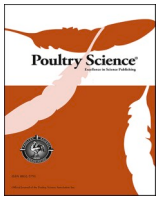
104 (2025) 104523

Contents lists available at ScienceDirect

Poultry Science

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



Effects of dietary dihydroartemisinin on growth performance, meat quality, and antioxidant capacity in broiler chickens

Xiaoxiao Pu, Yuxuan Liang, Jiafang Lian, Miaoxuan Xu, Yalan Yong, Hao Zhang, Lili Zhang, Jingfei Zhang *

College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095, PR China

ARTICLE INFO

Keywords:
broiler chicken
dihydroartemisinin
growth performance
meat quality
antioxidant capacity

ABSTRACT

This study aimed to investigate the effects of dietary dihydroartemisinin on the growth performance, meat quality, and antioxidant capacity of broiler chickens. Four-hundred one-day-old Arbor Acres male broilers were randomly assigned to five treatment groups with eight replicates and ten birds each. All broilers were fed a basal diet containing 0, 5, 10, 20 or 40 mg/kg dihydroartemisinin. The results showed that dihydroartemisinin at 10 mg/kg quadratically increased ADG, and dihydroartemisinin at 10 and 20 mg/kg quadratically increased ADFI during the days 1–21 period. Compared to the control group, dihydroartemisinin at 10 and 20 mg/kg quadratically decreased the drip loss at 24 h. Dihydroartemisinin linearly and quadratically decreased the L* value of breast muscles. Dihydroartemisinin at 20–40 mg/kg linearly and quadratically decreased the MDA concentrations at D5 and D 7 of postmortem storage. Dihydroartemisinin linearly and quadratically increased the ABTS scavenging activity at D 7 of postmortem storage. Dietary 20 mg/kg dihydroartemisinin at 21 days and 40 mg/kg dihydroartemisinin at 42 days linearly and quadratically increased serum glutathione concentrations. Dihydroartemisinin at 5–40 mg/kg linearly increased serum total superoxide dismutase activity at 42 days. Dihydroartemisinin at 10–20 mg/kg quadratically decreased serum malondialdehyde contents at 42 days. At 21 days, 20 mg/kg dihydroartemisinin quadratically increased hepatic glutathione concentrations and catalase activities. Compared to the control group, 40 mg/kg dihydroartemisinin linearly and quadratically decreased hepatic malondialdehyde contents. At 42 days, 20 mg/kg dihydroartemisinin quadratically increased catalase activities and reduced the malondialdehyde contents in liver. Dihydroartemisinin quadratically increased the hepatic mRNA expression of *Nrf2*. Compared to the control group, dihydroartemisinin at 10 and 20 mg/kg quadratically induced the hepatic mRNA expression of *HO-1*. Dihydroartemisinin at 10–40 mg/kg linearly and quadratically increased the mRNA expression of *CAT* in liver. These results showed that dihydroartemisinin improved growth performance, meat quality, and antioxidant capacity of broiler chickens, especially at 10 and 20 mg/kg.

Introduction

In China, poultry is the second most important livestock after pigs, helping to meet the growing demand for meat and eggs. Modern broiler chicken production is characterized by rapid growth and high-density feeding. However, broilers are vulnerable to intensive production, various diseases, and stress, which can lead to suboptimal performance and economic losses. For many years, antibiotics have been used as growth promoters to reduce the occurrence of diseases and improve animal performance (Cheng et al., 2014). However, over the last 100 years, the overuse of antibiotics in poultry has exacerbated drug resistance, increasing the risk to public health and food safety (Kim and Ahn, 2022). Thus, there is an urgent need for safe and environmentally friendly alternatives to antibiotics. In China, the inclusion of dietary antibiotics in feed was banned on July 1, 2020, which further promoted the already burgeoning research on antibiotics alternatives to ensure livestock health and enhance production efficiency. Plant extracts, especially those with individually isolated active components, are among the most promising alternatives (Lillehoj et al., 2018).

Artemisia annua L. is an annual herb belonging to the *Asteraceae* family that is widely distributed worldwide. As a medicine plant native to China, *Artemisia annua* L. is traditionally used to treat fever and

* Corresponding author.
E-mail address: zhangjingfei@njau.edu.cn (J. Zhang).

https://doi.org/10.1016/j.psj.2024.104523
Received 3 August 2024; Accepted 5 November 2024
Available online 10 November 2024

malaria (Brisibe et al., 2009). Our previous studies found that enzymatically treated *Artemisia annua* L. not only effectively alleviated the growth depression of broilers under heat stress, but also repaired the intestinal injury in pigs (Wan et al., 2018; Xiong et al., 2022; Zhang et al., 2020). The active ingredients derived from *Artemisia annua* L. consist of volatile essential oils, sesquiterpenes, phenols, monoterpenes and coumarins (Septembre-Malaterre et al., 2020). Artemisinin was discovered in *Artemisia annua* L. and used as an effective antimalarial drug by Youyou Tu in 1972. Artemisinin is an enantiomerically pure sesquiterpene that plays an essential role in antioxidant, anti-inflammatory, antitumor, antidiabetic, antimalarial, neuroprotective, and hepatoprotective activities (Bisht et al., 2021). As the most studied constituent of *Artemisia annua* L., artemisinin has been used in medicine, function foods, and dietary supplements in feed. However, poor solubility in water, low oral bioavailability, and a short half-life severely compromises its efficiency; moreover, increasing resistance has limited its application in animal production in recent years.

