

Effects of natural capsicum extract on growth performance, nutrient utilization, antioxidant status, immune function, and meat quality in broilers

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ABSTRACT This research was conducted to determine the effects of natural capsaicin extract (NCE) as an alternative to the antibiotic (chlortetracycline, CTC) on growth performance, antioxidant capacity, immune function, and meat quality of broiler chickens. A total of 168 one-day-old Arbor Acre male broiler chickens with an average weight of 46.4 ± 0.6 g were randomly allotted to 3 dietary treatments, with 7 replicates per treatment and 8 broilers per pen. These 3 dietary treatments included a corn-soybean meal basal diet (CON), a basal diet + 75 mg/kg CTC (CTC), and a basal diet + 80 mg/kg NCE (NCE). Broilers from the NCE group showed higher average daily gain compared to broilers from the CON group at all stages ($P < 0.05$). On d 42, NCE supplementation improved dietary nitrogen-corrected apparent metabolizable energy compared to nonsupplemented or CTC-supplemented diets ($P < 0.05$). The digestibility of organic matter and crude protein were higher in the NCE diet than in the CON or CTC diets ($P < 0.05$). Higher relative weight of bursa of Fabricius was observed in broilers fed NCE diets

compared with CON ($P < 0.05$). Pancreatic trypsin and lipase activities were significantly increased in the NCE group compared with those in the CON group ($P < 0.05$). The value of lightness (L*) of breast muscles from broilers fed NCE diets was significantly lower compared to those fed CON diets ($P < 0.05$). Broilers fed NCE diets also had higher levels of serum total antioxidant capacity, glutathione peroxidase, superoxide dismutase, and lower levels of interleukin-1 β , and tumor necrosis factor- α compared with broilers fed CON diets ($P < 0.05$). The liver catalase activity of broilers was also significantly increased in the NCE group than the CON group ($P < 0.05$). In addition, broilers from NCE group had lower concentrations of serum urea-N, low-density lipoprotein cholesterol, and total cholesterol, and higher concentration of growth hormone compared with those from the CON group ($P < 0.05$). Therefore, we concluded that supplementation of 80 mg/kg of NCE in diets could improve growth performance, nutrient digestibility, antioxidant status, immune function, and meat quality in broilers.

Key words: broiler, natural capsicum extract, immune function, antioxidant capacity, meat quality

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INTRODUCTION

Antibiotic growth promoters (AGP) have been widely applied in poultry diets to improve growth performance and health status of broilers (Cervantes, 2015; Castanon, 2007). However, the application of AGPs in animal feed has been banned in the European Union, United States, China, and other countries due to the risk of drug resistance bacteria and antibiotic residues in animal products (Van den Bogaard et al., 2002; Barug et al., 2006; Singer and Hofacre, 2006). Overall,

the development of alternatives to AGPs is necessary, and numerous bioactive compounds from plants have been proved to improve growth performance, antioxidant status, and immune function of animals, thus the bioactive compounds from plants are considered as potential alternatives to AGPs (Liu et al., 2020; Hu et al., 2017).

Chili pepper (*Capsicum annuum*) is widely planted throughout the world, and applied for food and traditional medicine (Hernandez-Perez et al., 2020). Capsaicin, the origin of the spicy taste of chili pepper, has been proved to play an important role in the improvement of antioxidant capacity and anti-inflammatory activity, relief of pain, and modulation of lipid metabolism and intestinal microbial community (Lee et al., 2003; Aasvang et al., 2008; Prakash and Srinivasan, 2010; Liang et al., 2013). Thus, natural capsicum extract (NCE), whose main component is capsaicin, might

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positively affect poultry production. For the last few years, some studies have shown that supplementing plant extract mixture (including NCE) to diets could improve growth performance, nutrient digestion, digestive enzyme activities, and modulate the gut microbiota to benefit the health of broilers (Jamroz et al., 2005; Pirgozliev et al., 2019). However, to the best of our knowledge, studies that focused on effects of NCE alone as an antibiotic substitute on broilers growth performance and health status are limited, and the potential mechanism of action of NCE in broilers still need to be further explored. Thus, this experiment was conducted to assess the effects of NCE as a substitute for AGP on growth performance, nutrient digestion, antioxidant status, immune function, and meat quality in broilers.

