Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers

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ABSTRACT Heat stress impairs growth performance and alters body protein and amino acid metabolism. This study was investigated to explore how body protein and amino acid metabolism changed under heat stress (**HS**) and the stress adaptation mechanism. A total of 144 broilers (28 d old) were divided into 3 treatment groups for 1 wk: HS group (32°C), normal control group (22°C), and pair-feeding group (22°C). We found that HS elevated the feed-to-gain ratio, reduced the ADFI and ADG, decreased breast muscle mass and plasma levels of several amino acids (glycine, lysine, threonine, and tyrosine), and increased serum glutamic oxaloacetic transaminase (**GOT**) activity and corticosterone (**CORT**) level and liver GOT and glutamic pyruvic transaminase activities. Heat stress elevated muscle atrophy F-box mRNA expression and reduced mRNA expression of the 70-kD ribosomal protein S6 kinase in the breast muscle of broilers. Broilers in the HS group exhibited striking increases of mRNA expressions of solute carrier family 1 member 1, family 3 member 1, family 7 member 1, and family 7 member-like in the liver and liver gluconeogenesis genes (*PCKc*, *PCKm*, *PC*, and *FBP1*) in comparison with the other 2 groups. In conclusion, HS increased the circulating CORT level and subsequently caused muscle protein breakdown to provide amino acid substrates to liver gluconeogenesis responsible for energy supply.

Key words: heat stress, broiler, corticosterone, amino acid metabolism, protein metabolism

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INTRODUCTION

With the rising severity of global warming, heat stress (**HS**) triggered by high ambient temperature is the main environmental factor causing enormous economic loss in poultry production. Broiler chickens covered with feather possess underdeveloped sweat glands, and the long-standing genetic breeding for meat production causes elevated body heat production (He et al., 2018a). For these characteristics, broilers are susceptible to HS. Broilers suffered from HS exhibit physiological dyshomeostasis and poor performance, which has been considered as the result of the reduction of the feed intake (Habashy et al., 2017). The decreased uptake of energy substance and the increased body energy expenditure induce insufficient energy supply in heat-stressed broilers, subsequently decomposing body glycogen to supply energy for energy utilization (Akşit et al., 2006). Fat is mobilized for energy supply under normal physiological conditions. The process of fat mobilization is suppressed; meanwhile, fat synthesis and deposition are promoted in heat-stressed broilers (Lu et al., 2017), which aggravates body energy expenditure. Amino acid (**AA**) is mainly used for growth and development of tissues and also utilized as metabolic fuel to supply energy by liver gluconeogenesis. However, it remains to be elucidated whether AA is used for energy supply in broilers under HS.

Corticosterone (**CORT**), as a downstream hormone of the hypothalamic-pituitary-adrenal axis, regulates body multiple critical physiological processes and energy distribution, and it is also a main biological indicator of the HS response (Cockrem, 2007; Iyasere et al., 2017). It is well established that CORT plays a critical role in the development of skeletal muscle (Biedasek et al.,

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2011; Morgan et al., 2016). Corticosterone drives muscle atrophy through suppressing protein synthesis and accelerating protein breakdown (Dong et al., 2007; Biedasek et al., 2011). In addition, the key enzyme of gluconeogenesis possesses glucocorticoid receptor-binding sites on its promoter, and thus, CORT plays a critical role in regulating gluconeogenesis (Imai et al., 1993; Lin et al., 2004). Heat stress triggers skeletal muscle catabolism and changes of AA metabolism (Tabiri et al., 2002; Ma et al., 2018). However, it is unknown whether gluconeogenesis is activated in heat-stressed broilers and what role CORT plays in gluconeogenesis.

Insulin-like growth factor-1 (**IGF-1**) has pleiotropic effects including the regulation of body growth and mediation of the energy metabolism (Park et al., 2020; Riis et al., 2020). The level of circulating IGF-1 possesses a positive regulation on body growth achieved by binding IGF-1 receptor (**IGF-1R**) and activating its downstream signaling pathway (Elis et al., 2010; François et al., 2017). Mammalian target of rapamycin, the main downstream target of IGF-1R (Bibollet-Bahena and Almazan, 2009), participates in protein metabolism and muscle growth via activating the 70-kD ribosomal protein S6 kinase (**S6K1**) and eukaryotic translation initiation factor 4E-binding protein-1. A previous study indicated that there was a negative interrelation between the circulating level of IGF-1 and the concentration of CORT (Gayan-Ramirez et al., 1999; Lodjak et al., 2016). Conversely, other research manifested that experimental improvement of circulating CORT did not affect circulating IGF-1 in passerine birds, and the interrelation between CORT and IGF-1 was dependent on the physiological status (Tóth et al., 2018). The changes of circulating IGF-1 and CORT as well as their functions in protein and AA metabolism under HS remain poorly understood.