Dihydroartemisinin is a semisynthetic derivative of artemisinin and its main active metabolite *in vivo*. Pharmacokinetics studies have shown that dihydroartemisinin has a longer half-life, is more stable, and is more active than artemisinin (Batty et al., 2002). Currently, dihydroartemisinin is attracting increasing attention as a first-line treatment for malaria and as an alternative to antibiotics in animal production. Dihydroartemisinin has been proven to have growth-promoting, antioxidant, anti-inflammatory, and immunomodulatory effects. One study noted that dihydroartemisinin increased nutrient digestibility, improved the intestinal structure, and promoted growth performance in weaned piglets (Niu et al., 2020). Dihydroartemisinin also stimulates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, regulates superoxide dismutase (SOD) activity and malondialdehyde (MDA) contents, and attenuates weaned stress syndrome in piglets. However, little is known about the effects of dihydroartemisinin on broiler chicken growth and health. Therefore, this study aimed to evaluate the effects of various levels of dihydroartemisinin on the growth performance, meat quality, meat shelf life and antioxidant capacity of broiler chickens and to determine optimal supplementation levels. It was hypothesized that dihydroartemisinin supplementation within the appropriate dose range might have beneficial effects on growth performance, meat quality and antioxidant capacities of broiler chickens.

Materials and methods

The experimental protocols were performed in accordance with the Guidelines for Experimental Animals of the Ministry of Science and Technology. All animal experiments were approved by the Nanjing Agricultural University Animal Care and Use Committee (SYXK (Su) 2017-0007).

Materials

The dihydroartemisinin (purity > 98%) was purchased from Guangzhou Kehu Biotechnology Co., Ltd. (Guangzhou, China).

Experimental design and diets

Four-hundred one-day-old male Arbor Acres (AA) broilers, with an average body weight (BW) of 44.52 ± 0.67 g, were purchased from a local hatchery (Hefei Arbor Acres Poultry Breeding Company, Anhui, China). All chickens were randomly divided into five treatment groups with eight replicates of ten chicks each, and received a basal diet containing 0, 5, 10, 20, or 40 mg/kg dihydroartemisinin. The basal diet was formulated according to the American National Research Council (1994) broiler feeding standard. The composition and nutrient levels of the basal diet are shown in Table S1. Broiler chickens were raised in three-layer cages (120 × 70 × 60 cm) and the experimental room were equipped with a 23L:1D lighting program and an environmentally controlled system. As our previously described, the room temperature was set at 32–34°C for 14 days and finally reached 25 ± 1°C by decreasing 2 to 3°C per week (Zhang et al., 2015b). During the entire experimental period, broilers had free access to mash feed and water.

Sample collection

At 21 and 42 days of age, one chick per replicate was randomly selected and weighted. Blood samples were taken via the wing vein into an anticoagulant-free plain tube and reclined for 1 h at 4°C. Serum was harvested by the centrifugation at 3,500 rpm for 15 min and stored at -20°C until analysis. Then the chicks were sacrificed by cervical dislocation. The bilateral breast meats (*pectoralis major*) were dissected (connective and adipose tissues removed). The right portion of breast meat was immediately stored at -80°C for the measurement of antioxidant capacity. A part of the left portion of breast meat was stored at 4°C for meat quality measurement, while the remaining pieces of the left portion were packaged with plastic wrap individually and stored at 4°C to determine the lipid peroxidation and free radicals scavenging activities at D 0, 3, 5 and 7 of postmortem storage. The chopped liver samples were put into two sterile tubes, immediately snap-frozen in liquid nitrogen, and stored at -80°C for the determination of antioxidant capacity and the mRNA expression.

Measurement of growth performance

At 0, 21 and 42 day of age, body weight and feed of the chickens were weighted per replicate. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratios (FCR) were calculated for the days 1-21, days 22–42, and over all experimental periods (days 1–42), respectively.

Measurement of meat quality

The pH values were determined at 45 min and 24 h postmortem using a hand-held pH meter (HI9125, HANNA Instruments, Padova, Italy) at three different locations per breast meat sample according to the previously reported (Zhang et al., 2015a).

Drip loss was determined as our previous study described (Zhang et al., 2015a). Briefly, a small piece of meat was weighted, suspended by paper clips in the box that was covered by the plastic wrap, and stored at 4 °C. The meat samples were dried with filter paper and weighted at 24 and 48 h postmortem, respectively. Drip loss was calculated as follows: Drip loss 24 h (%) = [(initial weight - weight 24 h)/initial weight] × 100; Drip loss 48 h (%) = [(initial weight - weight 48 h)/initial weight] × 100.

To measure the cooking loss, about 15 g breast muscle samples were weighted and then kept in a plastic bag. An 80 °C thermostatic waterbath was performed until the internal temperature of the cooked meat samples were arrived at 70 °C. After cooling by running water, the samples were wiped off and reweighted. Cooking loss was calculated as reported by Zhang et al. (2015a). Cooking loss (%) = [(initial weight-final weight)/initial weight] × 100.

After determining cooking loss, each meat sample were cut into the size of 3 cm × 1 cm × 1 cm. Each sample was sheared perpendicular to the muscle fibers direction in triplicate using a C-LT3B digital-display muscle tenderness determination device (Tenovo, Harbin, China).

Meat color was detected with a colorimeter (Minolta CR-10, Konica Minolta, Japan) according to the CIELAB system (L*= Lightness, a*=redness, b*=yellowness).

Determination of free radical scavenging activity

Breast muscle was homogenized in ice-cold 0.86% sodium chloride buffer (w/v, 1:9) and centrifuged at 3500 rpm for 10 min at 4°C. The supernatant was used for the determination of 2,2-Dipheny-L-

picrylhydrazyl (DPPH) and 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activities according to our previous methods (Zhang et al., 2014). The protein concentration of the homogenate was measured using a bicinchoninic acid assay kit (Beyotime Institute of Biotechnology, Shanghai, China). The results were expressed as the DPPH and ABTS radical scavenging activities per milligram protein tissue.

