The alleviative effects and related mechanisms of taurine supplementation on growth performance and carcass characteristics in broilers exposed to chronic heat stress

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ABSTRACT To investigate the alleviative effects and molecular mechanisms of taurine supplementation on growth performance and carcass characteristics in broilers exposed to chronic heat stress, 144 male Arbor Acres broilers (28 d old) were randomly distributed to positive control (PC, 22°C, basal diet), heat stress (HS, consistent 32°C, basal diet), or heat stress + taurine (HS + T, consistent 32°C, basal diet + 5.00 g/kg taurine) groups, with 6 cages per group and 8 birds per cage. Chronic heat stress significantly decreased body weight, average daily gain, and average daily feed intake, and increased cloacal temperature and feed conversion ratio (FCR, $P < 0.05$). Though taurine supplementation tended to decrease the FCR in the HS + T group compared with the HS group after 14 d of heat exposure ($P = 0.071$), there were no significant alleviative effects of taurine supplementation on the increased cloacal temperature and decreased growth performance in chronic heat-stressed broilers ($P > 0.05$). After 7 and 14 d of heat exposure, taurine supplementation significantly increased the proportion of breast muscle and hormone-sensitive lipase activity in the abdominal fat ($P < 0.05$), and decreased the mRNA expressions of muscle atrophy F-box protein (MAFbx) and muscle ring-finger protein-1 (MuRF1) in breast muscle compared with the HS group ($P < 0.05$). After 7 d of heat exposure, taurine supplementation significantly increased serum non-esterified fatty acid concentration ($P < 0.05$), and decreased the mRNA expressions of acetyl-CoA carboxylase 1c (ACC) and muscular isoform of carnitine palmitoyl transferase 1 (M-CPT1) compared with the HS group ($P < 0.05$). In addition, the mRNA expressions of M-CPT1 and ribosomal protein S6 kinase, 70 kDa (p70S6K) in the HS + T group were significantly higher than those of the other two groups after 14 d of heat exposure ($P < 0.05$). In conclusion, taurine supplementation can improve carcass characteristics of chronic heat-stressed broilers by facilitating lipolysis for energy, enhancing protein synthesis, and suppressing protein degradation of the breast muscles.

Key words: broiler, carcass characteristics, chronic heat stress, growth performance, taurine

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INTRODUCTION

In past decades, the genetic selection for broilers has drastically enhanced their growth performance and feed conversion efficiency to meet the increasing demands of poultry meat, but that also made modern broilers more sensitive to environmental challenges, especially high temperature (Tan et al., 2010). Due to abundant feathers, lack of sweat gland, and high metabolic activity, broilers are especially vulnerable to heat stress (Varasteh et al., 2015). Heat stress can cause huge economic losses via impairing production performance and meat quality, and has become a serious problem encountered in the poultry industry (Lu et al., 2017; Luo et al., 2018). In addition, a previous study in our laboratory found that chronic heat stress could affect the carcass characteristics of broilers, decrease the proportion of breast muscle, and increase the proportion of abdominal fat (Lu et al., 2018), which may further impair the economic benefits of the poultry industry during the processing of carcass and meat (Han et al., 2016).

Taurine (2-aminoethanesulfonic acid), a semi-essential beta-amino acid, is found in all animal cells at high concentrations (Ripps and Shen, 2012). Taurine has various biological functions and is considered a basic regulator of cell homeostasis though it is not involved in protein synthesis or energy supply (Murakami, 2017). Many functions of taurine are closely related to stress alleviation including osmoregulation, anti-inflammation, cell membrane stabilization,

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anti-oxidation, and neuromodulation (Cassol et al., 2010). A previous study reported that taurine could decrease sudden death syndrome and total mortality of broilers (Blair et al., 1991). However, there is extremely limited information on the effects of taurine supplementation on growth performance and carcass characteristics in broilers exposed to chronic heat stress; although a few researchers indicated that 0.5% and 1.0% taurine-supplemented diet could enhance the body weight gain of chronic heat-stressed broilers (Shim et al., 2006).

Thus, we hypothesized that taurine supplementation can alleviate the negative effects of heat stress on growth performance and carcass characteristics in broilers. In this study, we measured the cloacal temperature, growth performance, carcass characteristics, serum biochemical parameters, lipid metabolic enzyme activities and related regulatory gene expressions to investigate the alleviative effects and molecular mechanisms of taurine supplementation on growth performance and carcass characteristics in broilers exposed to chronic heat stress.