MATERIALS AND METHODS

Experimental Design and Diets

The procedures of this experiment were approved by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China). A total of 168 one-day-old male Arbor Acre broiler chickens (with initial body weight of 46.4 ± 0.6 g) were bought from Arbor Acres Poultry Breeding Company (Beijing, China). All broilers were raised in wire-floored cages ($0.9 \times 0.6 \times 0.4$ m) in an environmentally controlled room. These cages were equipped with 2 nipple drinkers and a feeder, and broilers had free access to feed and water throughout the experiment. In the first 3 d, the room temperature was maintained at 34°C . Then, the room temperature decreased by 3°C per week until 23°C . All broilers were randomly allotted into 3 treatments, with 7 replicates per treatment and 8 broilers per cage. The 3 dietary treatments were as follows: CON (corn-soybean meal basal diet), CTC (basal diet + 75 mg/kg chlortetracycline), and NCE (basal diet + 80 mg/kg NCE). This experiment consisted of 2 periods (d 1–21 and d 22–42), and 2 types of diets were formulated to meet nutrient levels recommended by National Research Council (National Research Council, 1994). The composition and nutrient levels (as-fed basis) of basal diets were shown in Table 1. The NCE used in this experiment was supplied by Leader Bio-Technology Co., Ltd (Guangzhou, China), and the main bioactive compound of NCE was natural capsaicin (2%). The chlortetracycline was purchased from Tonglixingke Agricultural and Technology Co., Ltd (Beijing, China).

Growth Performance and Nutrient Digestibility

On d 21 and d 42, broilers were fasted for 12 h, and then individual body weight and feed consumption were weighed to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F:G). Approximate 2 kg of the representative feed samples were randomly collected from the bags at varying locations

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

Items	Period 1 (d 1–21)	Period 2 (d 22–42)
Corn	58.17	64.26
Soybean meal	30.44	24.05
Corn gluten meal	2.00	2.50
Fish meal	2.00	2.00
Soybean oil	3.38	3.60
Dicalcium phosphate	1.50	1.04
Limestone	1.30	1.35
Salt	0.30	0.30
L-Lys, 98%	0.01	0.08
Methionine 98%	0.14	0.04
Threonine, 98%	0.01	0.03
Chromic oxide	0.25	0.50
Premix ²	0.50	0.25
Total	100	100
Analyzed values		
Dry matter	88.52	90.81
Crude protein	21.19	19.12
Calcium	0.96	0.85
Available phosphorus	0.64	0.58
Calculated value ³		
Metabolizable energy, MJ/kg	12.76	13.18
Total phosphorus	0.70	0.59
Digestible lysine	0.86	0.73
Digestible methionine	0.30	0.28
Digestible cysteine	0.27	0.25
Digestible threonine	0.63	0.56
Digestible methionine and cysteine	0.57	0.53

¹NCE (natural capsicum extract, 80 mg/kg) and CTC (chlortetracycline, 75 mg/kg) were mixed with premix and then mixed with each basal diet.

²Premix supplied per kg 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).

with a bag trier for further analysis. From d 39 to d 42, collection plates were placed underneath each cage for collecting excreta. Approximately 50 g of excreta was collected twice a day with feathers and feed residues removed, then immediately stored at -20°C . At the end of the collection period, excreta were thawed and mixed thoroughly. Excreta samples were dried for 72 h in an oven at 65°C , then all these feed and excreta samples were ground to pass through a 40 mesh (1-mm) screen. The dry matter (DM), ether extract (EE), crude protein (CP) and ash content in feed and excreta samples were analyzed following the Association of Official Agricultural Chemists (AOAC, 2004). The gross energy (GE) of samples was determined by an automatic adiabatic oxygen bomb calorimeter (Parr 6300 Calorimeter, Moline, IL). The organic matter (OM) content was obtained as follows: OM = 1 - ash content (DM-Base). The chromium (Cr) concentration of feed and excreta samples was measured via atomic absorption spectrometry (Z-5000; Hitachi, Tokyo, Japan) according to the instructions described by Williams et al. (1962). The apparent total tract digestibility (ATTD) of nutrients was calculated as follows:

ATTD (%) = $[1 - (\text{content of Cr in diets} \times \text{content of nutrients in excreta}) / (\text{content of Cr in excreta} \times \text{content of nutrients in diets})] \times 100\%$.

