Chronic heat stress alters hypothalamus integrity, the serum indexes and attenuates expressions of hypothalamic appetite genes in broilers

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ABSTRACT

The hypothalamus is crucial to ensure the functionality of the entire organisms, such as body temperature, feed intake and energy regulation. Exposing broilers to high ambient temperature usually induces lower feed intake and energy imbalance. We investigated the molecular mechanisms by which heat stress impairs the appetite via dysfunction in hypothalamus of the broilers. Broilers were allocated to three groups: the normal control (NC) group, and fed ad libitum; heat-stress (HS) group, and fed ad libitum; pair-fed (PF) group, which received the feed intake equal to HS group. Experiment lasted from the age of 28 to 42 d. The results showed that HS increased the head surface temperature of broiler and changed hypothalamic ultrastructure. HS treatment also increased the serum corticosterone in the broilers after 7 days of heat stress, elevated the FT4 and FT3 after 14 days of heat stress. Heat stress of 14 days showed a tendency to increase the leptin. However, the serum corticosterone in the HS group had no significant difference after 14 days of heat stress. In addition, HS treatment decreased the expression of orexigenic gene *neuropeptide Y (NPY)* after 14 days of heat stress, while HS treatment had no effect on the reactive oxygen species (ROS), as well as the gene expression of *AMPKα1* and *LKB1* in the hypothalamus. In conclusion, HS increased the surface temperature of head in broiler, and then altered the integrity of hypothalamus. Meanwhile, HS increased the serum corticosterone which may ascribe to the activation of HPA axis in the broilers. In addition, chronic heat stress decreased the expression of orexigenic gene *NPY*, which may cause the broiler to reduce feed intake.

1. Introduction

Seasonal heat waves can cause huge loses especially in the tropical countries of poultry production (Nardone et al., 2010). It is an annoying issue with an increased rate of mortality for broilers because of high temperature exposure. Around the world, this problem continues to aggregate as the global temperature is gradually rising (Bayer Altın and Barak, 2017). Heat stress has substantially negative effects on profitability due to lower performance (Pearce et al., 2014; He et al., 2018a). Under high temperature, broilers are more prone to display symptoms of heat stress, such as reducing feed intake and decreasing the secretion level of some hormones, which eventually alter the metabolic rate of

the body, leading to less heat production (Lara and Rostagno, 2013).

The central nervous system (CNS) is involved in the control of energy homeostasis, hunger and satiety feeling (Morton et al., 2006). It can sense and combine the signals from the periphery, then convey pertinent information regarding short-term and long-term energy status of the individual (Richards and Proszkowiec-Weglarz, 2007). Energy imbalance status is associated with reduced appetite and abnormal metabolic rate, which causes anorexia or obesity, as well as impair the health of the organism (Minokoshi et al., 2004; He et al., 2018b). As an important part of central nervous system, hypothalamus has many capabilities, such as regulating feed intake, manipulating body temperature and endocrine through different pathways. It is also involved

with emergency responses to stressors in the environment (Luquet and Magnan, 2009; Liu et al., 2017). The formation of appetite is very complex, and hypothalamus is the main integration center of various appetite regulatory signals, consisted of mainly two opposite branches of neurons. One neuron is orexigenic neuron, called NPY/AgRP. Another neuron is anorexigenic neuron, named POMC/CART, which is responsible for suppress appetite (Liu et al., 2017).

To better understand the molecular basis for the effects of heat stress on hypothalamus function, serum parameters, gene expression of appetite in hypothalamus, appetite-related factors were measured in the hypothalamus. We previously found that long-term heat stress altered feed intake and the gene expression of appetite related neuropeptides in the intestinal tract of broilers (He et al., 2018a). However, the mechanism of chronic heat stress on feed intake regulatory peptides in hypothalamus remains scarce. Therefore, it is critical to reveal the heat-stress-mediated mechanisms of reducing feed intake for animal nutritionists. The objective of this study was to investigate the effects of chronic heat stress on the head temperature, ROS in the hypothalamus, as well as expression of appetite related and energy regulation genes in the hypothalamus of broilers.

2. Materials and methods

2.1. Broilers' management and sample collection

All experimental procedures involving the use of animals were conducted under the guidelines of Nanjing Agricultural University Institutional Animal Care and Use Committee.