Table 1. Composition and nutrient levels of the basal diets.[1]

Ingredients (g/kg)		Calculated nutrient levels	
Corn	622.7	ME (MJ/kg)[4]	13.13
Soybean meal	230.0	CP (g/kg)	195.7
Corn gluten meal[2]	60.0	Ca (g/kg)	9.5
Soybean oil	40.0	Available phosphorus (g/kg)	3.9
Limestone	14.0	Lysine (g/kg)	10.5
Dicalcium phosphate	16.0	Methionine (g/kg)	4.2
L-lysine	3.5	Methionine + cysteine (g/kg)	7.6
DL-methionine	0.8		
Salt	3.0		
Premix[3]	10.0		

[1]The nutrient levels were as-fed basis.
[2]Crude protein content was 60%.
[3]Premix provided per kilogram of diet: retinyl acetate for vitamin A, 12,000 IU; cholecalciferol for vitamin D3, 2,500 IU; DL-α-tocopheryl acetate for vitamin E, 20 IU; menadione sodium bisulfate, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8.0 mg; nicotinamide, 40 mg; choline chloride, 400 mg; calcium pantothenate, 10 mg; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamin), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8.0 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc sulfate), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.
[4]ME = metabolizable energy.

MATERIALS AND METHODS

Bird Management and Dietary Treatments

All experimental procedures involving the use of animals were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee. A total of 200 1-d-old male Arbor Acres broilers were obtained from a commercial hatchery and received commercial standard diets and managements from 1 to 27 d of age. At 28 d of age, a total of 144 chickens with similar body weights (1102.69±28.50 g) were selected and randomly allocated to three groups with 6 cages per group and 8 chickens per cage. In the positive control (**PC**) group, chickens were fed a basal diet and kept at 22°C ambient temperature (thermoneutral) during the whole experiment period; in the heat stress (**HS**) group, chickens were fed the basal diet and subjected to constant heat stress at 32°C ambient temperature; in the heat stress + taurine (**HS + T**) group, chickens were fed the basal diet supplemented with 5.00 g/kg taurine and subjected to the same constant heat stress as the HS group. The feed was produced as a single batch, and then an aliquot was supplemented with taurine. The compositions and nutrients of the basal diets are presented in Table 1. Taurine was obtained from NOW FOODS (Bloomingdale, IL, USA), and the purity of taurine was 1,000 mg/g. All groups were maintained at 55 ± 5% relative humidity, and provided *ad libitum* access to feed and water manually. The cloacal temperatures of 2 randomly selected birds from each cage were measured using a rectal probe (Shandong Shangnong Electronic Technology Co., Ltd, Linyi, China) after 2 h, 3, 7 and 14 d of heat exposure. Body weight (**BW**) and feed consumption on a replicate (cage) basis were recorded after 7 and 14 d of heat exposure to calculate the average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**, feed : gain, g : g).

Sample Collection

After 7 and 14 d of heat exposure, 2 birds per cage (close to the replicate average BW) were selected, and blood samples were drawn from the wing vein of broilers using the additive-free vacutainers. Serum was obtained by centrifugation at 4,000 × g for 10 min at 4°C and stored at −20°C. Immediately after the blood samples were taken, the broilers were slaughtered by cervical dislocation and exsanguinations. After bleeding, the broilers were defeathered, eviscerated, and discarded the head and feet to determine carcass weight. The abdominal adipose tissue (from the surrounding proventriculus and the gizzard down to the cloaca), breast muscle, and legs from each bird were collected and weighed to calculate the carcass characteristics including the proportions of carcass, breast muscle, legs and abdominal fat in BW by the method of Lu et al. (2007). Meanwhile, the breast muscle and abdominal fat samples were collected and stored in liquid nitrogen for further analysis.

Serum Analysis

The concentrations of uric acid (**UA**) and non-esterified fatty acids (**NEFA**) in the serum were measured using commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by a 1200 UV spectrophotometer (Mapada Instruments Co., Ltd., Shanghai, China) with detection wavelengths of 690 and 440 nm respectively, according to the manufacturer's instructions.

Table 2. Primer sequences for real-time quantitative PCR analysis.

Gene[1]	Accession no.	Primer sequence (5′ to 3′ direction)	Product size (bp)
p70S6K	NM001030721	Forward: AAGAGGTGCTTCTGCCAGCG Reverse: TCATGCGCAAGTGCTCTGGTC	200
MAFbx	NM001039309	Forward: CCAACAACCCAGAGACCTGT Reverse: GGAGCTTCACACGAACATGA	180
MuRF1	NM204639	Forward: ACCCCCAACCCCATGATCCAG Reverse: TACACTGCTGTGGCCCCCAT	160
ACC	NM205505	Forward: AACGAGTCGGGCTACTACCT Reverse: ATCAGCATCCCGTGAAGTGG	119
FAS	NM205155	Forward: GCAGCTTCGGTGCCTGTGGTT Reverse: GCTGCTTGGCCCACACCTCC	119
M-CPT1	DQ314726	Forward: GATTTCTGCTGCTTCCAATTCG Reverse: TGCAGCGCGATCTGAATG	92
GAPDH	NM204305	Forward: GAGGGTAGTGAAGGCTGCTG Reverse: CATCAAAGGTGGAGGAATGG	113

[1]p70S6K = ribosomal protein S6 kinase, 70 kDa; MAFbx = muscle atrophy F-box protein; MuRF1 = muscle ring-finger protein-1; ACC = acetyl-CoA carboxylase 1c; FAS = fatty-acid synthase; M-CPT1 = muscular isoform of carnitine palmitoyl transferase 1; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