Determination of antioxidant capacity

The hepatic homogenates were prepared by ice-cold 0.86% sodium chloride buffer and centrifuged at 3500 rpm for 10 min at 4°C. The supernatant was collected for antioxidant capacity analysis. The antioxidant capacities of serum and liver included the MDA, total superoxide dismutase (T-SOD), catalase (CAT) and reduced glutathione (GSH). The MDA content was determined using a commercial kit (Beijing Solarbio Science & Technology Co.,Ltd, Beijing, China). The T-SOD, CAT activity and GSH concentration were determined using commercial kits purchased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China). The protein concentration of hepatic homogenates was measured using a bicinchoninic acid assay kit (Beyotime Institute of Biotechnology, Shanghai, China) to adjust the results of hepatic antioxidant capacity.

Total RNA extraction and real-time quantitative PCR

Total RNA was extracted from liver sample using the TRizol reagent (Takara Biotechnology, Dalian, China) as previously descried (Zhang et al., 2018). The RNA quality and quantity were measured using a Nanodrop-1000 UV spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific Inc., Waltham, MA, USA), and the ratio of OD260/OD280) ranged between 1.8 to 2.0. Thereafter, extracted RNA was reverse transcribed into cDNA using a PrimeScript RT Reagent kit (Takara Biotechnology Co., Dalian, China).

Real-Time PCR reaction was performed in a QuantStudio® real-time PCR Design & Analysis system (Applied Biosystems, USA) using the SYBR® Premix Ex Taq™ Kit (Takara Biotechnology Co. Ltd., Dalian, China) according to the manufacturer's instructions. β-actin gene was used as the internal reference gene to normalize the mRNA expression levels of the target genes. The primer sequences of the target genes are shown in Table S2. The relative expressions of the target genes were calculated with the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (Ver.22.0 for windows, SPSS Inc., Chicago, IL). Polynomial orthogonal contrasts were used to determine linear and quadratic effects of dietary dihydroartemisinin levels. Duncan's multiple comparison test was applied to determine significant differences among all treatment groups. Data were expressed as means and pooled standard error of mean (SEM), and $P < 0.05$ were considered statistically significant.

Results

Growth performance

As shown in Table 1, during the days 1–21 period, dietary dihydroartemisinin supplementation quadratically increased ADG (10 mg/kg, $P < 0.001$) and ADFI (10 and 20 mg/kg, $P = 0.003$). Dietary 40 mg/kg dihydroartemisinin decreased ADG during the days 1–21 period (quadratic, $P < 0.001$), 21–42 period (linear, $P = 0.009$; quadratic, $P = 0.001$) and 1–42 period (linear, $P = 0.009$; quadratic, $P < 0.001$). Moreover, the ADFI was decreased quadratically by the dietary 40 mg/kg dihydroartemisinin during 1–21 period ($P = 0.003$) and 1–42 period ($P = 0.021$) (Table 2).

Meat quality

Compared to the control group, dietary dihydroartemisinin supplementation quadratically decreased the drip loss at 24 h (5–20 mg/kg, $P = 0.001$). Dietary dihydroartemisinin supplementation, ranging from 5 to 40 mg/kg, significantly decreased the L* value of breast muscles in broilers (linear, $P = 0.003$; quadratic, $P = 0.004$).

Shelf life

As shown in Table 3, compared to the control group, dietary dihydroartemisinin supplementation at 20 and 40 mg/kg decreased MDA concentrations in breast muscles at D 5 of postmortem storage (linear, $P = 0.001$; quadratic, $P = 0.006$). The MDA concentrations were significantly decreased by the dietary 10–40 mg/kg dihydroartemisinin (linear, $P < 0.001$; quadratic, $P < 0.001$) at D 7 of postmortem storage. Furthermore, dietary dihydroartemisinin supplementation, ranging from 5 to 40 mg/kg, significantly increased the ABTS scavenging activity at D 7 of postmortem storage compared with the control group (linear, $P = 0.003$; quadratic, $P < 0.001$).

Serum antioxidant capacity

Table 4 showed that dietary 20 mg/kg dihydroartemisinin supplementation increased serum GSH concentrations at 21 days of age (linear, $P = 0.007$; quadratic, $P = 0.014$) while 40 mg/kg dihydroartemisinin

Table 1
Effects of dietary dihydroartemisinin supplementation on growth performance of broiler chickens.[1]

Items	dihydroartemisinin supplemental levels, mg/kg						*P*-value		
	0	5	10	20	40	SEM	Treatment	Linear	Quadratic
days 1–21									
ADG	33.23^{b}	33.83^{b}	35.66^{a}	34.53^{ab}	31.57^{c}	0.324	<0.001	0.250	<0.001
ADFI	51.68^{b}	51.34^{bc}	54.29^{a}	53.80^{a}	49.34^{c}	0.450	<0.001	0.497	0.003
FCR	1.56	1.52	1.52	1.56	1.56	0.013	0.726	0.552	0.488
days 21–42									
ADG	86.05 ^{a}	87.54 ^{a}	87.54 ^{a}	84.73 ^{a}	76.78 ^{b}	1.190	0.012	0.009	0.001
ADFI	168.93	168.77	169.24	168.58	159.72	1.722	0.353	0.129	0.136
FCR	1.96	1.93	1.94	2.00	2.11	0.034	0.475	0.142	0.161
days 1–42									
ADG	59.65^{a}	60.68^{a}	61.60^{a}	59.63^{a}	54.17^{b}	0.677	0.001	0.009	<0.001
ADFI	110.30^{a}	110.05^{a}	111.76^{a}	111.19^{a}	104.53^{b}	0.868	0.049	0.090	0.021
FCR	1.85	1.82	1.82	1.87	1.94	0.023	0.397	0.138	0.122

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, the ration of the feed intake to the body gain;
[1] SEM =standard error of the mean.
[a–c] Means in a row with different superscripts are significantly different ($P < 0.05$).