Two hundred newly-hatched male Arbor Acres broilers were obtained from a commercial hatchery and raised in battery brooders. From 1 d to 27 d of age, the birds were received commercial standard diets and managements. At 28 d of age, a total of 144 chicks with similar body weights were selected and equally distributed into three groups with 6 cages per group and 8 chickens per cage (length × width × height: 1.0 m × 0.5 m × 0.4 m). In normal control (NC) group, chickens were kept at 22 °C ambient temperature; in heat stress (HS) group, chickens were reared at 32 °C constant temperature, both NC and HS groups provided ad libitum access to feed; in pair-fed (PF) group, chickens were maintained at 22 °C and received the amount of feed each day equal to the feed consumed in the previous day by the HS group. The PF group was designed to understand the effects of heat stress on the serum parameters and genes expressions in hypothalamus independent of the reduction in feed intake. All groups relative humidity was maintained at 55 ± 5%, received common commercial grower feed, and water was provided for ad libitum consumption. The lighting was provided 24 h per day throughout the experimental period. The compositions and nutrient levels of basal diet are shown in Table 1.

2.2. Infrared thermal image analysis

At 14 days of heat stress, temperatures were obtained from the thermal images using the Fluke Ti400 (Japan). The measurement regions are shown in Fig. 1. In chickens, the head and comb regions generally vary in temperature over their surface and so maximum head and maximum comb temperature were obtained by drawing polygons around either the whole head (including the comb) or the whole comb, which was according to the Cangar et al. (2008).

2.3. Sample collection and preparation

After 7 days and 14 days of heat stress, two broilers nearly the average weight of the replicate were randomly taken out from each cage and slaughtered via exsanguination without fasting. Blood samples were collected, and serum samples were separated, stored at −20 °C for further analysis. After that, the head of broiler was dissected cautiously, separated the hypothalamus, cut a small sample to detect the level of

Table 1
The compositions and nutrient levels of basal diet.

Ingredients (%)		Calculated nutrient levels (%)	
Corn	62.07	ME[b] (kcal/kg)	3152.41
Soybean meal	23.00	Crude protein	19.60
Corn gluten meal (60%)	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		
Common salt	0.30		
Premix[a]	1.00		

[a] Per kilogram of diet, the premix provided retinyl acetate for vitamin A, 12000 IU; cholecalciferol for vitamin D_3, 2500 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.
[b] ME, metabolizable energy.

ROS. Then we chopped a piece of major tissue into 1 mm^3 and fixed in 2.5% glutaraldehyde phosphate buffer saline (v/v, pH7.2) for the ultrastructural examination immediately. The remaining hypothalamus tissue was stored in liquid nitrogen for further analyses.

2.4. Serum indexes analysis

Serum corticosterone (CORT), Triiodothyronine (T3), Thyroxine (T4), Free triiodothyronine (FT3), Free thyroxine (FT4) and leptin concentrations were measured using a commercial ELISA kit, validated for use in chickens (Angle Gene Bioengineering Co., Ltd., Nanjing, China).

2.5. Detection of ROS

Reactive oxygen species (ROS) were measured vis utilizing an ROS measurement kit (E004, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) on the basis of the oxidation of 2′7′-dichlorodihydrofluorescein diacetate (DCFH-DA) to 2′7′-dichloro-fluorescein (DCF), referring to Sang et al. (2012). The fresh hypothalamus tissue samples were used to make single-cell suspensions for flow cytometry. The formation of DCF was monitored employing a FACS Calibur flow cytometer (FC500 MCL/MPL, Beckman Coulter Inc., CA) with the excitation at 488 nm and emission at 525 nm. ROS generation was quantified by the mean fluorescence intensity over that of the NC group.

2.6. Ultrastructural observation

The hypothalamus tissues fixed in 2.5% glutaraldehyde phosphate buffer saline (v/v, pH 7.2), post-fixed in 1% osmium tetroxide (v/v), and the samples were stained with 4.8% uranyl acetate. Then, we dehydrated the fixed samples in a graded series of ethanol and embedded in araldite. The samples were cut into the ultrathin (less than or equal to 90 nm) sections, mounted on coated copper grids, washed in propylene oxide and impregnated with epoxy resins, and post-stained with uranyl acetate and lead citrate. Specimens were observed via a transmission electron microscope (HT7700, Hitachi, Japan).