Therefore, our present study was conducted to determine the changes of circulating CORT and IGF-1 and their effects on protein and AA metabolism as well as liver gluconeogenesis under HS.

MATERIALS AND METHODS

Experimental Design

Two hundred chicks (1 d old, male, Arbor Acres) were purchased from Shan's Poultry Farming Professional cooperative (Chuzhou, Anhui, China) and received standard diets and management for 27 d. Our study on live birds and procedures met the guidelines of the Institutional Animal Care and Use Committee of Nanjing Agricultural University. At day 28, 144 chickens with the closed weight were divided into 3 environment-controlled chambers. Each chamber included 6 replicates (cages) with 8 birds per replicate (cage) (cage: length × width × height: 1.0 m × 0.5 m × 0.4 m). Chickens in the normal control (**NC**) group were maintained at 22°C and received feed and water with free access. Broilers in the HS group were kept at 32°C and received feed and water with free access. Chickens in pair-feeding (**PF**) group were provided with equal exact amount of ADFI of the HS group on the previous day and raised in the same ambient temperature (22°C) as the NC group with *ad libitum* water. The relative humidity was maintained at 55 ± 5%. All experimental treatments lasted for 1 wk. The corn-soybean meal basal diet was applied for 3 groups, and the composition is listed in Table 1. The feed intake was documented daily, and the BW was documented on 28 and 35 d of age. The ratio of the ADFI to ADG was calculated as the feed-to-gain ratio (**F/G**).

Sampling

On 35 d, 2 chickens with a BW closed to the average BW of each replicate were stunned by electric shock (50 V, alternating current; 400 Hz for 5 s), subsequently euthanized by exsanguination. Serum and plasma were obtained from blood samples. The whole breast muscle was immediately separated and weighed for calculating breast muscle yield on a live weight basis. The breast muscle (3-g) and liver (3-g) with the same location were sampled and frozen in liquid nitrogen immediately before storing in an ultralow-temperature freezer (−80°C) for further index detection.

Blood Parameters Analysis

Serum indexes including glutamic oxaloacetic transaminase (**GOT**) and glutamic pyruvic transaminase (**GPT**) activities and the level of total protein (**TP**) were detected by the reagent kit (the Nanjing Jiancheng Bioengineering Institute, Nanjing, China). We used the Elisa reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to determine the serum CORT level. The radioimmunoassay reagent kit (Beijing North Institute of Biotechnology Company, Beijing, China) was applied for measurement of serum IGF-1 level.

Table 1. The compositions of basal diet.

Ingredients (%)		Calculated nutrient levels (%)	
Corn	62.07	ME (MJ/kg)	13.19
Soybean meal	23.00	CP	19.60
Corn gluten meal[1]	6.00	Calcium	0.95
Soybean oil	4.00	Available phosphorus	0.39
Limestone	1.20	Lysine	1.05
Dicalcium phosphate	2.00	Methionine	0.42
L-lysine	0.35	Methionine + cysteine	0.76
DL-methionine	0.08		
Salt	0.30		
Premix[2]	1.00		

[1]The CP content was 60%.

[2]Per kilogram of the diet, the premix provided retinyl acetate for vitamin A, 12,000 IU; cholecalciferol for vitamin D_3, 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.

Table 2. Parameters of primer pairs for RT-PCR.