Table 2
Effects of dietary dihydroartemisinin supplementation on breast meat quality of broiler chickens.[1]

Items	dihydroartemisinin supplemental levels, mg/kg						*P*-value		
	0	5	10	20	40	SEM	Treatment	Linear	Quadratic
pH 45min	6.59	6.64	6.34	6.48	6.53	0.055	0.472	0.462	0.480
pH 24h	5.93	6.07	5.82	5.92	5.97	0.039	0.361	0.775	0.816
Drip loss 24h, %	5.47 ^{a}	3.91 ^{bc}	3.31 ^{c}	3.65 ^{c}	5.19 ^{ab}	0.280	0.017	0.694	0.001
Drip loss 48h, %	9.43 ^{ab}	8.01 ^{b}	8.36 ^{b}	9.31 ^{ab}	10.96 ^{a}	0.343	0.024	0.069	0.003
Cooking loss, %	12.12	12.1	11.84	11.79	11.88	0.311	0.996	0.725	0.930
Shear force, N	24.43	22.35	27.24	33.31	34.31	1.900	0.175	0.019	0.059
Color									
L*-lightness	55.84 ^{a}	50.04 ^{b}	50.70 ^{b}	49.11 ^{b}	48.58 ^{b}	0.769	0.011	0.003	0.004
a*-redness	5.32	5.47	5.79	5.74	5.65	0.168	0.912	0.446	0.631
b*-yellow	14.32	14.72	14.24	14.44	14.96	0.666	0.998	0.838	0.967

[1] SEM =standard error of the mean.
$^{a-c}$Means in a row with different superscripts are significantly different ($P < 0.05$).

Table 3
Effects of dietary dihydroartemisinin supplementation on shelf life of breast meat in broiler chickens.[1]

Items	dihydroartemisinin supplemental levels, mg/kg						*P*-value		
	0	5	10	20	40	SEM	Treatment	Linear	Quadratic
MDA, nmol/mgprot									
D 0	0.50	0.37	0.38	0.38	0.41	0.019	0.219	0.249	0.078
D 3	0.47 ^{ab}	0.58 ^{a}	0.38 ^{b}	0.39 ^{b}	0.33 ^{b}	0.030	0.050	0.028	0.076
D 5	0.72 ^{a}	0.63 ^{ab}	0.54 ^{ab}	0.43 ^{b}	0.38 ^{b}	0.043	0.053	0.001	0.006
D 7	0.66 ^{a}	0.71 ^{a}	0.50 ^{b}	0.24 ^{b}	0.22 ^{b}	0.061	0.001	<0.001	<0.001
DPPH, %/mgprot									
D 0	8.18	8.35	8.93	8.87	8.91	0.219	0.742	0.205	0.403
D 3	8.17	8.48	8.8	8.13	8.05	0.258	0.915	0.753	0.707
D 5	8.05	8.22	8.57	8.22	7.95	0.199	0.924	0.893	0.672
D 7	8.14	8.29	8.33	8.01	7.9	0.219	0.979	0.640	0.813
ABTS, %/mgprot									
D 0	6.69	7.56	8.02	8.72	8.19	0.474	0.751	0.221	0.392
D 3	6.26	7.42	7.95	8.22	8.12	0.443	0.680	0.155	0.284
D 5	6.29	7.67	7.98	7.63	7.61	0.270	0.343	0.184	0.119
D 7	5.91 ^{c}	7.14 ^{b}	7.7 ^{ab}	8.05 ^{a}	7.44 ^{ab}	0.211	<0.001	0.003	<0.001

Abbreviations: ABTS, 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DPPH, 2,2-Dipheny-L-picrylhydrazyl; MDA, malondialdehyde.
[1] SEM =standard error of the mean.
$^{a-c}$ Means in a row with different superscripts are significantly different ($P < 0.05$).

Table 4
Effects of dietary dihydroartemisinin supplementation on serum antioxidant capacity of broiler chickens [1]

Items	dihydroartemisinin supplemental levels, mg/kg						*P*-value		
	0	5	10	20	40	SEM	Treatment	Linear	Quadratic
21 days of age									
GSH, μmol/mL	16.86 ^{b}	18.58 ^{b}	20.77 ^{ab}	23.5 ^{a}	21.58 ^{ab}	0.777	0.050	0.007	0.014
T-SOD, U/mL	9.1	9.05	10.3	8.32	8.87	0.761	0.956	0.830	0.932
CAT, U/mL	5.05	5.65	5.73	4.48	4.37	0.286	0.440	0.218	0.245
MDA, nmol/mL	2.34	3.05	2.73	3.07	4.02	0.281	0.439	0.089	0.214
42 days of age									
GSH, μmol/mL	13.86 ^{b}	16.39 ^{b}	18.31 ^{b}	20.77 ^{ab}	27.32 ^{a}	1.447	0.026	0.001	0.004
T-SOD, U/mL	10.64 ^{c}	14.75 ^{a}	11.98 ^{bc}	13.87 ^{abc}	16.05 ^{a}	0.623	0.034	0.021	0.073
CAT, U/mL	2.9	4.76	5.36	3.72	2.59	0.511	0.385	0.656	0.143
MDA, nmol/mL	3.61 ^{a}	2.85 ^{ab}	2.29 ^{b}	2.47 ^{b}	3.64 ^{a}	0.180	0.032	0.806	0.005

Abbreviations: CAT, catalase; GSH, glutathione; MDA, malondialdehyde; T-SOD, total superoxide dismutase.
[1] SEM =standard error of the mean.
$^{a-c}$ Means in a row with different superscripts are significantly different ($P < 0.05$).

increased serum GSH concentrations at 42 days of age (linear, P =0.001; quadratic, P = 0.004). Compared to the control group, 5 and 40 mg/kg dihydroartemisinin linearly increased the T-SOD activity in serum (P = 0.021). Dietary dihydroartemisinin supplementation at 10 and 20 mg/kg quadratically decreased serum MDA concentrations (P = 0.005).