2.7. RNA extraction and real-time quantitative PCR

Total mRNA was isolated from the hypothalamus samples using TRIzol reagent according to the manufacturer's instructions (Takara
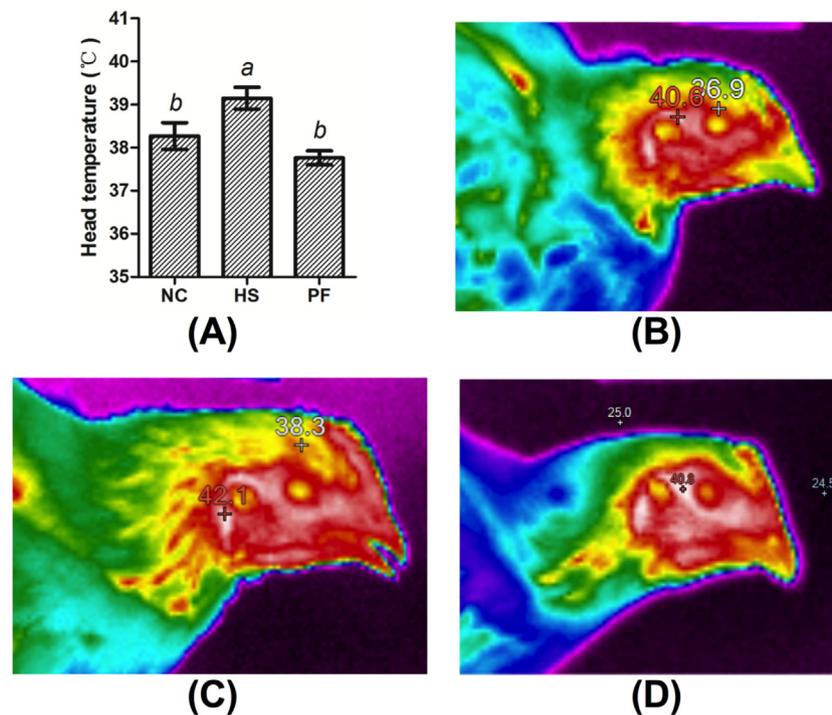
Fig. 1. Measuring the head temperatures in broilers after 14 days heat stress. The visible images show broilers in the normal control group(NC) (B); heat stress group (HS) (C); pair-fed group(PF) (D). The figure shows head temperatures of broilers among the three groups by using a thermal imaging camera.

Biotechnology Co. Ltd, Dalian, China). The RNA concentration was measured by an ultramicro-spectrophotometer (Thermo Scientific, Wilmington, DE, USA) at 260/280 nm. The quality of the RNA was estimated by detecting the number of bands by agarose gel electrophoresis. RNA was reverse transcribed into cDNA using a cDNA reverse transcription kit (Takara Biotechnology Co. Ltd, Dalian, China) and a thermal cycler according to manufacturer's instructions. The RT reaction was conducted in 20 μL of reaction mixture at 37 °C for 15 min, and terminated by heating at 85 °C for 5 s followed by cooling at 4 °C.

Quantitative real-time PCR was performed on an ABI PRISM 7500 Detection System (Applied Biosystems, Foster City, CA, USA). Reactions were performed with 2.0 μL of first-strand cDNA and 0.8 μL of sense and anti-sense primers in a final volume of 20 μL as recommended by the SYBR Premix Ex Taq kits (Takara Biotechnology Co. Ltd, Dalian, China). Primers were synthesized by Sangon Biotechnology (Shanghai, China) according to the sequences described in GenBank. The specific sequences of primers are listed in Table 2. The procedure of real-time PCR analyses was as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, then 95 °C for 15 s, 60 °C for 1 min and 95 °C for

15 s. All of the PCR reactions were performed in triplicate. The results were normalized on glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene expression, and melt curve analysis was performed to validated the specificity of the reactions. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method according to Livak and Schmittgen (2001), and normalized the relative abundance to the control (as 1).

2.8. Immunofluorescence of the whole brain

The whole brains were dissected and transferred immediately into a silver paper. The silver paper was snap frozen by above the liquid nitrogen for 60 s, then the completely frozen brain tissues were embedded with OCT compound. Frozen sections (8 μm) were placed at room temperature for 5 min, fixed in 4% neutral paraformaldehyde for 10 min, blocked with 3% bovine serum and then incubated with primary antibody overnight at 4 °C. Antibodies and their sources are NPY (DF6431, Affinity Biosciences, Cincinnati, OH, USA), diluted with 1:50. POMC (DF7154, Affinity Biosciences, Cincinnati, OH, USA) diluted with 1:100. The Alexa Fluor. 647 conjugated goat anti-rabbit IgG (ab150083,

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

gene[a]	primer sequences (5′→3′)	amplicon size (bp)	genBank number.
NPY	forward: TGCTGACTTTCGCCTTGTCG	148	M87294
	reverse: GTGATGAGGTTGATGTAGTGCC		
AgRP	forward: GGAACCGCAGGCATTGTC	163	NM 001031457
	reverse: GTAGCAGAAGGCGTTGAAGAA		
POMC	forward: CGCTACGGCGGCTTCA	88	NM 001031098
	revers e: TCTTGTAGGCGCTTTTGACGAT		
LKB1	forward: TGAGAGGGATGCTTGAATACGA	138	NM_001045833.1
	reverse: ACTTGTCCTTTGTTTCTGGGC		
AMPKα1	forward: ATCTGTCTCGCCCTCATCCT	125	NM_001039603.1
	reverse: CCACTTCGCTCTTCTTACACCTT		
GAPDH	forward: GAGGGTAGTGAAGGCTGCTG	113	NM_204305.1
	reverse: CATCAAAGGTGGAGGAATGG		

[a] NPY, neuropeptide Y; AgRP, Agouti-related peptide; POMC, proopiomelanocortin; LKB1, liver kinase B1; AMPKα1, adenosine 5'-monophosphate-activated protein kinase α1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

abcam, USA) diluted with 1:400, employed as the second antibody. DAPI was used as a marker for cell nuclei.