The nitrogen-corrected apparent metabolizable energy (**AMEn**) of feed was calculated as follows:

AMEn (MJ/kg)

$$= GE_{\text{diet}} - (GE_{\text{excreta}} \times Cr_{\text{diet}}) / Cr_{\text{excreta}} \\ - (34.49 \times N_{\text{retained}}) / 1000$$

$$N_{\text{retained}} = N_{\text{diet}} - (N_{\text{excreta}} \times Cr_{\text{diet}}) / Cr_{\text{excreta}}$$

GE_{diet} and GE_{excreta} (MJ/kg) are the gross energy of the diet and excreta, respectively. Cr_{diet} and Cr_{excreta} (%) are the content of chromic oxide of the diet and excreta, respectively. 34.39 (MJ/kg) is the energy value of uric acid. N_{retained} (g/kg) is the nitrogen retained by the broilers. N_{diet} and N_{excreta} (g/kg) are nitrogen content of the diet and excreta, respectively.

Meat Quality and Relative Organ Weight

On d 42 of this experiment, one broiler (close to the average body weight of the pen) was slaughtered by using cervical dislocation ($n = 7$), then liver, spleen, thymus, and bursa of Fabricius were removed and weighed to calculate the relative organ weight.

On d 42, the right breast muscle samples ($n = 7$) were immediately collected for analyzing pH and meat color, and samples were determined in triplicate at 3 different locations. The pH meter (pH-star; Thermo Fisher, Mathaus, Germany) was used to determine pH values, 45 min and 24 h after slaughter. Meat color was measured using a Chroma Meter (CR-410, Konica Minota, Tokyo, Japan), and scores of meat color were expressed as lightness (L^*), redness (a^*), and yellowness (b^*). Approximately 30 g samples ($n = 7$) of left breast muscle were collected to measure drip loss as previously described (Mohamed et al., 2020). The samples were cut into 1-cm thickness slices, weighted, and then placed in a nitrogen filled container at 4°C for 24 h. Then, the samples were carefully cleaned with filter paper and weighed again. Drip loss was calculated based on the differences in the weight before and after hanging.

Digestive Enzyme Activities

The pancreas samples ($n = 7$) were obtained from slaughtered broilers, snap frozen in liquid nitrogen, and stored at -80°C. Before analysis, the pancreas samples were thawed at 4°C and homogenized in 9 volumes of ice-cold saline. Tissue homogenates were centrifuged at 15,000 × g for 15 min at 4°C to obtain the supernatant for analyzing digestive enzyme activities. Corresponding kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China) and UV-VIS spectrophotometer (UV1100, MAPADA, Shanghai, China) were used to determine activities of α -amylase, trypsin and lipase in pancreas of broilers.

Analysis of Antioxidant Capacities

On d 42, blood samples ($n = 7$) were obtained by wing vein puncture and then centrifuged at 3,000 × g for 15 min at 4°C to collect the supernatant. The collected serum samples were stored at -20°C until further analysis. After slaughter, the liver samples were collected, immediately frozen in liquid nitrogen, and then stored at -80°C. Before analysis, frozen liver samples were thawed at 4°C, homogenized with ice-cold saline, then centrifuged at 15,000 × g for 15 min at 4°C to collect the supernatant. The contents of total antioxidant capacity (**T-AOC**), glutathione peroxidase (**GSH-Px**), catalase (**CAT**), superoxide dismutase (**SOD**), and malondialdehyde (**MDA**) were determined by an automatic biochemical analyzer (RA-1000, Bayar Corp., Tarrytown, NY) using the corresponding reagent kits (Zhongsheng Biochemical Co., Ltd., Beijing, China).