Genes[1]	GenBank number	Sequence (5′→ 3′)	Product size (bp)
GAPDH	NM_204305.1	Forward: GAGGGTAGTGAAGGCTGCTG	113
		Reverse: CATCAAAGGTGGAGGAATGG	
IGF-I	NM_001004384.2	Forward: CACTGTGTGGTGCTGAGCTG	139
		Reverse: TGGAAGCAGCATTCATCCAC	
IGF-IR	NM_205032.1	Forward: GTCCAGGAACGATGGAGGAG	127
		Reverse: CAGGCTCTCTTGCCACATGA	
S6K1	NM_001030721.1	Forward: TGGACCATGGAGGAGTTGG	111
		Reverse: AGCACTCCGGTCGGATCTT	
4EBP1	XM_424384.5	Forward: GTTCCTGATGGAGTGCCGTA	80
		Reverse: GGCTGGTAACACCTGGAATG	
MAFbx	NM_001030956.1	Forward: CCTTCACAGACCTGCCATTG	140
		Reverse: GCAGAGCTTCTTCCACAGCA	
MuRF1	XM_015297755.1	Forward: GCTGGTGGAGAACATCATCG	123
		Reverse: TCGCAGGTGACGCAGTAGAT	
SLC1A1	XM_424930.6	Forward: CCTGTCACCTTCCGCTGTGC	200
		Reverse: GCTGCTGTTGCTGTGACACTAATG	
SLC6A19	XM_419056.6	Forward: GGGTTTTGTGTTGGCTTAGGAA	60
		Reverse: TCCATGGCTCTGGCAGAGAT	
SLC3A1	XM_004935370.3	Forward: GGCTTACCAATGGAGCTGAGTGAG	100
		Reverse: CTTGGCTGCTGGTGTCAGTATCC	
SLC7A9	NM_001199133.1	Forward: CAGGTGGGCCTGATTAGTGG	108
		Reverse: AGGACCCACAGCTCCAACAT	
SLC7A1	XM_015277945.2	Forward: CAAGAGGAAAACTCCAGTAATTGCA	75
		Reverse: AAGTCGAAGAGGAAGGCCATAA	
SLC6A14	XM_025150098.1	Forward: CTCCAGTGGGCTGGATGAGA	88
		Reverse: CAAGGCAGCTCCAACGATCA	
SLC7AL	XM_418326.6	Forward: CAGAAAACCTCAGAGCTCCCTTT	71
		Reverse: TGAGTACAGAGCCAGCGCAAT	
SLC7A6	XM_025154295.1	Forward: AAGGAACTTGCCGCTGGCTATTG	129
		Reverse: GTCACTGCCACAGCATCACTAGAG	
SLC38A2	NM_001305439.1	Forward: ATGAACATCGAAGAGAACGCAGGAC	80
		Reverse: AGAATGAGGATAACAGACACCAACAGC	
PCKc	NM_205471.1	Forward: GAGAGCCTGCCTCCACAA	284
		Reverse: CGACCCAGCTGGCTACTT	
PCKm	NM_205470.1	Forward: CCGAGCACATGCTGATTTT	294
		Reverse: CGTTGGTGAAGATGGTGTTG	
PC	NM_204346.1	Forward: AAGACGCTGCACATCAAAGC	113
		Reverse: TGGGTGTCTCTCACGAGGAT	
FBP1	NM_001278048.1	Forward: ATGACCCGCTTCGTGATG	87
		Reverse: CCAGCAATCCCATACAGGTT	

[1]IGF-I, insulin like growth factor I; IGF-IR, insulin like growth factor I receptor; S6K1, the 70-kD ribosomal protein S6 kinase; 4EBP1, eukaryotic translation initiation factor 4E-binding protein-1; MAFbx, muscle atrophy F-box; MuRF1, muscle RING finger 1; SLC1A1, solute carrier family 1 member 1; SLC6A19, solute carrier family 6 member 19; SLC3A1, solute carrier family 3 member 1; SLC7A9, solute carrier family 7 member 9; SLC7A1, solute carrier family 7 member 1; SLC6A14, solute carrier family 6 member 14; SLC7AL, solute carrier family 7 member-like; SLC7A6, solute carrier family 7 member 6; SLC38A2, solute carrier family 38 member 2; PCKc, phosphoenolpyruvate carboxykinase–cytosolic form; PCKm, phosphoenolpyruvate carboxykinase–mitochondrial form; PC, pyruvate carboxylase; FBP1, fructose-1,6-bisphosphatase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Plasma AA concentration was measured by using the L-8900 amino acid detection instrument (Hitachi, Tokyo, Japan). Briefly, 50-μL plasma was placed in 150-μL 10% sulfosalicylic for precipitating protein at 4°C for 15 min. Then, the supernatant was obtained and isolated from the mixture by centrifugation (25,000 rpm, 4°C, 35 min) and subsequently filtrated by a 0.22-μm disposable filter membrane for AA determination.

Liver Aminotransferase Activity Analysis

The compound consisted of approximately 1-g frozen liver tissue, and 9-mL chilled saline was homogenized and centrifuged to collect the supernatant for analysis of TP and aminotransferase activities. The level of TP was detected using the protein assay kit (Beyotime Biotechnology, Shanghai, China). We used a commercial reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to measure the liver GOT and GPT activities in accordance with manufacturer's recommendations.