Hepatic antioxidant capacity

As shown in Table 5, dietary 20 mg/kg dihydroartemisinin supplementation quadratically increased hepatic GSH concentrations (P = 0.007) in broilers at 21 days of age. Compared to the control group, 10 and 20 mg/kg dihydroartemisinin quadratically increased hepatic CAT activity at 21 days of age (P = 0.010) while only 20 mg/kg dihydroartemisinin quadratically increased hepatic CAT activity at 42 days of age (P = 0.041). The hepatic MDA concentrations were increased by dietary 40 mg/kg dihydroartemisinin supplementation (linear, P =0.041; quadratic, P = 0.013) at 21 days of age. In addition, dietary 10 and 20 mg/kg dihydroartemisinin supplementation quadratically

Table 5
Effects of dietary dihydroartemisinin supplementation on hepatic antioxidant capacity of broiler chickens [1]

Items	dihydroartemisinin supplemental levels, mg/kg						*P*-value		
	0	5	10	20	40	SEM	Treatment	Linear	quadratic
21 days of age									
GSH, μmol/mgprot	282.52 [b]	379.41 [ab]	383.74 [ab]	420.09 [a]	292.02 [b]	17.029	0.024	0.629	0.007
T-SOD, U/mgprot	34.41	34.89	32.13	37.11	34.29	1.210	0.811	0.822	0.970
CAT, U/ mgprot	12.89 [b]	18.82 [ab]	25.64 [a]	22.58 [a]	17.4 [ab]	1.463	0.048	0.224	0.010
MDA, nmol/mgprot	8.17 [b]	11.28 [b]	8.05 [b]	7.12 [b]	18.21 [a]	1.114	0.003	0.041	0.013
42 days of age									
GSH, μmol/mgprot	233.36 [b]	228.53 [b]	322.8 [a]	233.23 [b]	230.75 [b]	12.159	0.046	0.995	0.217
T-SOD, U/mgprot	38.61 [ab]	36.85 [b]	43.6 [a]	43.34 [a]	37.76 [ab]	0.938	0.040	0.480	0.135
CAT, U/mgprot	22.23 [b]	36.36 [ab]	33.59 [ab]	46.05 [a]	29.24 [b]	2.636	0.048	0.209	0.041
MDA, nmol/mgprot	68.23 [a]	66.89 [a]	60.97 [b]	60.39 [b]	65.26 [ab]	0.983	0.023	0.073	0.014

Abbreviations: CAT, catalase; GSH, glutathione; MDA, malondialdehyde; T-SOD, total superoxide dismutase.
[1] SEM =standard error of the mean.
[a–b] Means in a row with different superscripts are significantly different ($P < 0.05$).

decreased hepatic MDA concentrations ($P = 0.014$) in broilers at 42 days of age.

Hepatic antioxidant gene mRNA expression

As indicated in Table 6, dihydroartemisinin supplementation at 5–40 mg/kg quadratically increased the mRNA expression of *Nrf2* ($P = 0.014$). Compared to the control group, the mRNA expression of *HO-1* was quadratically increased by 10 and 20 mg/kg dihydroartemisinin supplementation ($P = 0.003$). Additionally, 10-40 mg/kg dihydroartemisinin increased the mRNA expression of *CAT* (linear, $P = 0.013$; quadratic, $P = 0.006$) in liver.

Discussion

The administration of dried Artemisia annua leaves has been reported to improve the growth performance of broiler chickens and hens (Brisibe et al., 2008). Artemisinin derived from enzymatical treatment is believed to be responsible for the abovementioned benefits in broilers (Wan et al., 2017). Our previous study found that dietary inclusion of 1, 000 mg/kg enzymatically treated *Artemisia annua* L. significantly enhanced ADG but did not affect ADFI or F/G in broilers (Wan et al., 2016). Dihydroartemisinin, an artemisinin derivative, is one of the most important active metabolites *in vivo*. Dihydroartemisinin exhibits more than 10 times oral bioavailability than that of artemisinin (Ho et al., 2014). In the present study, the higher ADG and ADFI in dihydroartemisinin-treated groups (except 40 mg/kg) were found during the days 1-21 period. Niu et al. (2020) reported that dihydroartemisinin increased nutrition utilization, including protein and fat utilization, confirming our assumption. We assumed that the enhanced small intestine absorption promoted FCR, resulting in improving growth performance. At the same time, we found that 40 mg/kg dihydroartemisinin has a significant negative effect on the ADG and ADIF, suggesting that dihydroartemisinin might have a growth-inhibiting effect in overfeeding.

Consumer preferences regarding poultry meat are influenced by sensory properties, such as water capacity, color, and tenderness, which are indicators of meat quality. Meat quality is sensitive to supplementation with dietary feed additives, making it easy to manipulated through nutritional intervention. Some studies have reported that broilers fed artemisinin and its analogs exhibited better meat quality, although there were many controversial results regarding the color, pH, and water-holding capacity of the meat. In the present study, we employed the CIE color system, which consisted of L* (Lightness), a* (redness) and b* (yellowness), to determine the meat color. The results showed that the L* values of breast muscle exhibited significantly linear and quadratic decreases following dietary dihydroartemisinin supplementation; however, there were no differences in the a* and b* values between the control and dihydroartemisinin-treated groups. A lower L* value indicated a better sensory quality of breast muscle within the 48–53 range (Zhuang and Savage, 2010). Generally, consumers prefer less light-colored and redder breast muscle, which results from increased contents of myoglobin and myohemoglobin (Bianchi et al., 2006). However, few studies have demonstrated the effects of dihydroartemisinin on breast muscle lightness and its possible mechanism. The L* value depends on pH, which is mechanically related to postmortem glycolytic metabolism. In the present study, there were no significant differences in the pH values following dietary dihydroartemisinin supplementation. We found that dihydroartemisinin quadratically decreased drip loss postmortem. Wan et al. (2017) found that drip loss in breast and thigh muscles showed linear and quadratic decreases following supplementation with dietary enzymatically treated

