doi: 10.17221/67/2015-CJAS

Effects of γ-aminobutyric acid on the thymus tissue structure, antioxidant activity, cell apoptosis, and cytokine levels in chicks under heat stress

C. Liang, X.Z. Xie, Y.W. Zhou, Y.Y. Jiang, L.J. Xie, Z. Chen

Ministry of Education Key Laboratory for Tropical Animal and Plant Ecology, College of Life Sciences, Hainan Normal University, Haikou, P.R. China

ABSTRACT: This study aims to investigate the effect of dietary γ-aminobutyric acid (GABA) on the development of thymus tissue structure and function in chicks under heat stress. One-day-old male Wenchang chicks were randomly divided into control group (CK), heat stress group (HS), and GABA+HS group. The chicks from GABA+HS group were administered 0.2 ml of GABA solution daily by oral gavage (50 mg/kg of body weight). Chicks from HS and GABA+HS groups were subjected to heat stress treatment at 40 ± 0.5°C for 2 h every day. Blood and thymus tissue were collected from the chicks at the end of weeks 1–6. Results showed that the thymus weight and index, thickness of cortex, cortex/medulla ratio, number of lymphocytes, activity of superoxide dismutase, total antioxidant capacity, and glutathione peroxidase, and plasma level of tumor necrosis factor-α in HS group were significantly lower than in CK group ($P < 0.05$). The Toll-like receptor 2 (TLR2) expression in the late stage of heat stress, malondialdehyde (MDA) content, thymocyte apoptosis rate, number of lymphocytes in the S and G2/M phases, and plasma levels of interleukin-4 and interferon-γ in HS group were significantly higher than in CK group ($P < 0.05$). In contrast, the integrity of thymus tissue structure of GABA+HS group was improved compared with HS group. The TLR2 expression in the early stage of heat stress and the activity of antioxidant enzymes in GABA+HS group were significantly higher than in HS group ($P < 0.05$), and the MDA content, thymocyte apoptosis rate, number of lymphocytes in the S and G2/M phases, and plasma level of IL-4 and IFN-γ in GABA+HS group were significantly lower than in HS group ($P < 0.05$). We concluded that heat stress caused structure damage to thymus tissue of chicks, changed the plasma levels of cytokines, reduced the antioxidant activity, and increased cell apoptosis in chick thymus. GABA alleviated the negative effects on the development of chick thymus, improved the immune function of thymus, and played a protective role by regulating the plasma levels of cytokines and antioxidant activity of thymus tissue.

Keywords: heat shock; GABA; chick thymus; immune function; development

INTRODUCTION

Persistent high temperature in summer induces heat stress in domestic farming animals, leading to their body dysfunction that adversely affects reproduction and production performance. Wenchang chicks are native breed of Hainan Island, which has high nutritional and economic value. Heat stress during feeding brings about huge economic losses to the livestock industry, hindering sustainable and healthy development of animal husbandry. Therefore, heat stress is one of the key factors that affect productivity. Under high ambient temperature, the steady state in organism is disturbed, which causes metabolic disorders and affects the normal life (Borges et al. 2004). As a result, heat stress has a serious negative effect on the intestinal tissue structure of chickens (Chen et al. 2015). The dysfunction of intestinal mucosal barrier causes inflammatory infiltration, which

Supported by the National Natural Science Foundation of China (grant NSFC31260555).

doi: 10.17221/67/2015-CJAS

affects the absorption and utilization of nutrients. Heat stress not only influences the growth performance of chickens, but also greatly affects the immune system. It causes immune system disorders, resulting in damages in the structure and function development of the immune organs (Tang and Chen 2015).

Thymus is one of the most important lymphoid organs in the central immune system. It is the main site of T cell differentiation and development. The functional development of the thymus tissue directly affects the immune function. Early growth period is important in the development of immune organs in chicks, and the thymus development is completed by the time of sexual maturity. Studies have demonstrated that heat stress can inhibit the development of central immune organs and cause structural changes of the thymus tissue, such as reduction in thymus weight, congestion and swelling of thymus tissue, and reductions in thymic cortex and cortical lymphocytes, resulting in karyopyknosis and karyorrhexis (Ghazi et al. 2012). Under long-term heat stress, T lymphocyte subsets undergo changes (reduction of the CD^{4+} T lymphocytes number and of the CD^{4+}/CD^{8+} T lymphocytes ratio). This suggests that the change of T lymphocyte subsets induced by heat stress is one of the important factors of immune suppression in the body under high temperature (Xiang et al. 2011). Besides, the decrease of T lymphocytes also indicates the apoptosis and death of thymus cells. In the development of thymus cells, apoptosis occurs in a small amount in order to maintain the normal physiological function, and it is positively correlated with the growth cycle of cells. Studies have shown that heat stress accelerates the apoptosis of thymus cells, leading to a gradual increase in the apoptosis rate, and typical apoptotic bodies can be observed by electron microscopy (Gu et al. 2014). Therefore, heat stress has a great influence on the thymus tissue structure, i.e. it directly causes a decline in immune function, resulting in a large number of inflammatory cytokines produced. Han (2010) reported that high temperature induces changes in the levels of interleukin-2 (IL-2) and other inflammatory cytokines in immune organs of broiler chickens, leading to the immune function disorder. Therefore, it is crucial to investigate the deleterious effects of heat stress on the immune function development of chicks and to reduce them. At present, various measures have been taken in relieving heat stress.