Real-Time Quantitative PCR

The relative gene expressions in the breast muscle and liver of broilers were detected by real-time quantitative PCR (**RT-PCR**) using the 7,500 sequence detection machine (Applied Biosystems, Foster City, CA). 50-mg of frozen tissue was lysed and homogenized in 1,000-μL ice-cold TRIzol Reagent (Thermo Fisher Scientific Inc., Shanghai, China) for RNA extraction. The samples with the 260/280 ratios of 1.8 to 2.0 and the 260/230 ratios of 2.0 to 2.2 were chose to carry out the PCR experiment. The 500-ng RNA was applied for cDNA synthesis by using the Reverse-Transcription Master Mix reagent

(Takara Biomedical Technology Company, Beijing, China) at 37°C for 15 min, followed by 85°C for 5 s, terminated by 4°C for subsequent RT-PCR. The reaction system was carried out with 1-μL cDNA and 0.4-μL forward and reverse primers in an ultimate volume of 20-μL in accordance with the TB green Premix Ex Taq reagent kit recommendations (Takara, Beijing, China). The primers used were designed based on chicken (gallus) sequence in GenBank and synthesized commercially (Sangon Biotechnology, Shanghai, China). The detailed information of primer is provided in Table 2. The reaction circumstance of RT-PCR was established at 95°C for 30 s, then 40 cycles of 95°C for 5 s, and 60°C for 30 s. Results of target gene expressions were normalized by glyceraldehyde-3-phosphate dehydrogenase and calculated by the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001).

Statistical Analysis

The replicate was regarded as a statistic unit for analysis of growth performance (ADFI, ADG, and F/G), and the average of 2 birds per replicate was applied for parameter analysis of all other variables. Results were presented as the mean ± SE. We used the Kolmogorov–Smirnov test to test the normal distribution of the data. We used SPSS software (version 20.0, SPSS Inc.) and used one-way ANOVA to assess statistical significance, and $P < 0.05$ was identified as significance.

RESULTS

Growth Performance

Birds in the HS group exhibited the reduction of the ADG, ADFI, and breast muscle mass and exhibited the elevation of the F/G compared with birds in the NC group (Table 3, $P < 0.05$). There was no significant difference in the ADG, ADFI, F/G, and breast muscle mass between the HS and PF groups (Table 3, $P > 0.05$).

Serum Parameters

As shown in Table 4, the serum GOT activity and CORT level in birds from the HS group were significantly elevated compared with that in the other 2 groups ($P < 0.05$).

As shown in Table 5, HS decreased plasma concentrations of glycine, lysine, threonine, and tyrosine compared with the NC group ($P < 0.05$). There were no significant differences in plasma concentrations of glycine, lysine, threonine, and tyrosine between the HS and PF groups ($P > 0.05$).

Liver Aminotransferase Activities

Table 6 displays that birds in the NC group showed lower liver GOT and GPT activities than those of the HS group ($P < 0.05$). There was no significant change in the liver GOT and GPT activities between the HS and PF groups ($P > 0.05$).

Gene Expressions in Breast Muscle

Birds in the HS group displayed significant reduction in mRNA expression of S6K1 and elevation in mRNA expression of muscle atrophy F-box (**MAFbx**) in the breast muscle, compared with the other 2 groups (Figure 1, $P < 0.05$).

Expressions Analysis of Amino Acid Transporters in the Broiler Liver

As shown in Figure 2, HS significantly increased mRNA expressions of solute carrier family 1 member 1 (**SLC1A1**), solute carrier family 3 member 1 (**SLC3A1**), solute carrier family 7 member 1 (**SLC7A1**), and solute carrier family 7 member-like in the broiler liver compared with those of the other 2 groups ($P < 0.05$). Chickens from the PF group exhibited lower mRNA expressions of solute carrier family 6 member 14 and solute carrier family 7 member 6 in the liver than those of the other 2 groups ($P < 0.05$).

Expressions Analysis of Gluconeogenesis in the Broiler Liver

Figure 3 shows that HS significantly increased mRNA expressions of phosphoenolpyruvate carboxykinase–cytosolic form (**PCKc**), phosphoenolpyruvate carboxykinase–mitochondrial form (**PCKm**), pyruvate carboxylase (**PC**), and fructose-1,6-bisphosphatase (**FBP1**) in the broiler liver compared with the NC and PF groups ($P < 0.05$).