2.9. Statistical analyses

One-way analysis of variance was performed for the data via statistical software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Differences among means were tested using Duncan's multiple range tests. The data were analyzed with the cage as the experimental unit (n = 6). Data were expressed as means ± standard errors and significance was set at $P < 0.05$.

3. Results

3.1. Head temperature

After 14 days of heat stress, the head surface temperature of HS group was significantly elevated compared to the other two groups ($P = 0.001$, Fig. 1).

3.2. Serum indexes

The plasma levels of CORT, FT4, FT3, T4, T3 and leptin were shown in Table 3. The plasma concentrations of CORT in the HS broiler chickens were significantly elevated after 7 days of heat stress compared with the NC and PF group chickens ($P = 0.001$). The concentration of CORT in HS group displayed no significant difference after 14 days of heat stress among the three groups. The FT4 ($P = 0.010$) and FT3 ($P = 0.023$) level of HS group were significantly elevated compared with the NC group after 14 days of heat stress. The concentration of leptin in the HS group showed an increasing tendency in comparison with NC group.

3.3. ROS levels

As shown in Fig. 2, there was no significant difference of ROS levels in the hypothalamus among the three groups after 14 days of heat stress.

3.4. Ultrastructural alterations

As shown in Fig. 3, the envelope of blood vessel in the neuron looked loose and disintegrate under heat stress condition, and the

Fig. 2. Effect of chronic heat stress on ROS generation in the hypothalamus of broilers after 14 days of heat stress. Each result represents the mean value ± standard error (SE); Data are means of 6 replicates of 2 birds per cage (n = 6). NC, normal control group; HS, heat stress group; PF, pair-fed group.

cytoplasm of the hypothalamus neurons had some empty aspects. It was indicated that heat stress disintegrated the ultrastructure in the hypothalamus (Fig. 3B).

3.5. Gene expressions

As shown in Fig. 4, after 14 days of heat stress, the mRNA expression of NPY in HS group was significantly lower than that of the other two groups ($P = 0.018$). The mRNA expressions of *AgRP* ($P < 0.001$) and *POMC* ($P = 0.002$) in PF group were significantly higher than those of NC group after 14 days of heat stress. Meanwhile, the mRNA expressions of *POMC* in the HS group has a tendency to increase compared to the NC group. There was no significant difference in the mRNA expression of *AgRP* between HS group and NC group, and no significant difference was observed in the mRNA expression of *NPY* between PF group and NC group after 14 days heat stress. In addition, the mRNA expression of *AMPKα1* and *LKB1* among the three groups showed no significant differences.

3.6. Immunofluorescence analysis

Figs. 5 and 6 revealed the protein expression of NPY and POMC in the brain of broilers. The protein level of the NPY in the HS group was lower than that in the other two groups (Fig. 5). In addition, the protein level of the POMC in the NC group was less than that in the other two groups (Fig. 6).

4. Discussion

4.1. Head temperature and serum indexes

Broilers respond to heat stress usually with increased rectal temperature, respiratory rate, and surface head temperature. They convert their body composition by altering the metabolism rates, increasing the adipose-to-muscle ratio (Lu et al., 2017; He et al., 2018a). The central nervous system (CNS) could detect signals from the periphery regarding metabolic status, process and integrate this information in a coordinated manner and exert a role of thermal regulation and energy regulation (Serge et al., 2005). In our previous study, we found that the respiratory rates of birds in high temperature were significantly accelerated, and broilers were suffering from panting (He et al., 2018a). We also observed this phenomenon in the infrared thermal image, revealing the broiler open its mouth to breath (Fig. 1C), and the head temperature in the HS group was significantly higher than the other two groups, which suggested the broiler in heat stress may suffer intracranial over heat.