Lipid-Metabolism Parameters

The frozen breast muscle sample was weighed and homogenized in 9 volumes of ice-cold physiological saline (7.5 g/L NaCl diluent, w/v) in a chilled homogenizer. Immediately, the supernatant was collected by centrifugation for 15 min at 4,000 × g at 4°C for future analysis. The content of triglyceride (**TG**) in the breast muscle and the activity of lipoprotein lipase (**LPL**) in the abdominal fat were measured using commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) by a 1200 UV spectrophotometer (Mapada Instruments Co., Ltd., Shanghai, China) with detection wavelengths of 500 and 550 nm respectively. The activity of hormone-sensitive lipase (**HSL**) in the abdominal fat was measured by enzyme immunoassay using a commercial kit (Hengyuan Biological Technology Co., Ltd, Shanghai, China) by a Microplate Reader (Thermo Scientific, Wilmington, DE, USA) with a detection wavelength of 450 nm. All the parameters were measured according to the manufacturers' instructions.

Real-Time PCR Analysis

Total RNA was extracted from frozen breast muscle sample using RNAiso Plus reagent (Takara Biotechnology Co. Ltd, Dalian, China) according to the manufacturer's instructions. Briefly, about 30 mg frozen breast muscle sample was transferred into mortar, added liquid nitrogen, and then crushed with pestle to homogenize until powdery. The homogenized sample was transferred into a centrifuge tube with 1 mL RNAiso Plus immediately, and then was centrifuged at 12,000 × g for 5 min at 4°C. Then the chloroform and isopropanol were used to extract total RNA. After cleaning by 75% cold ethanol, the purity and quantity of total RNA were measured by ultramicrospectrophotometer (Thermo Scientific, Wilmington, DE, USA). Then the total RNA was treated with DNase I (Takara Biotechnology Co. Ltd.) to remove DNA and was reverse transcribed to cDNA (10 μL reaction system for maximum use of 500 ng of total RNA) using a PrimeScript RT Master Mix kit (Takara Biotechnology Co. Ltd.) according to the manufacturer's instructions. The reverse transcription reactions were incubated for 15 min at 37°C, followed by 5 s at 85°C to inactivate the reverse transcription enzyme. The reverse transcription products (cDNA) were stored at −20°C for real-time PCR.

Quantitative real-time PCR analyses were performed using SYBR Premix Ex Taq kits (Takara Biotechnology Co. Ltd, Dalian, China) on an ABI PRISM 7500 Detection System (Applied Biosystems, Foster City, CA, USA) as follows: 1 cycle at 95°C for 30 s; 40 cycles at 95°C for 5 s, and 60°C for 30 s. All of the specific sequences of primers are listed in Table 2. All of the samples were run in triplicate, and the expressions of target genes relative to GAPDH were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical Analysis

The statistical analyses of the data were performed using statistical software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The growth performance data were analyzed with the cage as the experimental unit, and other traits were analyzed with the mean of the 2 birds in each cage (n = 6). The data were checked for normal distribution and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. All data were normally distributed and exhibited homogeneity of variance, and then were analyzed using one-way analysis of variance (**ANOVA**) followed by a Tukey's post-hoc test. Data were expressed as means ± standard error (SE), and significance was set at $P < 0.05$.

RESULTS

Cloacal Temperature

As shown in Figure 1, cloacal temperatures of the HS and HS + T groups were significantly higher than those of the PC group at all 4 different points in time

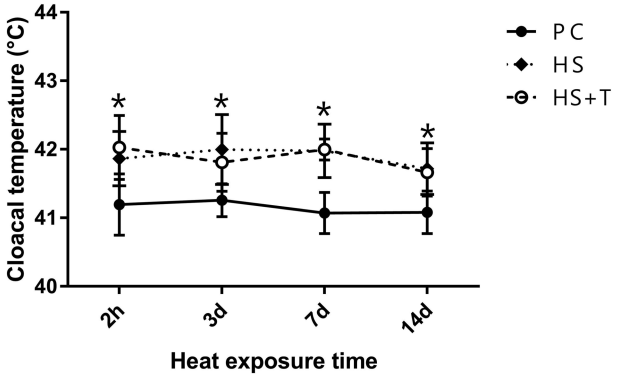

Figure 1. Effects of dietary supplementation of taurine on the cloacal temperature of broiler chickens exposed to chronic heat stress. Symbols and error bars represent means ± standard error (SE; n = 6). PC = positive control group; HS = heat stress group; HS + T = heat stress + taurine (5.00 g/kg diet) group. *Significant difference between the PC group and the other two groups ($P < 0.05$).

($P < 0.05$; Figure 1), and there was no significant difference in the cloacal temperatures of the HS and HS + T groups at the same point in time ($P > 0.05$).