Analysis of Serum Immune, Lipid Indices and Growth Hormone

The serum immunoglobulins (**IgA**, **IgG** and **IgM**) and cytokines (**TNF- α** and **IL-1 β**) concentrations were measured using the commercially available ELISA kits (Shanghai Junshi Biosciences Co. Ltd., Shanghai, China), and absorbance was measured at 450 nm. The concentrations of total cholesterol (**TC**), total triglyceride (**TG**), low density lipoprotein (**LDL**) cholesterol, high density lipoprotein (**HDL**) cholesterol and urea nitrogen (**urea-N**) in serum samples were analyzed by an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, NY) following the instructions of the commercial kits (Zhongsheng Biochemical Co., Ltd., Beijing, China). The concentration of growth hormone (**GH**) in serum samples was determined by using a radioimmunoassay (Sn-96513, Shanghai, China).

Statistical Analysis

The collected data were subjected to one-way Analysis of Variance (ANOVA) by the GLM procedures of SAS. The pen was an experimental unit for performance and nutrient digestibility, and the individual broiler was an experimental unit for other data. Data were expressed as mean ± SEM. $P < 0.05$ was considered significant, and $0.05 \leq P < 0.10$ was defined as a tendency for significance.

RESULTS

Growth Performance

On d 21 and d 42, broilers from NCE group showed higher body weight compared with broilers from the CON or CTC groups ($P < 0.05$). NCE supplementation significantly increased ADG compared with broilers from the CON group in all phases ($P < 0.05$). However, no differences in ADG were observed between broilers from NCE and CTC groups in phase 2 and overall.

Table 2. Effects of natural capsicum extract on growth performance of broilers.

Items	CON	CTC	NCE	SEM	P-value
Period 1 (d 1–21)					
Initial body weight (g)	46.66	46.30	46.22	0.58	0.85
Final body weight (g)	609.11 ^b	608.78 ^b	658.72 ^a	12.95	0.03
Average daily weight gain (g)	27.24 ^b	27.06 ^b	29.36 ^a	0.56	0.03
Average daily feed intake (g)	36.13	36.05	37.61	0.62	0.19
Feed-to-gain ratio (g/g)	1.33	1.34	1.28	0.03	0.42
Period 2 (d 22–42)					
Initial body weight (g)	609.11 ^b	608.78 ^b	658.72 ^a	12.95	0.03
Final body weight (g)	1924.91 ^b	1980.10 ^b	2083.04 ^a	26.24	< 0.01
Average daily weight gain (g)	67.74 ^b	71.04 ^{ab}	73.22 ^a	1.35	0.04
Average daily feed intake (g)	103.31	106.10	108.97	3.00	0.44
Feed-to-gain ratio (g/g)	1.50	1.50	1.49	0.04	0.99
Overall period (d 1–42)					
Average daily weight gain (g)	45.81 ^b	47.53 ^{ab}	49.69 ^a	0.74	< 0.01
Average daily feed intake (g)	68.90	70.22	72.42	1.59	0.33
Feed-to-gain ratio (g/g)	1.50	1.48	1.46	0.04	0.69

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; SEM, pooled standard error of the means.

Table 3. Effects of natural capsicum extract on nutrient digestibility of broilers (%).

Items	CON	CTC	NCE	SEM	P-value
Dry matter	74.88	74.29	75.32	0.51	0.39
Organic matter	78.25 ^b	78.39 ^b	79.50 ^a	0.30	0.03
Crude protein	64.14 ^b	64.82 ^b	68.83 ^a	0.86	< 0.01
AMEn (MJ/kg)	13.04 ^b	12.99 ^b	13.38 ^a	0.09	0.03
Ether extract	91.03 ^b	91.82 ^{ab}	92.83 ^a	0.46	0.07

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; SEM, pooled standard error of the means.

Similarly, there were no differences in ADFI and F:G among all groups (Table 2).

Nutrient Digestibility

On d 42, NCE supplementation improved dietary AMEn compared to nonsupplemented or CTC-supplemented diets ($P < 0.05$). The digestibility of OM and CP in broilers from NCE group was higher than the CTC and CON groups. ($P < 0.05$). Meanwhile, broilers fed NCE diets tended to show higher EE digestibility than those from the CON group ($P = 0.07$) (Table 3).