CORT is a sensitive indicator of heat stress response, which involves in metabolic rate and heat production of broilers (Lin et al., 2006; Lu et al., 2017). In our study, serum CORT was significantly increased after

Table 3
Effects of chronic heat stress on the serum indicators in broilers.

item	treatment[1]			SEM	P-value
	NC	HS	PF		
after 7 days of heat stress					
CORT, ng/mL	75.84[b]	90.26[a]	65.39[c]	3.10	0.001
leptin, ng/mL	6.35	7.01	6.14	0.23	0.302
T4, pmol/L	125.20	118.22	118.15	2.39	0.408
T3, pmol/L	2.81	3.18	2.68	0.09	0.071
FT4, nmol/L	27.67	30.09	29.83	0.64	0.254
FT3, nmol/L	28.74	29.07	28.92	0.08	0.328
after 14 days of heat stress					
CORT, ng/mL	90.21	84.10	87.64	2.71	0.681
leptin, ng/mL	5.19	6.32	6.08	0.21	0.057
T4, pmol/L	104.91	105.44	112.98	4.22	0.709
T3, pmol/L	2.61	2.77	2.74	0.07	0.683
FT4, nmol/L	25.14[b]	28.93[a]	28.01[a]	0.57	0.010
FT3, nmol/L	28.58[b]	29.57[a]	29.10[ab]	0.15	0.023

[1] NC, normal control group; HS, heat stress group; PF, pair-fed group. The results are presented as the mean value ± standard error, the data are the means of six replicates of two birds per cage (n = 6). Mean values with different letters were significantly different ($P < 0.05$).
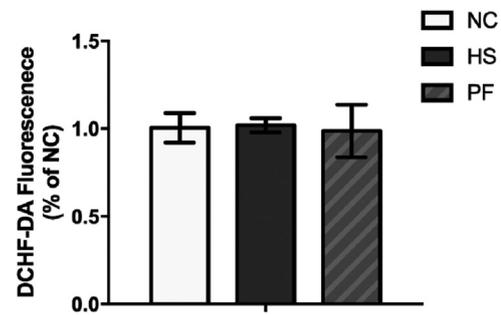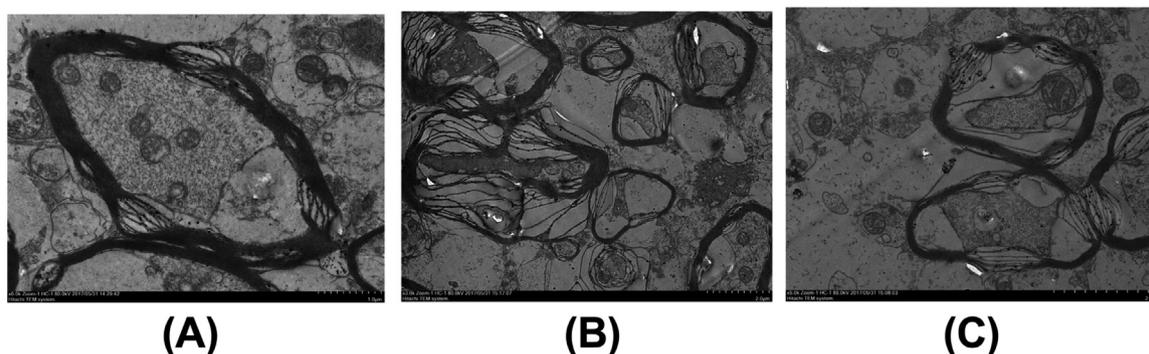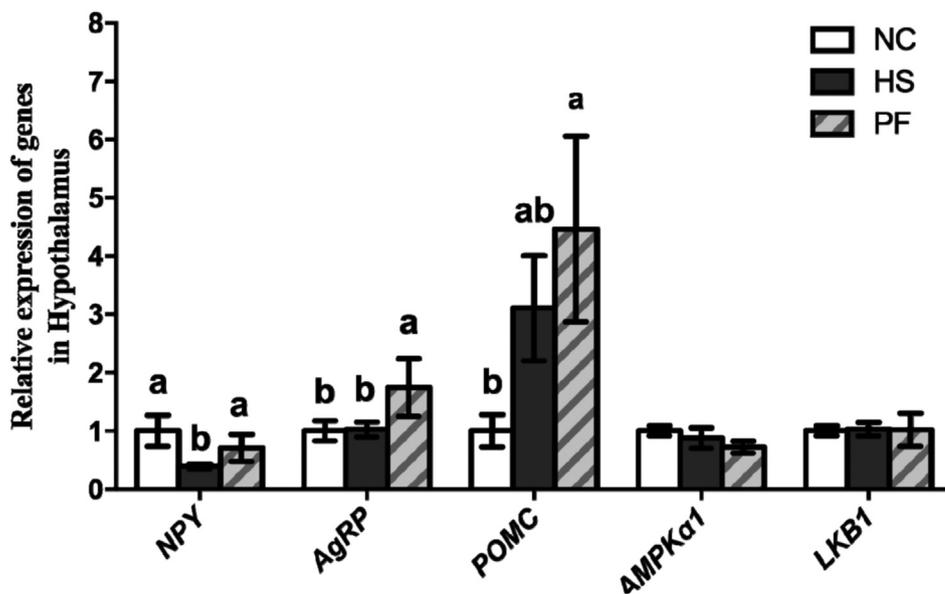
Fig. 3. Effect of chronic heat stress on ultrastructure of the hypothalamus in broilers after 14 days of heat stress. mitochondria in the hypothalamus of broilers from normal control group (A), heat stress group (B), pair-fed group (C). Transmission electron microscopy images at original magnifications of × 7000 (B) and (C), and × 8000 (A).