Growth Performance

As shown in Table 3, BW, ADG and ADFI in the PC group were significantly higher than those of the HS and HS + T groups after 7 and 14 d of heat exposure, respectively ($P < 0.05$). Compared with the PC group, 7 and 14 d of heat exposure significantly increased the FCR in the HS group ($P < 0.05$). After 7 d of heat exposure, the FCR in the HS + T group was similar to the PC and HS groups ($P > 0.05$). In addition, after 14 d of heat exposure, there was a tendency for the FCR to decrease in the HS + T group compared with the HS group ($P = 0.071$).

Carcass Characteristics

As shown in Table 4, the proportion of abdominal fat in the PC group was significantly lower than that of the HS and HS + T groups after 7 and 14 d of heat exposure, respectively ($P < 0.05$), and there was a tendency for the proportion of abdominal fat to decrease in the HS + T group compared with the HS group after 14 d of heat exposure ($P = 0.074$). Compared with the PC and HS + T groups, 7 and 14 d of heat exposure significantly decreased the proportion of breast muscle in the HS group ($P < 0.05$), and there was no significant difference in the proportion of breast muscle between the PC and HS + T groups ($P > 0.05$). After 7 and 14 d of heat exposure, there was no significant difference in the proportions of carcass and legs among the three groups ($P > 0.05$).

Serum Analysis

As shown in Figure 2, after 7 d of heat exposure, the concentration of NEFA in the PC group was significantly higher than that of the other two groups ($P < 0.05$), and NEFA concentration in the HS + T group was significantly higher than that in the HS group ($P < 0.05$, Figure 2B), and there was no significant difference in the concentration of UA among the three groups ($P > 0.05$, Figure 2A). After 14 d of heat exposure, the concentration of UA in the HS group was significantly higher than that in the PC group ($P < 0.05$), and the concentration of UA in the HS + T group was similar to the PC and HS groups ($P > 0.05$, Figure 2A). After 14 d of heat exposure, the concentration of NEFA in the PC group was significantly higher than that of the other two groups ($P < 0.05$), and there was no significant difference in the NEFA concentrations of the HS and HS + T groups ($P > 0.05$, Figure 2B).

Table 3. Effects of dietary supplementation of taurine on the growth performance in broiler chickens exposed to chronic heat stress.[1]

Items[3]	Treatments[2]		
	PC	HS	HS + T
Initial BW (28 d old, g/bird)	1098.37 ± 11.46	1108.92 ± 11.55	1100.78 ± 16.61
After 7 d of heat exposure			
BW (35 d old, g/bird)	1521.66 ± 33.06^{a}	1371.71 ± 20.00^{b}	1381.50 ± 17.66^{b}
ADG (g/bird/d)	60.47 ± 4.33^{a}	37.54 ± 2.56^{b}	40.10 ± 1.00^{b}
ADFI (g/bird/d)	115.61 ± 4.12^{a}	89.89 ± 1.45^{b}	84.75 ± 2.71^{b}
FCR (feed : gain, g : g)	1.94 ± 0.09^{b}	2.45 ± 0.16^{a}	2.12 ± 0.10^{a,b}
After 14 d of heat exposure			
BW (42 d old, g/bird)	1901.46 ± 35.92^{a}	1674.10 ± 28.31^{b}	1741.40 ± 17.80^{b}
ADG (g/bird/d)	57.36 ± 2.64^{a}	40.37 ± 1.81^{b}	45.76 ± 2.45^{b}
ADFI (g/bird/d)	120.26 ± 2.06^{a}	100.98 ± 2.14^{b}	100.58 ± 1.78^{b}
FCR (feed : gain, g : g)	2.11 ± 0.07^{b}	2.52 ± 0.12^{a}	2.21 ± 0.08^{a,b}

[1]Each result represents the mean value followed by the standard error (SE); the data were analyzed with a cage as the experimental unit (n = 6).

[2]PC = positive control group; HS = heat stress group; HS + T = heat stress + taurine (5.00 g/kg diet) group.

[3]BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

[a,b]Means in a row without a common superscript letter significantly differ ($P < 0.05$).

Table 4. Effects of dietary supplementation of taurine on the carcass characteristics in broiler chickens exposed to chronic heat stress.[1]

Items[3]	Treatments[2]		
	PC	HS	HS + T
After 7 d of heat exposure			
Carcass (g/100 g of BW)	73.56 ± 0.38	73.89 ± 0.37	73.44 ± 0.65
Breast muscle (g/100 g of BW)	18.03 ± 0.27[a]	16.08 ± 0.30[b]	17.17 ± 0.21[a]
Abdominal fat (g/100 g of BW)	1.06 ± 0.14[b]	1.52 ± 0.10[a]	1.52 ± 0.12[a]
Legs (g/100 g of BW)	15.80 ± 0.16	15.51 ± 0.29	15.81 ± 0.09
After 14 d of heat exposure			
Carcass (g/100 g of BW)	74.61 ± 0.67	74.87 ± 0.61	76.36 ± 0.28
Breast muscle (g/100 g of BW)	18.63 ± 0.41[a]	16.55 ± 0.24[b]	18.17 ± 0.49[a]
Abdominal fat (g/100 g of BW)	1.37 ± 0.08[b]	1.94 ± 0.05[a]	1.75 ± 0.03[a]
Legs (g/100 g of BW)	15.98 ± 0.20	16.52 ± 0.21	16.66 ± 0.27

[1]Each result represents the mean value followed by the standard error (SE); the data were means of 6 replicates (cages) of 2 birds per cage (n = 6).
[2]PC = positive control group; HS = heat stress group; HS + T = heat stress + taurine (5.00 g/kg diet) group.
[3]BW = body weight.
[a,b]Means in a row without a common superscript letter significantly differ ($P < 0.05$).