Relative Organ Weight

On d 42, broilers from NCE group showed increase in weight of bursa of Fabricius relative to body weight compared with broilers from CTC or CON groups ($P < 0.05$), while there was no differences in other organ relative weight among 3 treatments (Table 4).

Digestive Enzyme Activities

The activity of lipase in pancreas of broilers from the NCE group were higher than those from the CON or CTC groups ($P < 0.05$). Moreover, NCE supplementation increased trypsin activity in pancreas of broilers compared with CON ($P < 0.05$) (Table 5).

Table 4. Effects of natural capsicum extract on relative organ weight of broilers (% live weight).

Items	CON	CTC	NCE	SEM	P-value
Liver	1.90	1.92	1.96	0.04	0.58
Spleen	0.12	0.12	0.13	0.01	0.66
Thymus	0.25	0.21	0.25	0.03	0.52
Bursa of Fabricius	0.20 ^b	0.23 ^{ab}	0.26 ^a	0.01	0.04

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; SEM, pooled standard error of the means.

Table 5. Effects of natural capsicum extract on digestive enzyme activities of the pancreas in broilers.

Items	CON	CTC	NCE	SEM	P-value
α -amylase (U/g)	74.60	77.96	87.96	4.89	0.17
Trypsin (U/mg)	138.26 ^b	206.19 ^a	188.71 ^a	6.14	< 0.01
Lipase (U/mg)	40.08 ^c	49.29 ^b	55.28 ^a	1.20	< 0.01

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; SEM, pooled standard error of the means.

Meat Quality

On d 42, breast muscle samples of broilers fed NCE diets showed lower L^* values compared with CON and CTC ($P < 0.05$). In addition, samples of broilers fed CTC diets exhibited higher pH values at 24 h compared with broilers fed CON diets ($P < 0.05$). However, NCE supplementation did not affect drip loss, a^* and b^* values of breast muscle samples in broilers (Table 6).

Antioxidant Capacities

On d 42, compared with CON, NCE supplementation significantly increased contents of serum T-AOC, GSH-Px and SOD ($P < 0.05$). NCE supplementation tended to decrease content of serum MDA in broilers compared with CON ($P = 0.08$). NCE supplementation did not

Table 6. Effects of natural capsicum extract on meat quality of broilers.

Items	CON	CTC	NCE	SEM	<i>P</i> -value
pH at 45 min	6.47	6.74	6.50	0.10	0.12
pH at 24 h	5.66 ^b	5.97 ^a	5.80 ^{ab}	0.08	0.04
ΔpH	0.82	0.77	0.70	0.12	0.77
L*	48.71 ^a	48.36 ^a	44.11 ^b	0.91	< 0.01
a*	2.54	2.99	3.13	0.30	0.34
b*	9.18	9.69	8.79	0.41	0.36
Drip loss (%)	2.91	2.83	2.76	0.34	0.95

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; L*, lightness; a*, redness; b*, yellowness; ΔpH = pH 45 min- pH 24 h; SEM, pooled standard error of the means.

significantly increase serum CAT activity, but the addition of NCE increased serum CAT activity by 11.5%. The CAT activity in the liver was significantly higher in broilers fed NCE diets compared with those from the CON and CTC groups ($P < 0.05$) (Table 7).

Immune Indices

Compared with CON, supplementing NCE resulted in lower concentrations of serum TNF- α and IL-1 β in broilers ($P < 0.05$). Nevertheless, no differences were observed in contents of serum IgA, IgG and IgM among 3 treatments (Table 8).

Metabolic Functions

The serum urea concentration was lower in broilers fed NCE diets compared with CON. NCE supplementation induced a remarkable reduction in LDL and TC concentrations compared to CON ($P < 0.05$). No differences among 3 treatments in TG level were observed. The broilers from the NCE and CTC groups showed higher concentrations of serum GH compared with CON ($P < 0.05$) (Table 9).

Table 7. Effects of natural capsicum extract on antioxidant capacities of broilers.