7 days of heat stress, however there was no difference of serum CORT after 14 days of heat stress, which is consistent with the observation made by Gu et al. (2012). The increased CORT indicated that the HPA system was activated in response to the heat stress (Keller-Wood, 2015). Broilers cope with heat stress via decreasing the secretion rate of thyroid hormones, which is associated with a decreasing in the basic metabolic rate and heat production (Slimen et al., 2016). However, in our study, after 7 days of heat stress there was no effect on the concentration of T4 in serum, whereas the concentrations of FT4 and FT3 in serum were significantly elevated after 14 days of heat stress, respectively, which seemed discordance with previous study. Some researcher demonstrated that there was a negative relation between thyroid hormones and ambient temperature (Williamson et al., 1985; Sohail et al., 2010; Mahmoud et al., 2014). Another presumably reason was that genetic selection for growth may alter birds' thyroid hormone response to the environmental temperature (Chiang et al., 2008). Leptin is an anorexigenic peptides, mainly produced by fat tissues, which plays a key role not only in the regulation of energy homeostasis but thermoregulation as well, via reducing energy intake and stimulating energy expenditure (Cowley et al., 2001). Many evidences suggested that heat stress can elevate the mRNA of leptin and leptin receptor genes, which are considered as an adaptive response that further alter the carbohydrate and lipid metabolism (Margetic et al., 2002; Morera et al., 2012; Van Swieten et al., 2014). The present study indicated that chronic heat stress showing a tendency to increase serum leptin, suggested that leptin may be responsible for long term energy homeostasis

and feed intake regulation.

4.2. ROS levels

ROS is mainly produced and accumulated in mitochondria, which is an indispensable substance in organisms. It plays an important physiological role at a low concentration. Many researches demonstrated that heat stress induces oxidative stress by generating excessive ROS (Lin et al., 2006; Zhang et al., 2016; Lu et al., 2017). In the present study, the head temperatures of broilers in HS group were significantly elevated after 14 days of heat stress, however, the ROS production had no significant difference among the three groups, which is seemed inconsistent with previous studies. Many previous studies reported that adverse condition especially heat stress could induce oxidative stress, resulting in the generation of more ROS in the body (Mujahid et al., 2005; Wang et al., 2009; Lu et al., 2017). This indicated there may exist a compensation or buffering mechanism that hypothalamus can eliminate part of the excessive ROS. POMC neurons use glucose as the primary energy source, while NPY/AgRP take the fatty acids as the primary energy substrate (Parton et al., 2007). Under the energy imbalance status, the NPY/AgRP neurons will be activated, driving by long-chain fatty acids, and ROS levels will not increase in these cells even the utilization of energy substrates increased (Horvath et al., 2008).