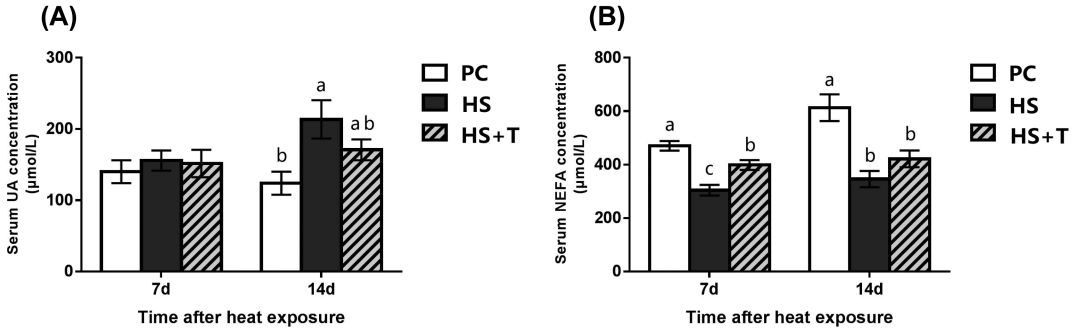

Figure 2. Effects of dietary supplementation of taurine on the serum UA (A) and NEFA (B) concentrations of broiler chickens exposed to chronic heat stress. Each result represents the mean value ± standard error (SE); Data are means of 6 replicates (cages) of 2 birds per cage (n = 6). Means without a common letter differ, $P < 0.05$. PC = positive control group; HS = heat stress group; HS + T = heat stress + taurine (5.00 g/kg diet) group. UA = uric acid; NEFA = non-esterified fatty acids.

Lipid-Metabolism Parameters

As shown in Figure 3, after 7 d of heat exposure, the content of TG in breast muscle of the HS group was significantly higher than that of the PC group ($P < 0.05$), and TG content in breast muscle of the HS + T group was similar to the PC and HS groups ($P > 0.05$, Figure 3A). The activity of LPL in abdominal fat of the PC group was significantly lower than that of the HS and HS + T groups ($P < 0.05$), and there was no significant difference in the LPL activities in abdominal fat of the HS and HS + T groups after 7 d of heat exposure ($P > 0.05$, Figure 3B). After 14 d of heat exposure, the content of TG in breast muscle and the activity of LPL in abdominal fat of the PC group were significantly lower than those of the other two groups ($P < 0.05$), and there was no significant difference in those parameters between the HS and HS + T groups ($P > 0.05$, Figures 3A and 3B). After 7 and 14 d of heat exposure, the activity of HSL in abdominal fat of the HS + T group was significantly higher than that of the other two groups ($P < 0.05$), and the enzymatic activity in the PC group was significantly higher than that in the HS group ($P < 0.05$, Figure 3C).

Gene Expressions

As shown in Table 5, the mRNA expressions of acetyl-CoA carboxylase 1c (**ACC**), muscular isoform of carnitine palmitoyl transferase 1 (**M-CPT**), muscle atrophy F-box protein (**MAFbx**), and muscle ring-finger protein-1 (**MuRF1**) in breast muscle of the HS group were significantly higher than those of the other two groups after 7 d of heat exposure ($P < 0.05$), and the mRNA expressions of ACC and MAFbx in the HS + T group were significantly higher than those of the PC group ($P < 0.05$). After 7 d of heat exposure, the mRNA expression of ribosomal protein S6 kinase, 70 kDa (**p70S6K**) in the PC group was significantly higher than that of the other two groups ($P < 0.05$), and there was no significant difference in the mRNA expression of fatty-acid synthase (**FAS**) among the three groups ($P > 0.05$). After 14 d of heat exposure, the