γ-Aminobutyric acid (GABA) is an inhibitory neurotransmitter widely present in animal bodies. GABA is widely used in food industry and animal husbandry as a safe food additive to improve the immunity and antioxidant capacity of the body. It is especially effective in alleviating the effects of heat stress on physiological function of livestock. Our previous studies showed that GABA alleviates the negative effect of heat stress in broiler chickens, increases the body weight and red blood cell count, and decreases the feed conversion ratio and respiratory frequency (Chen et al. 2002). Under heat stress, it improves the development level of digestive enzymes in the small intestine such as small intestinal mucosa amylase and disaccharidase (Chen et al. 2014). It alleviates the damage of heat stress on the antioxidant system in broiler chicken intestinal mucosa to a certain extent, and has protective effect on the antioxidant function of intestinal mucosa (Chen et al. 2013). In this study, we aim to investigate the effects of GABA on the thymus tissue structure, cytokine levels, antioxidant activity, and cell apoptosis in chicks reared under conditions of heat stress, and to provide experimental evidence for the application of GABA in overcoming heat stress.

MATERIAL AND METHODS

Animals and feeding management. Healthy 120 one-day-old male Wenchang chicks obtained from Hainan YongJi Live Stock Co. Ltd., Hainan, China were used in the study. The birds were weighed, coded, and randomly divided into three groups of 40 birds: control group (CK), heat stress group (HS), and GABA+HS group. There was no significant difference in body weight and feed intake between the groups. The chicks from the CK and HS groups were administered 0.2 ml of physiological saline solution daily. The chicks from GABA+HS group were administered 0.2 ml of GABA solution daily by oral gavage (50 mg/kg of body weight (BW)). Normal feeding was carried out in each group, and chicks were given *ad libitum* access to diets and distilled water. The feeding room (7 × 3.5 × 3.5 m) had natural ventilation and illumination (14 h light : 10 h darkness). The test period was 6 weeks.

Heat stress treatment. Chicks from HS and GABA+HS groups were subjected to heat stress treatment at 40 ± 0.5°C with a relative humidity of 70–80% in LRH-800-GS artificial climate

doi: 10.17221/67/2015-CJAS

chamber (Tomorrow Environmental Protection Instrument Co. Ltd., Shaoguan, China) for 2 h (from 13:00 to 15:00) every day. Chicks from CK group were placed in room temperature unit for 2 h every day. After the treatment, the chicks were put back to the normal breeding cages.

Sample collection. At the end of each of six experimental weeks, 6 chicks were randomly chosen from each group and weighed after 2 h of different temperature treatments. Venous blood samples were taken and placed into heparin-coated centrifuge tubes for preparation of plasma. Thymus tissue was collected aseptically in a quick manner, and weighed after excess fat was removed. A portion of the thymus tissue was kept at –80°C for further analysis. The rest tissue was fixed by Bouin's fixation fluid for 12 h, and washed with 70% ethanol.

Determination of tissue structure and physiological and biochemical indexes

Index of thymus. Five thymus tissue sections were obtained from each of the 6 chicks and stained with the routine hematoxylin and eosin (H&E) staining. Three tissue sections were randomly chosen for observation with 5 different visual fields for each section, and the mean value was taken. The change in thymus tissue structure was examined by Olympus BX50F microscope (Olympus Optical Co. Ltd., Tokyo, Japan). The thickness of cortex, cortex/medulla ratio, and number of cortical lymphocytes were examined by MiE3.1 microscopic image analysis system (Shangdong Yichuang Electronics Ltd., Jinan, China). All tissue sections were photographed with YD400C digital camera (Shangdong Yichuang Electronics Ltd., Jinan, China).

Antioxidant activity. The thymus tissue was washed with ice-cold PBS to remove the blood, and wiped dry with filter paper. PBS pH 7.4 was added at a ratio of 9 : 1 of the tissue weight. The mixture was homogenized with cold glass homogenizer in ice bath, until the cells were completely broken and the colour of the liquid was light and uniform. Subsequently, the thymus tissue homogenate was centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was collected and stored at –20°C, for measuring activity of total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) and malondialdehyde (MDA) content. Measurements were done with reagent kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's protocol.