DISCUSSION

The thermosensitivity of modern broiler breeds is higher than previous genotypes because of frequent selection for fast-growing strains (Deeb and Cahaner, 2002). Thus, these fast-growing broilers are susceptible to HS in summer, especially in tropical and subtropical areas. In our study, HS triggered negative effects on the ADFI, ADG, and F/G. Although we found no differences in the ADFI and F/G between HS and PF groups, birds in the HS group displayed a decreasing trend ($P = 0.052$) in the ADG in comparison with birds in the PF group, which suggested that the impairment of growth performance caused by HS was partly independent of the decreased feed intake. Heat stress reduced the feed intake and caused the elevation of the respiratory rate in broilers, subsequently inducing glycogen depletion and negative energy balance (Akşit et al., 2006; He et al., 2018b). The heat-stressed broilers do not mobilize fat to supply energy, but instead promote fat deposition in the body composition (Lu et al., 2019). As the largest reservoirs of protein and AA, muscle protein metabolism directly affects the whole-body AA and protein metabolism. Under stress conditions,

Table 3. Effects of heat stress on growth performance in broilers.

Growth parameter[1]	Group[2] NC	HS	PF	*P*-value
Initial BW (g)	1,246.67 ± 7.14	1,268.54 ± 11.40	1,264.17 ± 11.80	0.313
Final BW (g)	1,833.33 ± 12.73[a]	1,663.54 ± 40.82[b]	1,694.58 ± 7.29[b]	0.001
ADFI (g/bird/d)	171.88 ± 3.09[a]	138.42 ± 3.03[b]	145.05 ± 0.43[b]	<0.001
ADG (g/bird/d)	83.81 ± 0.95[a]	56.43 ± 2.60[b]	61.49 ± 1.10[b]	<0.001
F/G (g/g)	2.05 ± 0.02[b]	2.46 ± 0.05[a]	2.36 ± 0.05[a]	<0.001
Breast muscle mass (g)	384.62 ± 14.19[a]	310.49 ± 16.89[b]	317.77 ± 14.54[b]	0.007
Breast muscle yield (%)	18.75 ± 0.69	18.70 ± 0.52	17.98 ± 0.15	0.508

[a,b]Differ according to one-way ANOVA followed by Duncan's test ($P < 0.05$).
Results are represented as the mean value ± SE (n = 6).
[1]F/G, feed to gain ratio.
[2]NC, normal control group; HS, heat-stress group; PF, pair-fed group.

the muscle is catabolized to AA substrates for supplying energy via liver gluconeogenesis (Wernerman, 1996; Pedroso et al., 2012; Sandri, 2016). But, it is unclear whether this situation also occurred in broilers under the HS condition. We noted that HS significantly reduced breast muscle mass, which suggested that breast muscle breakdown may be activated to provide AA substrates for energy supply in birds that experienced HS.

Muscle breakdown means protein catabolism. Insulin-like growth factor-1 is synthesized and secreted from the liver, subsequently transported to blood for regulating body growth and protein metabolism (Beccavin, 2001; Sacheck et al., 2004). It increases chicken muscle AA uptake and protein synthesis and suppresses protein catabolism via binding IGF-1R and triggering downstream effectors S6K1 and eukaryotic translation initiation factor 4E-binding protein-1 (Crossland et al., 2013; Ma et al., 2018). In the present study, no significant differences were found in serum IGF-1 level and mRNA expressions of IGF-1 and IGF-1R in the breast muscle among the 3 groups. However, HS reduced S6K1 mRNA expression and increased MAFbx mRNA expression in the breast muscle, suggesting that HS regulates broiler muscle protein metabolism through a mechanism independent of the IGF-1 pathway.

Corticosterone, the major glucocorticoids in animals, is a stress response hormone secreted from the adrenal cortex and transported to blood for regulating body energy resource allocation (Post et al., 2003; Lin et al., 2006; Tóth et al., 2018). The alternation of the circulating CORT concentration directly regulates the myofibrillar protein metabolism (Doi et al., 2002; McGhee et al., 2009). It is widely accepted that a high circulating CORT concentration suppresses muscle growth and protein synthesis (Doi et al., 2002; De Theije et al., 2018). Previous research showed that CORT treatment decreased C2C12 myotubes diameters and increased the protein degradation rate via upregulating expressions of MAFbx and muscle RING finger 1 (Menconi et al., 2008). In addition, CORT also negative regulates mammalian target of rapamycin and S6K1, whereafter a reduction of protein synthesis occurred (McGhee et al., 2009; Pazini et al., 2016). In the present study, the circulating CORT and breast muscle MAFbx expression in the HS group were significantly increased, and breast muscle S6K1 expression was significantly decreased in birds that experienced HS compared with the other 2 groups, which indicated that the elevation of circulating CORT caused broiler breast muscle protein breakdown through upregulating expression of MAFbx and downregulating expression of S6K1 under the HS condition.