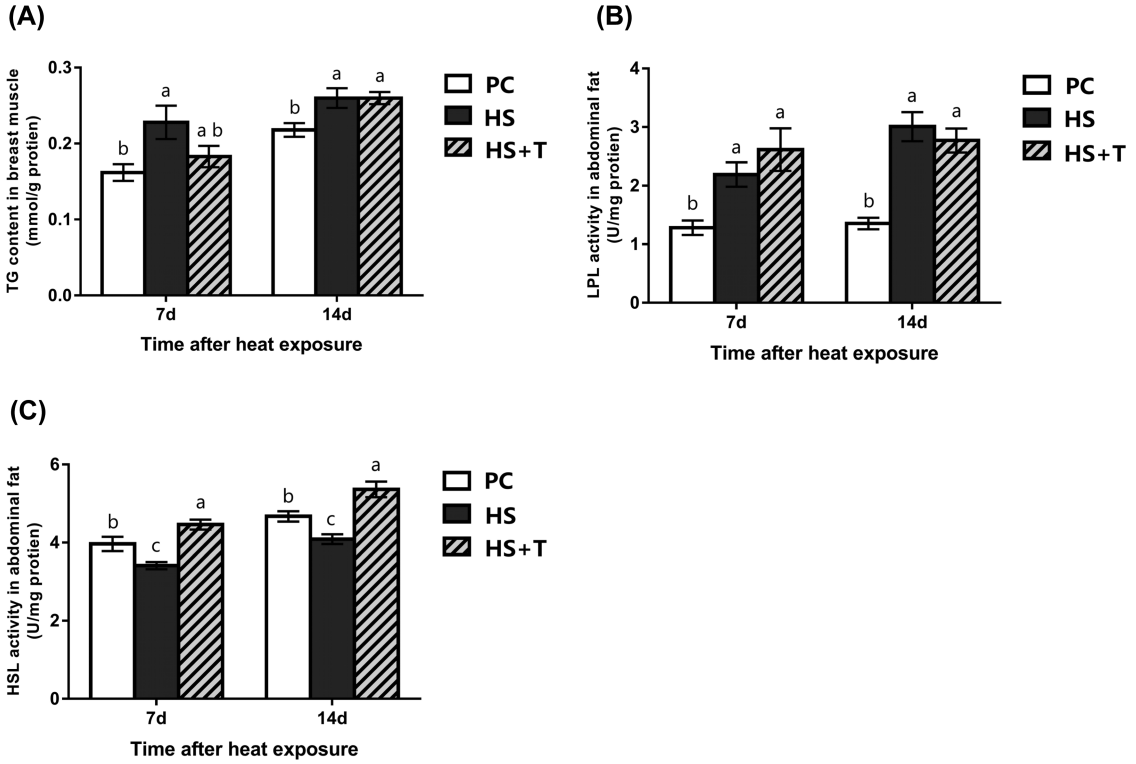

Figure 3. Effects of dietary supplementation of taurine on the TG content (A) in breast muscle and the activities of LPL (B) and HSL (C) in abdominal fat of broiler chickens exposed to chronic heat stress. Each result represents the mean value ± standard error (SE); Data are means of 6 replicates (cages) of 2 birds per cage (n = 6). Means without a common letter differ, $P < 0.05$. PC = positive control group; HS = heat stress group; HS + T = heat stress + taurine (5.00 g/kg diet) group. TG = triglyceride; LPL = lipoprotein lipase; HSL = hormone-sensitive lipase.

Table 5. Effects of dietary supplementation of taurine on the mRNA expressions in breast muscle of broiler chickens exposed to chronic heat stress.[1]

Items[3]	Treatments[2]		
	PC	HS	HS + T
After 7 d of heat exposure			
ACC	0.99 ± 0.05^{c}	4.37 ± 0.22^{a}	2.51 ± 0.26^{b}
FAS	1.00 ± 0.07	1.05 ± 0.15	0.97 ± 0.17
M-CPT1	1.13 ± 0.12^{b}	1.76 ± 0.16^{a}	1.05 ± 0.13^{b}
MAFbx	1.09 ± 0.19^{c}	4.14 ± 0.42^{a}	2.87 ± 0.26^{b}
MuRF1	1.22 ± 0.18^{b}	2.00 ± 0.11^{a}	1.44 ± 0.10^{b}
p70S6K	1.01 ± 0.03^{a}	0.74 ± 0.06^{b}	0.62 ± 0.08^{b}
After 14 d of heat exposure			
ACC	1.08 ± 0.14^{b}	1.86 ± 0.19^{a}	2.10 ± 0.26^{a}
FAS	1.05 ± 0.16^{b}	1.78 ± 0.12^{a}	1.16 ± 0.20^{b}
M-CPT1	1.27 ± 0.11^{b}	0.69 ± 0.04^{c}	2.00 ± 0.13^{a}
MAFbx	1.24 ± 0.13^{b}	3.24 ± 0.20^{a}	0.86 ± 0.06^{b}
MuRF1	1.01 ± 0.07^{b}	1.67 ± 0.24^{a}	0.99 ± 0.12^{b}
p70S6K	1.23 ± 0.13^{b}	1.26 ± 0.08^{b}	1.78 ± 0.19^{a}

[1]Each result represents the mean value followed by the standard error (SE); the data were means of 6 replicates (cages) of 2 birds per cage (n = 6).

[2]PC = positive control group; HS = heat stress group; HS + T = heat stress + taurine (5.00 g/kg diet) group.

[3]ACC = acetyl-CoA carboxylase 1c; FAS = fatty-acid synthase; M-CPT1 = muscular isoform of carnitine palmitoyl transferase 1; MAFbx = muscle atrophy F-box protein; MuRF1 = muscle ring-finger protein-1; p70S6K = ribosomal protein S6 kinase, 70 kDa.