Thymocyte apoptosis and cell cycle. Clean thymus tissue was placed in a petri dish with a small amount of PBS, and processed into homogenate with an ophthalmic scissor. The homogenate was filtered with 300-mesh nylon net, centrifuged at 1500 rpm for 10 min, and washed twice with PBS at 1000 rpm for 5 min each time. Supernatant was discarded, and thymocytes were collected and resuspended to the concentration of 1×10^6 cells /ml with PBS. Subsequently, 4.5 ml of the cell suspension was centrifuged at 1000 rpm for 5 min, and thymocytes were resuspended with 500 µl of binding solution. 5 µl of Annexin V-FITC and 5 µl of Proliferation index (PI) were added and mixed well, respectively. After incubation in dark at room temperature for 10 min, the thymocytes were examined for cell apoptosis with flow cytometer (BD FACSCalibur, Becton Dickinson Company, San Jose, USA). The cell suspension was fixed with absolute ethanol at –20°C for 1 h, and washed twice with cold PBS. It was then incubated in a 37°C waterbath for 1 h with 100 µl of RNase added, and subsequently stained with 400 µl of propidium iodide at 4°C for 30 min in dark. Cells were filtered with 300-mesh nylon net to the flow tube. CellQuest software (Becton Dickinson Company) was used to capture 10 000 cells automatically, and Modfit software (Verity Software House, USA) was used to analyze the DNA content. Analysis was conducted with cell apoptosis and cell cycle reagent kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocol.

Cytokine levels in plasma. The heparin-treated blood samples were centrifuged at 3000 rpm for 15 min. Plasma was collected for measuring the plasma levels of interleukin-4 (IL-4), interferon-γ (INF-γ), and tumor necrosis factor-α (TNF-α). The analysis was conducted with reagent kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's protocol. The absorbance (OD value) was measured at 450 nm wavelength with ELISA, and the cytokine levels were calculated based on the standard curve.

Expression of TLR2 in thymus tissue. Five serial sections of thymus tissue were obtained from each of the 6 chicks, followed by routine immunohistochemical procedures. The tissue sections were coloured by diaminobenzidine (DAB) and counterstained with hematoxylin before micro-

doi: 10.17221/67/2015-CJAS

scopic examination. Brown particles indicated Toll-like receptor 2 (TLR2) positive cells. Three tissue sections were randomly chosen for observation with 5 different visual fields for each section. The analysis was conducted with the primary and secondary antibody reagent kits from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China) according to the manufacturer's protocol. All tissue sections were photographed by YD400C digital camera (Shangdong Yichuang Electronics Ltd., Jinan, China). TLR2 positive cell count in the visual field and the integral optical density (IOD) values were obtained by Image-Pro Plus 6.0 image analysis software (Media Cybernetics, Inc., Rockville, USA).

Statistical analysis. Experimental data were analyzed using two-way ANOVA with SPSS software (Version 16.02, 2008) and using multiple comparisons with Duncan's method, respectively. $P < 0.05$ was considered statistically significant.

RESULTS

Effect of GABA on the development of thymus tissue structure in chicks under heat stress. Figure 1A shows that the CK group has an intact thymus tissue structure with clear medulla and coarctate cortical lymphocytes. In contrast, HS group suffered from severe damage in the thymus tissue structure, e.g. thymic lobule decreased, cortex dwindled, medulla was markedly enlarged, the number of thymic cortical lymphocytes decreased, and there was severe vacuolization. GABA+HS group suffered from less damage in thymus tissue structure as compared with HS group, e.g. thymic cortex incrassated, medulla was thinner, cortical lymphocytes became coarctate, and thymic lobule was markedly increased.

Table 1 shows that the thymus weight of CK group increased with growth in general, and reached the maximum at 5 weeks of age ($P < 0.05$). The index of thymus had a trend of decline after the first increase, and reached the maximum at 3 weeks of age ($P < 0.05$). Subjected to heat stress at 40 ± 0.5°C for 2 h every day, the weight and index of thymus of HS group were both decreased as compared with those of CK group. There was a significant difference in thymus weight in the first 5 weeks ($P < 0.05$), and in the index of thymus in weeks 1, 4, and 5 ($P < 0.05$). There was an interaction between age and treatment for these parameters ($P < 0.05$). Compared with HS group, GABA+HS group