Table 6
Effects of dietary dihydroartemisinin supplementation on hepatic mRNA expression of antioxidant-related genes of broiler chickens [1]

Items	dihydroartemisinin supplemental levels, mg/kg						*P*-value		
	0	5	10	20	40	SEM	Treatment	Linear	quadratic
NF-κB	1.00	0.80	0.74	0.77	0.79	0.059	0.678	0.279	0.323
Nrf2	1.00 [b]	1.61 [a]	1.90 [a]	1.57 [a]	1.65 [a]	0.096	0.034	0.061	0.014
HO-1	1.00 [b]	1.45 [ab]	1.85 [a]	1.62 [a]	1.37 [ab]	0.086	0.018	0.138	0.003
NQO1	1.00	1.10	1.02	0.92	1.13	0.049	0.682	0.825	0.870
CAT	1.00 [b]	1.29 [ab]	1.62 [a]	1.54 [a]	1.48 [a]	0.072	0.034	0.013	0.006
CuZnSOD	1.00	1.21	1.71	1.41	1.39	0.101	0.254	0.177	0.130
GPX1	1.00	1.20	1.28	1.25	1.28	0.103	0.919	0.421	0.636
GPX4	1.00	1.07	1.48	1.11	1.33	0.101	0.559	0.339	0.548
GSTA2	1.00	1.30	1.76	1.65	1.61	0.114	0.204	0.049	0.059

Abbreviations: *CAT*, catalase; *GPX1*, glutathion peroxidase1; *GPX4*, glutathion peroxidase4; *GSTA*2, glutathione S-transferase alpha2; *HO-1*, haem oxygenase-1; *NF-κB*, nuclear factor kappa-B; *NQO1*, NAD (P) H: quinone oxoreductase1; *Nrf2*, nuclear factor erythroid 2-related factor 2; *CuZnSOD*, copper and zinc superoxide dismutase.
[1] SEM =standard error of the mean.
[a–b] Means in a row with different superscripts are significantly different ($P < 0.05$).

Artemisia annua L. Under high-temperature condition, dietary enzymatically treated *Artemisia annua* L. also significantly reduced drip loss in broilers (Wan et al., 2018). The space between the myofibrils shrank when the myoglobulin was denatured, leading to water loss and increased drip loss. The myofibrils protein denaturation is closely associated with the presence of free radicals before and after slaughter (Zhang et al., 2011). We assumed that the reduced drip loss of breast muscle may be related to the antioxidant function of dihydroartemisinin. In poultry, breast muscle with higher lightness and lower water-holding capacity are prone to the formation of pale, soft, exudative (PSE) meat (Che et al., 2023). Our results showed that dietary dihydroartemisinin supplementation improved meat color and water-holding capacity, and could protect against the risk of PSE meat.

In addition to meat quality, the shelf life of raw meat is a variable that affects consumer's choice. Improving the shelf life of raw meat reduces waste and increases economic benefits. Many studies have shown that the redox status of the local microenvironment in breast muscle plays a crucial role in the shelf life of meat. In the present study, we stored the breast samples at 4°C for 0, 3, 5 and 7 days postmortem and then determined their redox indicators. The results showed that dihydroartemisinin supplementation linearly decreased MDA content of breast meat at D 5 and 7 of postmortem storage. MDA is recognized as one of the most important lipid peroxidation byproducts and is widely used as an indicator of the redox status of tissues and cells. Generally, a lower MDA content is linked to a lower degree of lipid oxidation and higher cellular antioxidant capacity, whereas is negatively correlated with the reactive oxygen species levels. In the present study, broilers administrated 10–20 mg/kg dihydroartemisinin exhibited a higher antioxidant capacity of breast muscle, which is consistent with the results reported by Liu et al (2019). We then selected two classical free radical systems, fat- and water-soluble, to measure the non-enzymatic antioxidant capacities of breast muscle. The results showed that dihydroartemisinin significantly increased the ABTS scavenging activity of breast muscle at D 7 of postmortem. Dihydroartemisinin is a water-soluble derivative of artemisinin, thus, its remarkably increased ABTS scavenging activity might be related to its water solubility. Although there is little literature on the effect of dihydroartemisinin on the shelf life of breast muscle, similar reduced MDA contents has been observed in the lung, small intestine, and liver in other studies, indicating that dihydroartemisinin could prevent lipid peroxidation (Liang et al., 2020; Huang et al., 2019; Zhao et al., 2019).

To fully evaluate the effect of various levels of dietary dihydroartemisinin supplementation on the antioxidant system of broilers, we collected serum and liver samples at 21 and 42 days of age. The results showed that dihydroartemisinin prevented lipid peroxidation in both serum and liver, and significantly reduced the MDA contents. Dihydroartemisinin supplementation increased GSH concentrations and CAT activities in liver, suggesting that dihydroartemisinin had an antioxidant effect both on antioxidant enzyme activities and non-enzymatic antioxidant capacities. CAT plays an important role in mediating the detoxification of hydrogen peroxide, whereas GSH is a critical non-enzymatic antioxidant responsible for the maintenance of the redox system. The increased CAT activity and GSH concentration worked together to improve the antioxidant capacity, which in turn explained the observed reduction in MDA contents. Although few studies have focused on the effects of dihydroartemisinin in poultry, research has been conducted on rodents and piglets, yielding results consistent with our findings (Li et al., 2022; Liu et al., 2019; Zhao et al., 2019). Dihydroartemisinin is structurally characterized by aromatic rings with two hydroxyl groups, that serve as electron donors to neutralize reactive oxygen species and other free radicals. The free radical scavenging activity of dihydroartemisinin might directly assist in decreasing the MDA contents, and provide an explanation for the increased ABTS scavenging activity following dihydroartemisinin supplementation.