[a-c]Means in a row without a common superscript letter significantly differ ($P < 0.05$).

mRNA expressions of FAS, MAFbx, and MuRF1 in the HS group were significantly higher than those of the PC and HS + T groups ($P < 0.05$), and the mRNA expression of ACC in the PC group was significantly lower than that of the other two groups ($P < 0.05$). Meanwhile, the mRNA expression of M-CPT1 in the HS + T group was significantly higher than that of the other two groups ($P < 0.05$), and this gene expression in the HS group was significantly lower than that of the other two groups ($P < 0.05$). After 14 d of heat exposure, the mRNA expression of p70S6K in the HS + T group was significantly higher than that in the other two groups ($P < 0.05$), and there was no significant difference in the mRNA expression of p70S6K between the PC and HS groups ($P > 0.05$).

DISCUSSION

It is now widely accepted that broilers cannot maintain normal body temperature and are considered to be heat-stressed when the ambient temperature exceeds the thermoneutral zone (16 to 26°C) (Diarra and Tabuaciri, 2014). In this study, chronic heat exposure caused significant increase in cloacal temperatures of broilers in the HS and HS + T groups at all 4 different points in time. The elevation in cloacal temperature reflects the chicken's inability to dissipate excessive heat, proving the plausibility of the chronic heat stress model (Edgar et al., 2013). As many amino acids are

regarded as important neurotransmitters in the thermoregulatory system, amino acids have received more and more attention with their thermoregulatory effects. The hypothalamus is the central organ of thermoregulation and contains high concentration of taurine (Ivanov, 1997). A previous study reported that taurine could reduce body temperature of rabbits exposed to heat stress (Frosini et al., 2000). However, in the present study, taurine supplementation did not alleviate the increase in cloacal temperatures of broilers exposed to chronic heat stress. The discrepancy in thermoregulatory effects might be related to the difference of species.

Numerous studies have shown that chronic heat stress negatively affected the production performance of broilers (Zhang et al., 2017). Oliveira et al. (2016) reported that the feed intake was decreased by 2.2% per degree centigrade increase in room temperature above the thermoneutral zone, which means the decreasing feed intake may be a mechanism to decrease the production of body heat (Sohail et al., 2013). In the present study, chronic heat stress significantly decreased BW, ADFI and ADG in the HS group compared with the PC group, which is in line with the results of Yi et al. (2016). However, in the present study, taurine supplementation did not alleviate the decrease in BW, ADFI and ADG caused by chronic heat stress. A previous study reported that taurine supplementation significantly enhanced body weight gain of heat-stressed broilers and did not affect the feed intake (Shim et al., 2006). In the present study, chronic heat stress significantly increased the FCR in the HS group compared with the PC group, and taurine supplementation tended to decrease the FCR in the HS + T group compared with the HS group after 14 d of heat exposure. Traditional studies indicated that the decreased production performance of heat-stressed broilers mainly resulted from the decline in nutrient uptake, but a recent study in our laboratory found that the decrease in production performance was caused by both the decline in feed intake and changes in nutrient postabsorptive metabolism (Lu et al., 2018). Thus, taurine supplementation might improve the nutrient metabolism of chronic heat-stressed broilers to affect the feed efficiency, though the treatment did not alleviate the decrease in feed intake caused by high temperature.

In addition to concerns about performance impairment, previous studies have reported that chronic heat stress significantly changed the carcass characteristics of broilers (Lu et al., 2007). Ain Baziz et al. (1996) reported that chronic heat stress could enhance the proportion of the carcass of broilers, which might be explained by the decrease in feather proportion to improve heat losses (Geraert et al., 1996). In the present study, chronic heat stress and taurine supplementation did not affect the carcass yield of broilers, which may be related to shorter duration of high temperature compared with the study of Ain Baziz et al. (1996). In the present study, chronic heat stress significantly decreased breast muscle yield in the HS group compared with the PC group, which is in line with the findings of Lu et al. (2007) and Ain Baziz et al. (1996). Muscle development is the combined effect of the synthesis and degradation of protein. Zuo et al. (2015) reported that chronic heat stress reduced protein deposition in the breast muscle of broilers mainly via restraining protein synthesis. p70S6K is an important protein mediating ribosomal protein mRNA translation and affecting total protein synthesis (Gingras et al., 2001). In the present study, the mRNA expression of p70S6K in the PC group was significantly higher than that of the HS and HS + T groups after 7 d of heat exposure, which suggests that 7 d of heat exposure significantly suppressed protein synthesis, and taurine supplementation did not alleviate the decrease in protein synthesis caused by high temperature. After 14 d of heat exposure, the mRNA expression of p70S6K in the HS + T group was significantly higher than that in the other two groups, which suggests that taurine supplementation promoted protein synthesis in the breast muscle at the later phase of chronic heat stress. Besides, there was no significant difference in the mRNA expression of p70S6K between the PC and HS groups after 14 d of heat exposure, which may be related to heat acclimation and compensatory growth (Zulkifli et al., 1994). Many previous studies showed that chronic heat stress greatly promoted protein degradation, which might be related to disordered energy metabolism (Lu et al., 2018). The ubiquitin-proteasome pathway is regarded as the main pathway of protein degradation (Mitch et al., 1999), and two muscle-specific ubiquitin ligases, MAFbx and MuRF1, play a vital role in the process of muscle degradation (Sacheck et al., 2004). In the present study, the mRNA expressions of MAFbx and MuRF1 in the HS group were significantly higher than those of the PC group after 7 and 14 d of heat exposure, which suggests that chronic heat stress enhanced protein degradation in the breast muscle of broilers. Those results are in agreement with the previous metabolomics analysis in our laboratory (Lu et al., 2018). Besides, the breast muscle proportion in the HS + T group was significantly higher than that of the HS group after 7 and 14 d of heat exposure, and the mRNA expressions of MAFbx and MuRF1 in the HS + T group were significantly lower than those of the HS group, which suggests that taurine supplementation effectively alleviated protein degradation in the breast muscle of heat-stressed broilers. The results that the serum UA concentration in PC group was significantly lower than that of the HS group and similar to that of the HS + T group further confirmed above findings. In the present study, chronic heat stress and taurine supplementation did not affect the leg yield of broilers. Similarly, the previous study in our laboratory also showed that chronic heat stress did not affect the leg yield of broilers, which may be related to the finding that the protein degradation for energy supply in chronic heat-stressed broilers mainly occurred in the breast muscles rather than the leg muscles (Lu et al., 2018).