Items	CON	CTC	NCE	SEM	<i>P</i> -value
Serum					
T-AOC (U/mL)	7.79 ^c	10.44 ^a	9.28 ^b	0.20	<0.01
CAT (U/mL)	55.94 ^b	66.39 ^a	62.38 ^{ab}	2.78	0.07
GSH-Px (U/mL)	825.40 ^c	934.65 ^a	880.45 ^b	14.31	<0.01
SOD (U/mL)	24.09 ^b	29.24 ^a	32.81 ^a	1.43	<0.01
MDA (nmol/mL)	5.62	5.52	3.91	0.53	0.08
Liver					
T-AOC (U/mg)	1.87	1.94	1.92	0.08	0.82
CAT (U/mg)	5.84 ^c	8.61 ^b	10.47 ^a	0.51	<0.01
GSH-Px (U/mg)	133.35 ^b	161.68 ^a	155.19 ^{ab}	7.77	0.07
SOD (U/mg)	14.83	15.51	15.55	0.48	0.53
MDA (nmol/mg)	0.68	0.62	0.65	0.07	0.88

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; T-AOC, total antioxidant capacity; CAT, catalase; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; SEM, pooled standard error of the means.

Table 8. Effects of natural capsicum extract on immune indices of broilers.

Item	CON	CTC	NCE	SEM	<i>P</i> -value
IgA (g/L)	4.27	4.25	4.33	0.08	0.73
IgG (g/L)	2.28	2.25	2.35	0.04	0.23
IgM (g/L)	1.65	1.68	1.68	0.04	0.78
TNF- α (pg/mL)	51.73 ^a	39.33 ^b	42.13 ^b	1.63	< 0.01
IL-1 β (pg/mL)	26.97 ^a	19.97 ^b	20.80 ^b	1.60	0.01

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL-1 β , interleukin1 β ; TNF- α , tumor necrotic factor- α ; SEM, pooled standard error of the means.

Table 9. Effects of natural capsicum extract on metabolic functions of broilers.

Item	CON	CTC	NCE	SEM	<i>P</i> -value
Urea-N (mmol/L)	1.24 ^a	1.18 ^{ab}	1.17 ^b	0.03	0.03
HDL (mmol/L)	0.74 ^b	1.13 ^a	0.96 ^{ab}	0.08	0.02
LDL (mmol/L)	0.36 ^a	0.27 ^{ab}	0.17 ^b	0.04	0.03
TC (mmol/L)	1.74 ^a	1.38 ^{ab}	1.02 ^b	0.13	<0.01
TG (mmol/L)	0.38	0.35	0.32	0.04	0.32
GH (ng/mL)	3.08 ^b	4.83 ^a	4.83 ^a	0.29	<0.01

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

Abbreviations: CON, control; CTC, antibiotic (chlortetracycline); NCE, natural capsicum extract; urea-N, urea- nitrogen; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; GH, growth hormone; SEM, pooled standard error of the means.

DISCUSSION

The current study demonstrated that supplementing NCE to broilers diets could improve ADG compared with CON, and this finding was similar to previous reports. [Atapattu and Belpagodagama \(2011\)](#) showed that 5% of chili powder supplementation to broilers diets could significantly increase BW at d 49 and ADG at d 30–49. [Pirgozliev et al. \(2019\)](#) also reported that 100 mg/kg of phytogetic feed additives with 2% of capsicum oleoresin could improve weight gain by 16.4%. [Liu et al. \(2013\)](#) found that the addition of 10 mg/kg of capsicum oleoresin resulted in an enhanced growth performance of piglets challenged with F-18 *E. coli*. The improvement in growth performance of broilers might be due to the capsaicin, capsaicin has been proved to have antioxidant capacities and anti-inflammatory activities ([Liu et al., 2012](#); [Nascimento et al., 2013](#)). In addition, it has been reported that capsaicin was beneficial to the gut health of animals ([Liu et al., 2013](#)). [Vicente et al. \(2007\)](#) showed that supplementing natural capsaicin to diets could significantly decrease the abundance of pathogenic bacteria in hens challenged by *Salmonella enteritidis*. [Jamroz et al. \(2003\)](#) also reported that 100 mg/kg of plant extract (including 1.98% capsaicin) could lower the abundance of *E. coli* and *Clostridium perfringens* in the rectum. At the same time, the enhanced GH level was observed in broilers fed NCE diets in the current study, and the increase of GH concentration may be also one of the reasons for the improvement of growth performance in broilers fed NCE

diets. Calcitonin gene-related peptide (**CGRP**) has been proved to promote the secretion of GH in the body (Fahim et al., 1990; Nakamura et al., 1998). Studies showed that capsaicin could stimulate capsaicin-sensitive afferent neurons after entering the body, thus leading to the secretion of endogenous CGRP (Evangelista, 2009).

