J. L. Heath, C. J. Wabeck, and J. A. Mench. 1997. Shackling of broilers: effects on stress responses and breast meat quality. *Br. Poult. Sci.* 38:323–332.
- Kumar, R., and S. Vaithyanathan. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. *Anim. Feed Sci. Technol.* 30:21–38.
- Kuntzel, A. 1954. Die quantitative Gerbstoffanalyse nach dem Filtterverfahren. *Das Leder* 5:S28–S31.
- Liu, H. W., F. Gai, L. Gasco, A. Brugiapaglia, C. Lussiana, K. J. Guo, J. M. Tong, and I. Zoccarato. 2009. Effects of chestnut tannins on carcass characteristics, meat quality, lipid oxidation and fatty acid composition of rabbits. *Meat Sci.* 83:678–683.
- Liu, H. W., D. W. Zhou, J. M. Tong, and V. Vaddella. 2012. Influence of chestnut tannins on welfare, carcass characteristics, meat

- quality, and lipid oxidation in rabbits under high ambient temperature. *Meat Sci.* 90:164–169.
- Liu, H. W., K. Li, J. S. Zhao, and W. Deng. 2018. Effects of chestnut tannins on intestinal morphology, barrier function, proinflammatory cytokine expression, microflora and antioxidant capacity in heat stressed broilers. *J. Anim. Physiol. Anim. Nutr.* 102:717–726.
- Li, M., S. F. Long, Q. Q. Wang, L. H. Zhang, J. X. Hu, J. Yang, Z. B. Cheng, and X. S. Piao. 2019. Mixed organic acids improve nutrients digestibility, volatile fatty acids composition and intestinal microbiota in growing-finishing pigs fed high-fiber diet. *Asian-Australas J. Anim. Sci.* 32:856–864.
- Liu, H. S., J. X. Hu, S. Mahfuz, and X. S. Piao. 2020. Effects of hydrolysable tannins as zinc oxide substitutes on antioxidant status, immune function, intestinal morphology, and digestive enzyme activities in weaned piglets. *Animals* 10:757.
- Lonergan, E. H., W. Zhang, and S. M. Lonergan. 2010. Biochemistry of postmortem muscle-lessons on mechanisms of meat tenderization. *Meat Sci.* 86:184–195.
- Long, S. F., L. Liu, S. J. Liu, S. Mahfuz, and X. S. Piao. 2019. Effects of Forsythia suspense extract as an antibiotics substitute on growth performance, nutrient digestibility, serum antioxidant capacity, fecal *Escherichia coli* concentration and intestinal morphology of weaned piglets. *Animals* 9:729.
- Mahfuz, S., H. Song, Y. Miao, and Z. Liu. 2018a. Dietary inclusion of mushroom (*Flammulina velutipes*) stem waste on growth performance and immune responses in growing layer hens. *J. Sci. Food Agric.* 99:703–710.
- Mahfuz, S. U., M. Chen, J. S. Zhou, S. Wang, J. Wei, Z. Liu, and H. Song. 2018b. Evaluation of golden needle mushroom (*Flammulina velutipes*) stem waste on pullet performance and immune response. *S. Afr. J. Anim. Sci.* 48:563–571.
- Mahfuz, S., T. F. He, S. J. Liu, D. Wu, S. Long, and X. S. Piao. 2019. Dietary inclusion of mushroom (*Flammulina velutipes*) stem waste on growth performance, antibody response, immune status, and serum cholesterol in broiler chickens. *Animals* 9:692.
- Molino, S., M. Fernández-Miyakawa, S. Giovando, and J. A. Rufián-Henares. 2018. Study of antioxidant capacity and metabolization of quebracho and chestnut tannins through in vitro gastrointestinal digestion-fermentation. *J. Func. Foods* 49:188–195.
- National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. National Academi Press, Washington, DC.
- Ozturk, E., N. Ocak, A. Turan, G. Erener, A. Altop, and S. Cankaya. 2011. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. *J. Sci. Food Agric.* 92:59–65.
- Pan, L., P. F. Zhao, X. K. Ma, Q. H. Shang, S. F. Long, Y. Wu, and X. S. Piao. 2018a. Forsythia suspensa extract protects broilers against breast muscle oxidative injury induced by corticosterone mimicked pre-slaughter acute stress. *Poult. Sci.* 97:2095–2105.
- Pan, L., X. K. Ma, P. F. Zhao, Q. H. Shang, S. F. Long, Y. Wu, and X. S. Piao. 2018b. Forsythia suspensa extract attenuates breast muscle oxidative injury induced by transport stress in broilers. *Poult. Sci.* 97:1554–1563.
- Rezar, V., J. Salobir, A. Levart, U. Tomažin, M. Škrlep, L. Batorek, N. Batorek-Lukač, and M. Čandek-Potokar. 2017. Supplementing entire male pig diet with hydrolysable tannins: effect on carcass traits, meat quality and oxidative stability. *Meat Sci.* 133:95–102.
- Schiavone, A., K. Guo, S. Tassone, L. Gasco, E. Hernandez, R. Denti, and I. Zoccarato. 2008. Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen balance of broiler chicks. *Poult. Sci.* 87:521–527.
- Smulikowska, S., B. Pastuszewska, E. Swiech, A. Ochtabin, A. Mieczkowska, V. C. Nguyen, and L. Buraczewska. 2001. Tannin content affects negatively nutritive value of pea for monogastrics. *J. Anim. Feed Sci.* 10:511–523.
- SPSS. 2006. *Statistical Software Package for the Social Sciences*. SPSS Inc., Chicago, IL, 20.0.
- Starčević, K., L. Krstulović, D. Brozić, M. Maurić, Z. Stojević, Ž. Mikulec, M. Bajić, and T. Mašek. 2015. Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. *J. Sci. Food Agric.* 95:1172–1178.
- Voljč, M., A. Levart, S. Zgur, and J. Salobir. 2013. The effect of α -tocopherol, sweet chestnut wood extract and their combination on oxidative stress in vivo and the oxidative stability of meat in broilers. *Br. Poult. Sci.* 54:144–156.
- Williams, C. H., D. J. David, and O. Iismaa. 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. Agric. Sci.* 59:381–385.
- Xing, T., G. Feng, R. K. Tume, G. Zhou, and X. Xu. 2019. Stress effects on meat quality: a mechanistic perspective. *Compr. Rev. Food Sci. Food Saf.* 18:380–401.
- Xiong, Y., S. Dong, X. Zhao, K. J. Guo, L. Gasco, and I. Zoccarato. 2016. Gene expressions and metabolomic research on the effects of polyphenols from the involucre of *Castanea mollissima* Blume on heat-stressed broilers chicks. *Poult. Sci.* 95:1869–1881.