On the other hand, the previous studies also showed that chronic heat stress could increase lipid deposition of broilers (Ain Baziz et al., 1996; Lu et al., 2007). Similarly, in this study, chronic heat exposure significantly increased the proportion of abdominal fat in the HS group compared with the PC group. Unlike in mammals, adipose tissue of birds serves only as a fat storage site, and the development of adipose tissue is dependent on the utilization of plasma triglycerides (Griffin et al., 1992). LPL is a key enzyme for the hydrolysis of plasma lipoproteins and is strongly associated with fat deposition and obesity (Cai et al., 2009). In the present study, chronic heat exposure significantly enhanced the LPL activity in abdominal fat of the HS group compared with the PC group, which is in line with the findings of Yuan et al. (2008). In addition, it is widely accepted that chronic heat-stressed animals are energy-deficient but unable to effectively mobilize fat (Rhoads et al., 2013), which may be explained by decreased lipolytic enzyme activity (Torlinska et al., 1987). In this study, chronic heat exposure significantly decreased the HSL activity in abdominal fat and NEFA concentration in serum of the HS group compared with the PC group, which is consistent with our pervious study and suggests that chronic heat exposure significantly suppressed lipolysis in abdominal fat (Lu et al., 2018). However, taurine supplementation significantly enhanced the HSL activity in abdominal fat and resulted in higher NEFA concentration compared with the HS group, which suggests that the treatment effectively facilitated lipolysis for energy. Moreover, there was a tendency for the proportion of abdominal fat to decrease in the HS + T group compared with the HS group after 14 d of heat exposure, which also proved that taurine supplementation promoted lipolysis to some extent. The previous study in our laboratory also found that chronic heat stress caused mitochondria to malfunction and suppressed lipid aerobic metabolism, resulting in increased TG content in muscles (Lu et al., 2017). Similarly, in this study, chronic heat stress significantly enhanced TG content in breast muscles and the fat synthesis key genes of ACC and FAS mRNA expressions compared with the PC group. On the other hand, M-CPT 1 is regarded as a key enzyme of mitochondrial oxidation of fatty acids. Though 7 d of heat exposure significantly enhanced the mRNA expression of M-CPT 1 for urgent energy demand, 14 d of heat exposure significantly decreased this mRNA expression compared with the PC group, which is similar with our previous study and related to mitochondrial dysfunction (Lu et al., 2017). In the present study, taurine supplementation did not alleviate the increased TG content and mRNA expression of ACC in breast muscles caused by chronic heat exposure. However, taurine supplementation significantly decreased the mRNA expression of FAS and increased the mRNA expression of M-CPT 1 compared with the HS group after 14 d of heat exposure, which suggests that taurine supplementation might enhance lipid aerobic metabolism in breast muscles. The mechanisms under these results may be related to the fact that taurine supplementation might protect mitochondria from oxidative stress at initial phase of heat stress and enhance lipid aerobic metabolism for energy (Shimada et al., 2015; Lu et al., 2017). Thus, taurine supplementation might alleviate the degradation of protein for energy production in breast muscles of chronic heat-stressed broilers by mobilizing fat effectively.

In conclusion, chronic heat stress significantly decreased production performance and impaired carcass characteristics of broilers. Taurine supplementation can improve carcass characteristics of chronic heat-stressed broilers by facilitating lipolysis for energy, enhancing protein synthesis, and suppressing protein degradation of breast muscles.

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