In this study, NCE supplementation improved dietary AMEn compared to nonsupplemented or CTC-supplemented diets, and the digestibility of organic matter and crude protein were higher in the NCE diet than in the CON or CTC diets. Isley et al. (2003) showed that plant extract including capsaicin could improve CP digestibility of sows on the 1st wk of lactation, and the addition of plant extract tended to increase DM and OM digestibility during this period. Jamroz et al. (2005) also reported that broilers fed plant extract containing 3% capsaicin tended to increase digestibility of crude fiber and protein compared to broilers from the control group. The improvement in nutrient digestibility might be related to the increase of digestive enzyme activities. In this study, we observed that NCE supplementation could increase trypsin and lipase activities in the pancreas of broilers. Platel and Srinivasan (2000) also reported that 1.5 mg/kg of capsaicin supplementation to Wistar rats' diets could significantly increase pancreatic lipase and trypsin activities. The possible underlying mechanism of increased digestive enzyme activities may be related to the increase of endogenous cholecystokinin (**CCK**) levels. Chronic CCK stimulation could improve pancreatic functions, and it has been proved that capsaicin could increase the concentration of CCK via stimulating capsaicin-sensitive afferent vagal pathway (Otsuki et al., 1983; Yamamoto et al., 2003). In addition, we speculated that higher CP digestibility was also associated with the decrease of serum urea-N concentration in broilers. High level of serum urea-N was considered to have adverse effects on growth performance of broilers as a stressor (Glantzounis et al., 2005; Pan et al., 2018).

In this study, NCE had positive effects on immune function of broilers. The relative weight of the bursa of Fabricius in broilers fed NCE diets were higher than those of CON. Tollba et al. (2007) also indicated that antioxidants (including capsaicin) supplementation to Egyptian chicken diets could increase the relative weight of bursa of Fabricius. The bursa of Fabricius is an essential immune organ in broilers, and the relative weight of bursa of Fabricius is a key indicator of the health status of animals (Mahfuz et al., 2018). The increase of the relative weight of the bursa of Fabricius demonstrated that NCE supplementation could efficiently improve some aspects of immune function in broilers. Furthermore, lower concentrations of TNF- α and IL-1 β were observed in broilers fed NCE diets compared with broilers from the CON group, and these results showed that NCE could exert an anti-inflammatory effect in broilers. Sarwa et al. (2015) reported that the injection of capsaicin could efficiently relieve inflammatory symptoms in a carrageenin-induced arthritic rat model. The anti-

inflammatory effects of capsaicin were mainly attributed to the effective regulation of the secretion of proinflammatory factors (Liu et al., 2012). Mendivil et al. (2019) reported that capsaicin could reduce mRNA expression of TNF- α , IL-1 β , and IL-6 in acetylsalicylic acid-induced gastritis in rats, and resulted in a reduction in the infiltration of inflammatory cells. Zhou et al. (2014) showed that capsaicin could modulate serum TNF- α , IL-1 β , IL-6, IL-1 and IL-10 concentrations through the pathway NF- κ B, and further studies found that the anti-inflammatory activity of capsaicin may be related to the significant reduction of *Cyclooxygenase-2* (**COX-2**) mRNA expression by capsaicin. The reduction of COX-2 mRNA expression might lead to the inhibition of the arachidonic acid pathway, and ultimately reduced the release of a proinflammatory mediator - prostaglandin E2 (Bartchewsky et al., 2009; Mendivil et al., 2019).