In addition to neutralizing free radicals, the dihydroartemisinin-mediated activation of antioxidant pathways plays an important role in redox regulation. Therefore, we measured the mRNA expression of antioxidant genes in the liver. Consistent with the results reported by Li et al. (2019), dihydroartemisinin upregulated the mRNA expression of the Nrf2-related pathway. Activated Nrf2 facilitates the transcription of downstream target genes, which enhance the antioxidant system and maintain the cellular redox status in animals (He et al., 2020). Nrf2 is a nuclear transcription factor that orchestrates the expression of a large number of antioxidant-related genes, including *HO-1, CAT, GST*, and *SOD*. Dihydroartemisinin counteracts oxidant stress and alleviates multi-organ injury by inducing the Nrf2 pathway in lupus, cardiomyocyte ferroptosis, and pulmonary fibrosis models (Li et al., 2019; Lin et al., 2024; Yang et al., 2018). Our results showed that the mRNA expression of *Nrf2* and the downstream genes *HO-1* and *CAT* were increased by dihydroartemisinin supplementation. The increased mRNA expression in the dihydroartemisinin-treated groups might explain the enhancement of CAT and GSH activities, and the inhibition of lipid peroxidation in the liver and serum. However, it was rarely reported whether dihydroartemisinin induced the Nrf2 pathway in un-challenged animals, especially broilers. Moreover, the minimum dietary dihydroartemisinin level required to induce antioxidant activity remains unknown.

Conclusion

In conclusion, the present study demonstrated for the first time that dietary dihydroartemisinin supplementation at 5–20 mg/kg, has beneficial effects on growth performance, meat quality, meat shelf life, and serum and hepatic antioxidant capacities of broiler chickens. Specifically, dietary dihydroartemisinin at 40 mg/kg had negative effects on the ADG and ADFI during all three experimental periods. Considering the different effects of various levels of dihydroartemisinin, 10 and 20 mg/kg dihydroartemisinin was recommended as the optimal dose, and 40 mg/kg dihydroartemisinin was assumed as overfeeding and to have the potential toxicity for growth.

Declaration of competing interest

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

Acknowledgments

This research was financially supported by Jiangsu Provincial Agricultural Science and Technology Independent Innovation Fund (NO: CX (23)3082) and National Natural Science Foundation of China (32272917).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104523.

References

Batty, K.T., Iletr, K.E., Powell, S.M., Martin, J., Davis, T.M., 2002. Relative bioavailability of artesunate and dihydroartemisinin: investigations in the isolated perfused rat liver and in healthy Caucasian volunteers. Am. J. Trop. Med. Hyg. 66, 130–136.

Bianchi, M., Petracci, M., Cavani, C., 2006. The influence of genotype, market live weight, transportation, and holding conditions prior to slaughter on broiler breast meat color. Poultry Sci 85, 123–128.

Bisht, D., Kumar, D., Kumar, D., Dua, K., Chellappan, D.K., 2021. Phytochemistry and pharmacological activity of the genus artemisia. Arch. Pharm. Res. 44, 439–474.

Brisibe, E.A., Umoren, U.E., Brisibe, F., Magalh?Es, P.M., Ferreira, J.F.S., Luthria, D., Wu, X., Prior, R.L., 2009. Nutritional characterisation and antioxidant capacity of different tissues of *Artemisia annua* L. Food Chem 115, 1240–1246.

Brisibe, E.A., Umoren, U.E., Owai, P.U., Brisibe, F., 2008. Dietary inclusion of dried *Artemisia annua* leaves for management of coccidiosis and growth enhancement in chickens. Afr. J. Biotechnol. 7, 4083–4092.