Fig. 4. Effect of chronic heat stress on the mRNA expression of *NPY, AgRP, POMC, AMPKα1* and *LKB1* after 14 days heat stress in the hypothalamus of broilers. Each result represents the mean value ± standard error (n = 6). Means without a common letter significantly differ (P < 0.05). *NPY*, neuropeptide Y; *AgRP*, Agouti-related peptide. *POMC*, proopiomelanocortin; *AMPKα1*, AMP-activated protein kinase *α1*; *LKB1*, liver kinase B1; NC, normal control group; HS, heat stress group; PF, pair-fed group.
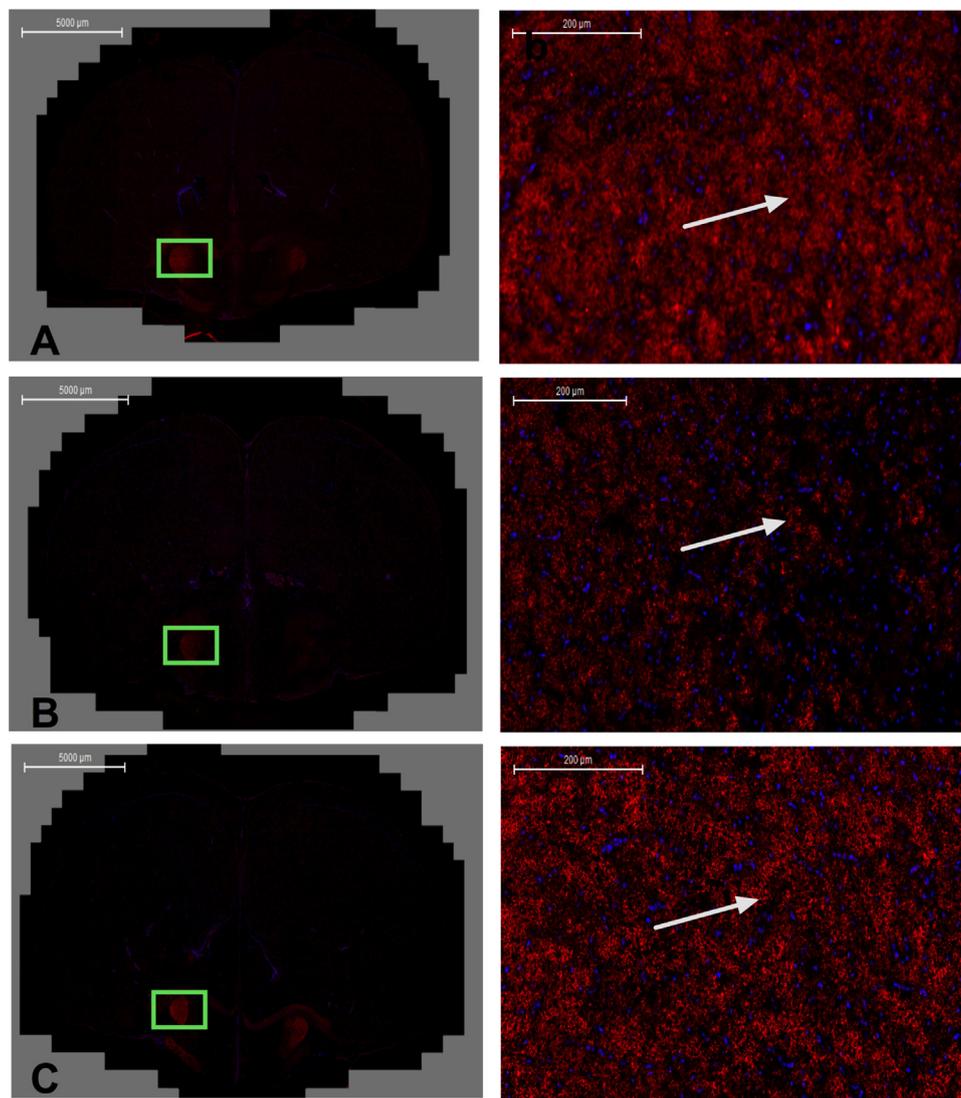
Fig. 5. Effect of chronic heat stress on protein levels of NPY in the hypothalamus of broilers after 14 days heat stress based on the whole-brain immunofluorescent method of the control group (A), heat stress group (B), pair-fed group (C). NPY, neuropeptide Y. White arrows on the right row panels indicates NPY proteins which colors are red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

4.3. Appetite-related genes expression

Neuropeptide Y is a highly conserved neuropeptide, which plays a role in regulating energy homeostasis and appetite (Schwartz et al., 2000). In the present research, the mRNA expression of NPY in the HS group was significantly decreased than the other two groups, combined with the whole-brain immunohistochemical results, indicated that chronic heat stress diminishes the feed intake of broilers via reducing the generation of NPY. Meanwhile, the mRNA expression of AgRP in the PF group was significantly upregulated than the other two groups, suggested that feed restriction may enhance the hunger feeling or induce more expression of AgRP for survival or compensation. Actually, the feeding status of broilers could also have influences on the expression of appetite-related peptides in hypothalamus. There are evidences reported that fasting induced *NPY* and *AgRP* genes upregulated in hypothalamus (Higgins et al., 2010; Dunn et al., 2013). In the present study, we made the sampling without fasting in order to eliminate the effects of feeding status in the result in order to better understand the hypothalamic appetite regulation in the broiler under heat stress.

POMC neurons are engaged in inducing satiety and elevating metabolic rate (Yoo et al., 2011; Joly-Amado et al., 2014). The POMC is a precursor peptide, expressed by the anorectic POMC/CART neurons, is processed to various important bioactive products, such as the MSHs, ACTH and β-endorphin (Zhou et al., 1993; Cone, 2005; Millington, 2007). In the present study, the mRNA expression of POMC in the PF group was significantly increased compared with the NC group, suggested that POMC might exert other role when animal suffered feed restriction.