It has been reported that intensive broiler rearing could lead to excess production of reactive oxygen species (**ROS**), and excessive production of ROS could result in lipid, protein, and DNA damage (Fulbert and Cals 1992; Dröge 2002). Antioxidant enzymes, such as SOD, CAT and GSH-Px, play an important role in protecting against oxidative damage (Pruchniak et al., 2016; Escorcia et al., 2020). MDA, a product of lipid peroxidation, the concentration of MDA could reflect the state of oxidative stress. In the present study, enhanced serum T-AOC, SOD and GSH-Px concentrations were observed in broilers fed NCE diets. The CAT level in liver was higher in broilers fed NCE diets than those fed nonsupplemented diets. Moreover, the addition of NCE tended to decrease serum MDA concentrations. These results suggested that NCE could improve antioxidant capacity in broilers. To our acknowledgment, capsaicin exhibited a strong antioxidant effect in vitro and in vivo. Liu et al. (2015) reported that capsaicin showed strong free radical scavenging capacity in vitro. Capsaicin also has been demonstrated to inhibit oxidative degradation, carbonylation modification, nitration modification, lipid peroxidation and DNA oxidative damage caused by free radicals (Zhang et al., 2017). Melekoglu et al. (2018) showed that supplementing 0.5 mg/kg capsaicin to rat diets could significantly reduce MDA levels and increase GSH and SOD levels in ovarian tissue to treat premature ovarian failure in rats induced by cyclophosphamide. The phenolic hydroxyl group of capsaicin can transfer hydrogen atoms from free radicals, thus efficiently reduced their activities (Tsai et al., 2006). Furthermore, phenolic hydroxyl could react with metal ions to inhibit the formation of free radicals required metal ions (Hua et al., 2010). Therefore, we speculated that the phenolic structure of capsaicin might be the reason why capsaicin exhibited antioxidant capacity.

In this study, meat quality of breast muscle of broilers fed NCE diets significantly improved compared to the control group, such as the decrease in L* value. The reason may also be related to the antioxidant and anti-inflammatory properties of capsaicin. Consistent with our current results, Behera et al. (2017) suggested that

capsaicin supplementation significantly improved lipid metabolism in White Pekin Ducks. [Atapattu and Belpagodagamage \(2011\)](#) also reported that broilers fed 1% chilli powder (**CHPW**) showed lower serum cholesterol levels than control group. Medical research has reported that capsaicin and related products could be used as drugs to regulate cardiovascular function. [Zhang et al. \(2013\)](#) indicated that supplementing capsaicinoids (15 mg/kg/d for 28 d) in the ovariectomized rats could significantly decrease plasma TC, TG and LDL concentrations compared with the control group, and the addition of capsaicinoids could also lower total lipid levels in the liver. [Pi et al. \(2017\)](#) reported that compared with the control group, supplementation of 15 mg/kg capsaicin could significantly decrease TC, TG and LDL contents, and increased HDL levels in C57BL/6 rats fed high-fat diets. In ovariectomized Sprague-Dawley rats, capsaicin prevented estrogen deficiency and hyperlipidemia induced by feeding a high-cholesterol diet. Capsaicin could increase total bile acid levels in the small intestine, and total bile acid and neutral sterol contents were also increased in feces. Meanwhile, capsaicin can significantly up-regulate *CYP7A1* gene expression in the liver of ovariectomized rats ([Zhang et al., 2013](#)). *CYP7A1* is a rate-limiting enzyme that transforms cholesterol into bile acid in the liver, and stimulating gene expression of *CYP7A1* in the liver is one common method for the treatment of cardiovascular diseases ([Shin et al., 2011](#)). Therefore, the potential mechanism of the effect of dietary capsaicin on lipid metabolism in animals may be associated with the promotion of synthesis of bile acids from cholesterol in the liver, thus resulted in an increase in excretion of total bile acids and neutral sterols in feces.

CONCLUSIONS

In this study, compared with broilers from the CON group, supplementing 80 mg/kg NCE to diets could improve growth performance, nutrient digestibility, digestive enzyme activities, meat quality, immune and antioxidant functions of broilers. Broilers fed NCE diets exhibited similar growth performance and health status to those fed CTC diets. Therefore, NCE is considered as an effective alternative to AGP in broilers.

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DISCLOSURES

There is no conflict of interest relevant to this publication.

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