The body pathophysiological changes induced by stress may elevate the demand of AA for energy supplementation and heat-shock protein synthesis (Harris et al., 1992; Hoskin et al., 2016). The plasma AA concentration is evaluated by difference between the inflow (AA intake, AA released from tissues) and outflow (AA catabolism, AA participation in protein synthesis, and it loss), and the rate of outflow is higher than that of inflow (i.e., muscle production) under stress conditions, which causes the decrease of the plasma AA

Table 4. Alterations of serum indicators in broilers that experienced heat stress.

Parameter[1]	Group[2] NC	HS	PF	*P*-values
GPT (U/L)	15.93 ± 1.11	16.18 ± 0.74	16.10 ± 0.87	0.313
GOT (U/L)	253.29 ± 12.31[b]	287.80 ± 9.37[a]	181.98 ± 9.40[c]	<0.001
TP (g/L)	38.47 ± 1.42	36.84 ± 0.94	40.51 ± 1.69	0.535
IGF-1 (ng/mL)	0.70 ± 0.03	0.74 ± 0.03	0.71 ± 0.01	0.637
CORT(ng/mL)	75.84 ± 3.18[b]	90.27 ± 3.23[a]	72.14 ± 6.23[b]	0.002

[a-c]Differ according to one-way ANOVA followed by Duncan's test ($P < 0.05$).
Results are represented as the mean value ± SE (n = 6).
[1]GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; TP, total protein; IGF-1, insulin like growth factor 1; CORT, corticosterone.
[2]NC, normal control group; HS, heat-stress group; PF, pair-fed group.

Table 5. Alterations of plasma levels of amino acids in broilers that experienced heat stress.

Amino acid (ng/μL)	Group[1] NC	HS	PF	*P*-values
Arginine	77.10 ± 6.41	70.54 ± 7.93	79.80 ± 8.95	0.143
Cysteine	13.01 ± 1.76	11.63 ± 1.77	12.82 ± 1.63	0.348
Glycine	45.30 ± 1.75[a]	35.44 ± 5.61[b]	37.54 ± 5.42[b]	0.005
Histidine	16.59 ± 1.92	15.52 ± 2.72	16.97 ± 2.23	0.547
Isoleucine	8.50 ± 1.16	8.96 ± 1.43	9.01 ± 1.46	0.773
Leucine	31.32 ± 4.60	27.65 ± 3.58	31.06 ± 4.23	0.256
Lysine	75.53 ± 10.54[a]	50.68 ± 7.91[b]	54.69 ± 8.03[b]	<0.001
Methionine	12.82 ± 1.57	11.73 ± 1.17	12.48 ± 2.05	0.516
Phenylalanine	24.92 ± 2.22	24.62 ± 3.23	25.19 ± 3.42	0.949
Threonine	30.04 ± 4.42[a]	24.14 ± 3.48[b]	25.23 ± 2.82[b]	0.030
Tyrosine	39.16 ± 5.11[a]	30.75 ± 4.17[b]	34.27 ± 4.10[a,b]	0.018
Valine	22.25 ± 3.31	19.02 ± 3.15	22.29 ± 3.48	0.181
Alanine	60.90 ± 9.35	57.57 ± 8.29	57.75 ± 8.59	0.764
Glutamic acid	127.74 ± 13.17	114.78 ± 10.39	113.55 ± 12.77	0.115
Serine	69.73 ± 8.92	66.38 ± 10.00	73.92 ± 9.85	0.417

[a,b]Differ according to one-way ANOVA followed by Duncan's test ($P < 0.05$).
Results are represented as the mean value ± SE (n = 6).
[1]NC, normal control group; HS, heat-stress group; PF, pair-fed group.

concentration (Cynober, 2002; Hoskin et al., 2016). In poultry, 3 AA (arginine, glycine, histidine) also belong to essential AA (**EAA**) (Wu, 2009). Among blood AA, levels of EAA are positively associated with the rate of muscle protein synthesis (Volpi et al., 2003; West et al., 2011). In our study, we found that HS reduced plasma levels of EAA (lysine, threonine, and glycine), which suggested that muscle protein synthesis was decreased under HS, and this was consistent with the aforementioned results. Threonine, glycine, and tyrosine belong to glucogenic AA. In the present study, HS decreased plasma levels of tyrosine, glycine, and threonine in broilers, probably because of the increased dependence on the liver glucose synthesis and output caused by HS, which was partly achieved by elevation of AA catabolism in the liver through gluconeogenesis (Baumgard and Rhoads, 2013; Gao et al., 2017).