Che, S., Susta, L., Barbut, S., 2023. Effects of broiler chilling methods on the occurrence of pale, soft, exudative (PSE) meat and comparison of detection methods for PSE meat using traditional and Nix colorimeters. Poultry Sci 102, 102907.
Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., Yuan, Z., 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front. Microbiol. 5, 217.
He, F., Ru, X., Wen, T., 2020. NRF2, a Transcription Factor for Stress Response and Beyond. Int. J. Mol. Sci. 21 (13), 4777.
Ho, W.E., Peh, H.Y., Chan, T.K., Wong, W.S., 2014. Artemisinins: pharmacological actions beyond anti-malarial. Pharmacol. Ther. 142, 126–139.
Huang, X.T., Liu, W., Zhou, Y., Hao, C.X., Zhou, Y., Zhang, C.Y., Sun, C.C., Luo, Z.Q., Tang, S.Y., 2019. Dihydroartemisinin attenuates lipopolysaccharide-induced acute lung injury in mice by suppressing NF-kappaB signaling in an Nrf2-dependent manner. Int. J. Mol. Med. 44, 2213–2222.
Kim, J., Ahn, J., 2022. Emergence and spread of antibiotic-resistant foodborne pathogens from farm to table. Food Sci. Biotechnol. 31, 1481–1499.
Li, D., Qi, J., Wang, J., Pan, Y., Li, J., Xia, X., Dou, H., Hou, Y., 2019. Protective effect of dihydroartemisinin in inhibiting senescence of myeloid-derived suppressor cells from lupus mice via Nrf2/HO-1 pathway. Free Radic. Biol. Med. 143, 260–274.
Li, J., Bai, Y., Ma, K., Ren, Z., Li, J., Zhang, J., Shan, A., 2022. Dihydroartemisinin alleviates deoxynivalenol induced liver apoptosis and inflammation in piglets. Ecotoxicol. Environ. Saf. 241, 113811.
Liang, R., Chen, W., Fan, H.N., Zhang, J., Zhu, J.S., 2020. Dihydroartemisinin prevents dextran sodium sulphate-induced colitisthrough inhibition of the activation of NLRP3 inflammasome and p38 MAPK signaling. Int. Immunopharmacol. 88, 106949.
Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez-Miyakawa, M.E., Chi, F., Cravens, R.L., Oh, S., Gay, C.G., 2018. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet. Res. 49, 76.
Lin, Z.H., Xiang, H.Q., Yu, Y.W., Xue, Y.J., Wu, C., Lin, C., Ji, K.T., 2024. Dihydroartemisinin alleviates doxorubicin-induced cardiotoxicity and ferroptosis by activating Nrf2 and regulating autophagy. FASEB J 38, e23677.
Liu, X., Lu, J., Liao, Y., Liu, S., Chen, Y., He, R., Men, L., Lu, C., Chen, Z., Li, S., Xiong, G., Yang, S., 2019. Dihydroartemisinin attenuates lipopolysaccharide-induced acute kidney injury by inhibiting inflammation and oxidative stress. Biomed. Pharmacother. 117, 109070.
National Research Council, 1994. Nutrient requirements of poultry: Ninth revised edition. National Academies Press, Washington, DC.
Niu, Y., Zhao, Y.W., He, J.T., Shen, M.M., Wang, T., 2020. Dietary dihydroartemisinin supplementation improves growth, intestinal digestive function and nutrient transporters in weaned piglets with intrauterine growth retardation. Livest. Sci. 241, 104264.
Septembre-Malaterre, A., Lalarizo Rakoto, M., Marodon, C., Bedoui, Y., Nakab, J., Simon, E., Hoarau, L., Savriama, S., Strasberg, D., Guiraud, P., Selambarom, J., Gasque, P., 2020. *Artemisia annua*, a traditional plant brought to light. Int. J. Mol. Sci. 21 (14), 4986.
Wan, X., Ahmad, H., Zhang, L., Wang, Z., Wang, T., 2018. Dietary enzymatically treated *Artemisia annua* L. improves meat quality, antioxidant capacity and energy status of breast muscle in heat-stressed broilers. J. Sci. Food Agr. 98, 3715–3721.
Wan, X.L., Niu, Y., Zheng, X.C., Huang, Q., Su, W.P., Zhang, J.F., Zhang, L.L., Wang, T., 2016. Antioxidant capacities of *Artemisia annua* L. leaves and enzymatically treated Artemisia annua L. in vitro and in broilers. Anim. Feed Sci. Tech. 221, 27–34.
Wan, X.L., Song, Z.H., Niu, Y., Cheng, K., Zhang, J.F., Ahmad, H., Zhang, L.L., Wang, T., 2017. Evaluation of enzymatically treated *Artemisia annua* L. on growth performance, meat quality, and oxidative stability of breast and thigh muscles in broilers. Poultry Sci 96, 844–850.
Xiong, L., Zhang, W., Zhao, H., Tian, Z., Ren, M., Chen, F., Guan, W., Zhang, S., 2022. Dietary Supplementation of enzymatically treated *Artemisia annua* L. improves lactation performance, alleviates inflammatory response of sows reared under heat stress, and promotes gut development in preweaning offspring. Front. Vet. Sci. 9, 843673.
Yang, D.X., Qiu, J., Zhou, H.H., Yu, Y., Zhou, D.L., Xu, Y., Zhu, M.Z., Ge, X.P., Li, J.M., Lv, C.J., Zhang, H.Q., Yuan, W.D., 2018. Dihydroartemisinin alleviates oxidative stress in bleomycin-induced pulmonary fibrosis. Life Sci 205, 176–183.
Zhang, J.F., Hou, X., Ahmad, H., Zhang, H., Zhang, L.L., Wang, T., 2014. Assessment of free radicals scavenging activity of seven natural pigments and protective effects in AAPH-challenged chicken erythrocytes. Food Chem 145, 57–65.
Zhang, J.F., Hu, Z.P, Lu, C.H., Bai, K.W., Zhang, L.L., Wang, T., 2015a. Effect of various levels of dietary curcumin on meat quality and antioxidant profile of breast muscle in broilers. J. Agric. Food Chem. 63, 3880–3886.
Zhang, J.F., Bai, K.W., Su, W.P., Wang, A.A., Zhang, L.L., Huang, K.H., Wang, T., 2018. Curcumin attenuates heat-stress-induced oxidant damage by simultaneous activation of GSH-related antioxidant enzymes and Nrf2-mediated phase II detoxifying enzyme systems in broiler chickens. Poult Sci 97, 1209–1219.
Zhang, J.F., Hu, Z.P., Lu, C.H., Yang, M.X., Zhang, L.L., Wang, T., 2015b. Dietary curcumin supplementation protects against heat-stress-impaired growth performance of broilers possibly through a mitochondrial pathway. J. Anim. Sci. 93, 1656–1665.
Zhang, W., Heng, J., Kim, S.W., Chen, F., Deng, Z., Zhang, S., Guan, W., 2020. Dietary enzymatically-treated Artemisia annua L. supplementation could alleviate oxidative injury and improve reproductive performance of sows reared under high ambient temperature. J. Therm. Biol. 94, 102751.
Zhang, W., Xiao, S., Lee, E.J., Ahn, D.U., 2011. Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. J. Agric. Food Chem. 59, 969–974.
Zhao, Y., Niu, Y., He, J., Zhang, L., Wang, C., Wang, T., 2019. Dietary dihydroartemisinin supplementation attenuates hepatic oxidative damage of weaned piglets with intrauterine growth retardation through the Nrf2/ARE signaling pathway. Animals (Basel) 9 (12), 1144.
Zhuang, H., Savage, E.M., 2010. Comparisons of sensory descriptive flavor and texture profiles of cooked broiler breast fillets categorized by raw meat color lightness values. Poultry Sci 89, 1049–1055.