There are mechanisms developed by the brain cells monitoring energy availability in the extracellular space. Among these mechanisms, the Adenosine 5′-Monophosphate-Activated Protein Kinase (AMPK) pathway is responsible for the energy status via detecting the ratio of AMP/ATP (Hardie and Carling, 1997). AMPKα1 and LKB1 as the molecules in the AMPK pathway are expressed ubiquitously in the body (Steinberg and Kemp, 2009). Our previous study found that chronic heat stress could damage the small intestinal epithelium cells associated with the AMPK pathway (He et al., 2018b). Recent studies indicated that the CNS neurons have a similar nutrient-sensing mechanism as the other organs in the animal (Wortman et al., 2003; Bloueta and Schwartz, 2010). In the present study, the results showed that the mRNA expression of *AMPKα1* and *LKB1* have no significant difference among the three groups. However, whether the *AMPKα1* and *LKB1* involve in the heat stress or feed restriction in the hypothalamus needs the further exploration of protein expression.
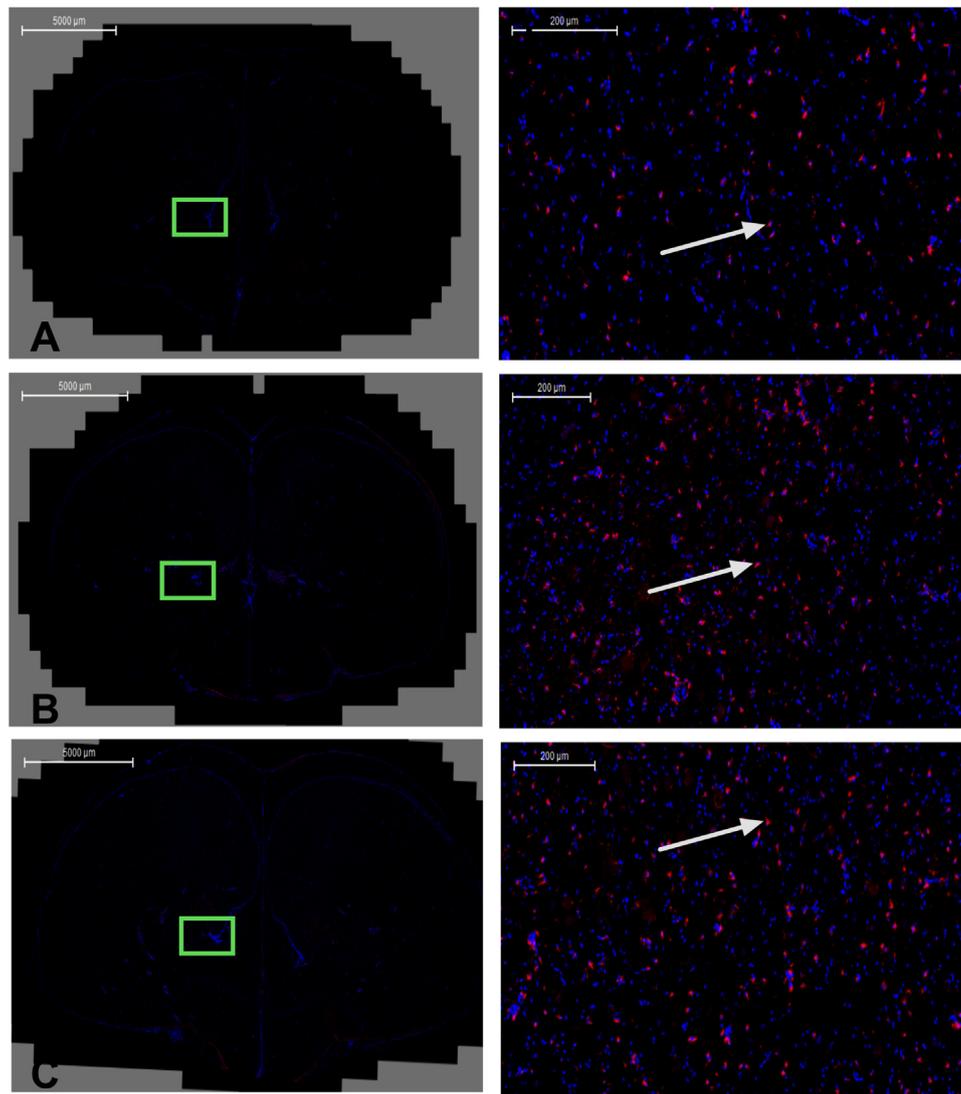
Fig. 6. Effect of chronic heat stress on protein levels of POMC in the hypothalamus of broilers after 14 days heat stress based on the whole-brain immunofluorescent method of the control group (A), heat stress group (B), pair-fed group (C). POMC, proopiomelanocortin; White arrows on the right panels indicates POMC proteins which colors are red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

5. Conclusions

In conclusion, HS increased the surface temperature of head in broiler, and then altered the integrity of hypothalamus. Meanwhile, heat stress increased the serum corticosterone which may ascribe to the activation of HPA axis in the broilers. In addition, chronic heat stress decreased the expression of orexigenic gene *NPY*, which may cause the broiler to reduce feed intake.

Acknowledgements

Conflict of interest

The authors declare that they have no conflicts of interest.

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