The liver is a highly metabolic organ for energy resource allocation via regulation of the AA metabolism and gluconeogenesis as well as other metabolic processes (Taub, 2004). Transdeamination of AA is carried out almost exclusively by the liver for gluconeogenesis and energy supply (Brosnan, 2000). The uptake of AA by the liver is achieved by AA transporters. Solute carrier family 7 member 1 is Na+ -independent cationic AA transporter, and it promotes the uptake of arginine and lysine (Fernandez et al., 2001). Solute carrier family 7 member-like is identified as the catalytic light chain of heterodimeric AA transporter complex and is shown to function of neutral AA uptake when coexpressed with 4F2hc (Verrey et al., 2004; Sperandeo et al., 2008). Solute carrier family 3 member 1 is a heavy subunit of heteromeric transporter systems responsible for transportation of cystine and cationic AA when SLC3A1 is covalently linked to rBAT and coexpressed with rBAT (Di Giacopo et al., 2013). Solute carrier family 1 member 1, also referred to as excitatory amino acid transporter 3 (**EAAT3**), transports excitatory AA by translocating the substrate binding site of the transport domain through the plane of the plasma membrane (Bjørn-Yoshimoto and Underhill, 2016). A previous study reported that stress increased the uptake of AA by the liver (Tang et al., 1994). Our research showed that HS elevated mRNA expressions of SLC1A1, SLC3A1, SLC7A1, and solute carrier family 7 member-like in the broiler liver, indicating that AA uptake is increased in the broiler liver under HS, and this may result in the decrease of plasma AA concentration. Amino acids are deaminized by aminotransferase (mainly including GOT and GPT) in the liver, thereby providing gluconeogenesis precursors. Our present study found that HS elevated liver GOT and GPT activities in broilers, which manifested that HS elevated the broiler liver AA catabolism. PCK, PC, and FBP1 are key regulatory enzymes of gluconeogenesis. The promoter of *PCK* gene possesses glucocorticoid receptor binding

Table 6. Effects of heat stress on the activities of liver GOT and GPT in broilers.

Parameter[1]	Group[2] NC	HS	PF	*P*-values
GPT (U/g of protein)	16.33 ± 1.15[b]	23.46 ± 0.91[a]	26.89 ± 1.32[a]	<0.001
GOT (U/g of protein)	23.39 ± 1.16[b]	28.05 ± 1.53[a]	29.38 ± 1.25[a]	0.015

[a,b]Differ according to one-way ANOVA followed by Duncan's test ($P < 0.05$).
Results are represented as the mean value ± SE (n = 6).
[1]GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase.
[2]NC, normal control group; HS, heat-stress group; PF, pair-fed group.

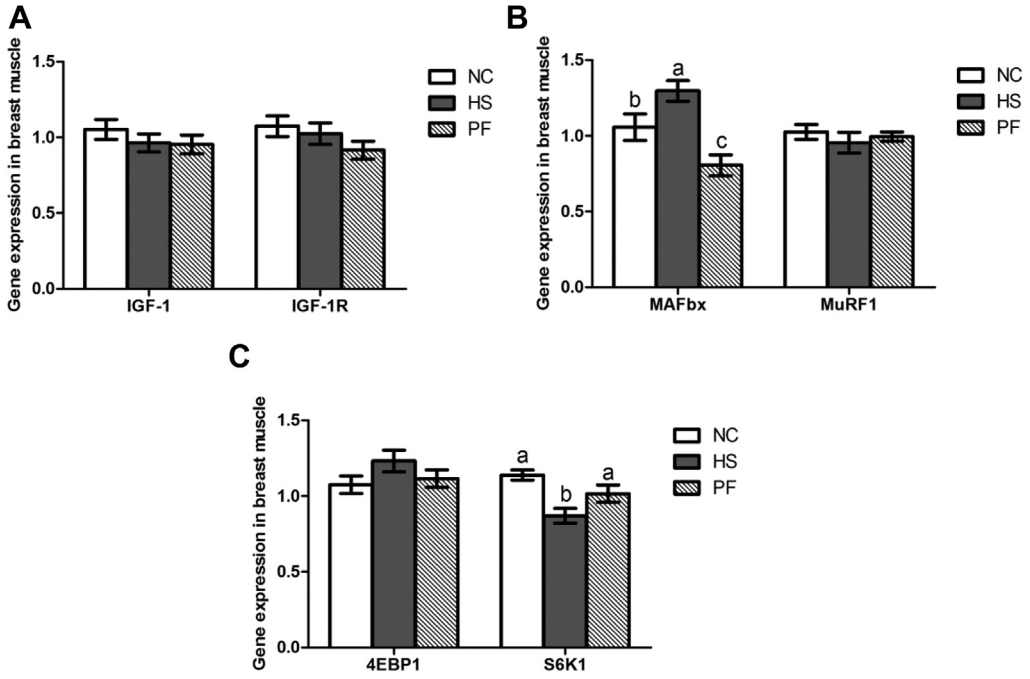

Figure 1. Alterations of mRNA expressions of IGF-1 and IGF-1R (A), and protein metabolism (B and C) in the breast muscle of broilers that experienced heat stress. Results are represented as the mean value ± SE (n = 6). Bars without identical superscripts were regarded as the significant difference ($P < 0.05$). Abbreviations: 4EBP1, eukaryotic translation initiation factor 4E-binding protein-1; HS, heat-stress group; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor; MAFbx, muscle atrophy F-box; MuRF1, muscle RING finger 1; NC, normal control group; PF, pair-fed group; S6K1, the 70-kD ribosomal protein S6 kinase.

areas, and CORT could elevate hepatic gluconeogenesis by upregulating *PCK* gene expression (Cawley, 2012; Kinote et al., 2012). *PCK* has 2 genotypes including *PCKc* (located in the cytosol) and *PCKm* (located in the mitochondrial matrix) (Parsanejad et al., 2003). In our study, HS increased mRNA expressions of *PCKc*, *PCKm*, *PC*, and *FBP1* in the broiler liver, which indicated that the liver gluconeogenesis was elevated by increase of circulating CORT for body energy supply under the HS condition.

In conclusion, the 7-d HS exposure caused the alteration of muscle protein metabolism and liver gluconeogenesis through elevating the circulating CORT concentration in broilers. The increase in the circulating CORT level decreased muscle protein synthesis and accumulated muscle protein breakdown to supply AA substrates to the liver and subsequently elevated liver gluconeogenesis through regulating key gluconeogenic enzymes for achieving energy supply via AA catabolism under the HS condition.

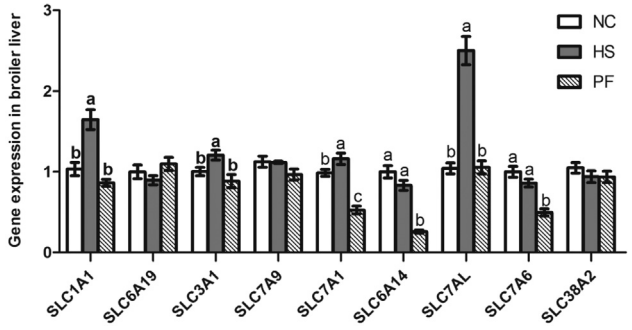

Figure 2. Alterations of mRNA expressions of amino acid transporters in the liver of broilers that experienced heat stress. Results are represented as the mean value ± SE (n = 6). Bars without identical superscripts were regarded as the significant difference ($P < 0.05$). Abbreviations: HS, heat-stress group; NC, normal control group; PF, pair-fed group; SLC1A1, solute carrier family 1 member 1; SLC3A1, solute carrier family 3 member 1; SLC38A2, solute carrier family 38 member 2; SLC6A14, solute carrier family 6 member 14; SLC6A19, solute carrier family 6 member 19; SLC7A1, solute carrier family 7 member 1; SLC7A6, solute carrier family 7 member 6; SLC7A9, solute carrier family 7 member 9; SLC7AL, solute carrier family 7 member-like.

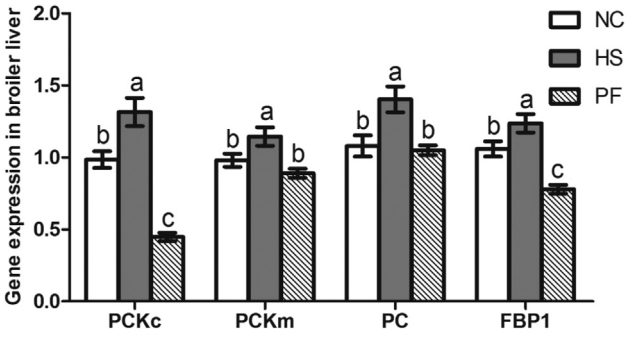

Figure 3. Alterations of relative mRNA expressions of liver gluconeogenesis in broilers that experienced heat stress. Results are represented as the mean value ± SE (n = 6). Bars without identical superscripts were regarded as the significant difference ($P < 0.05$). Abbreviations: FBP1, fructose-1,6-bisphosphatase 1; HS, heat-stress group; NC, normal control group; PC, pyruvate carboxylase; PCKc, phosphoenolpyruvate carboxykinase–cytosolic form; PCKm, phosphoenolpyruvate carboxykinase–mitochondrial form; PF, pair-fed group.

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DISCLOSURES

All authors declare no conflicts of interest.

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