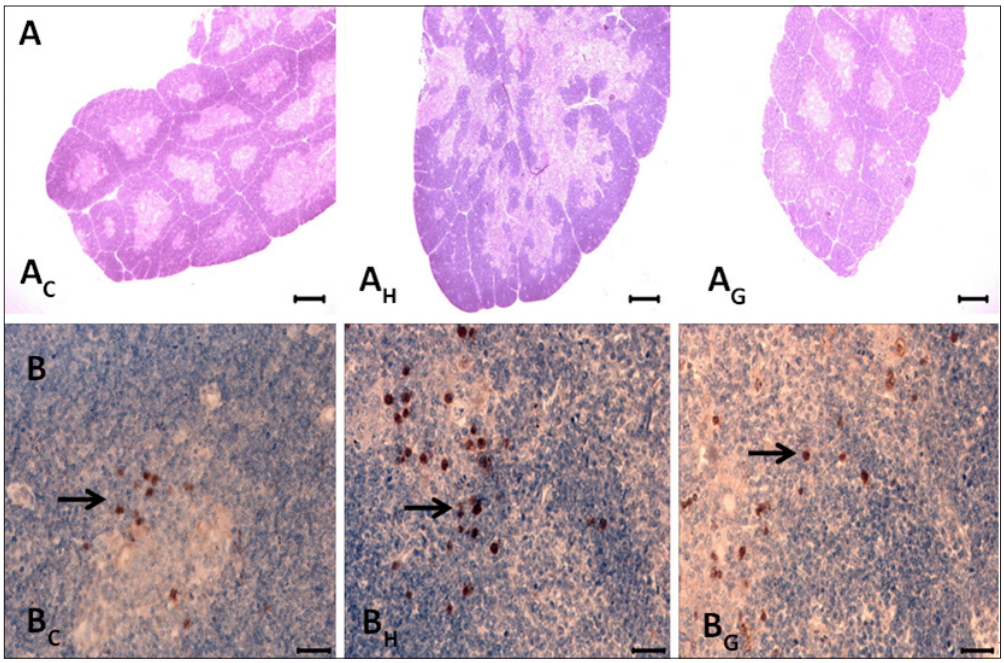

Figure 1. Effects of GABA on the thymus tissue structure and the thymus Toll-like receptor 2 expression in five-week-old chicks under heat stress

(**A**) tissue structure of chick thymus (A_C = control group, A_H = HS group, A_G = GABA+HS group), bar = 100 μm; (**B**) distribution of the TLR2 positive cells in chick thymus (B_C = control group, B_H = HS group, B_G = GABA+HS group), bar = 30 μm arrows indicate positive cells

doi: 10.17221/67/2015-CJAS

Table 1. Effects of GABA on the development of thymus tissue structure in chicks under heat stress

Parameter	Group	Age (week)						SEM
		1	2	3	4	5	6	
Thymus weight (g)	CK	$0.33^{a,C}$	$0.62^{a,C}$	$1.47^{a,C}$	$2.34^{a,B}$	$3.63^{a,A}$	2.91^{B}	0.22
	HS	$0.20^{b,C}$	$0.41^{b,C}$	$1.03^{b,B}$	$1.39^{b,B}$	$1.44^{c,B}$	2.33^{A}	0.13
	GABA+HS	$0.22^{b,B}$	$0.54^{ab,B}$	$0.90^{b,B}$	$1.98^{ab,A}$	$2.31^{b,A}$	2.82^{A}	0.19
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: < 0.001		
Index of thymus (g/kg)	CK	$4.00^{a,B}$	4.10^{B}	$6.29^{a,A}$	$5.94^{a,A}$	$5.86^{a,A}$	4.44^{B}	0.24
	HS	$2.95^{b,C}$	3.59^{BC}	$4.78^{ab,A}$	$4.43^{b,AB}$	$2.95^{b,C}$	3.83^{ABC}	0.17
	GABA+HS	3.90^{a}	3.89	4.10^{b}	5.35^{ab}	5.20^{a}	4.12	0.21
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: 0.047		
Thickness of cortex (μm)	CK	$189.22^{a,C}$	$206.36^{a,B}$	$217.90^{a,A}$	$200.08^{a,B}$	$185.96^{a,C}$	$150.19^{a,D}$	3.76
	HS	$178.33^{b,B}$	$179.62^{b,B}$	$208.63^{b,A}$	$170.48^{c,C}$	$163.67^{c,C}$	$139.72^{c,D}$	3.60
	GABA+HS	$181.51^{ab,C}$	$199.84^{a,B}$	$209.01^{b,A}$	$184.03^{b,C}$	$174.31^{b,D}$	$145.29^{b,E}$	3.54
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: < 0.001		
Cortex/medulla	CK	5.30^{A}	4.72^{B}	2.91^{C}	2.47^{D}	1.74^{E}	0.96^{F}	0.26
	HS	$5.05^{b,A}$	$4.36^{b,B}$	$2.80^{b,C}$	$1.98^{c,D}$	$1.22^{c,E}$	$0.89^{b,F}$	0.24
	GABA+HS	$5.21^{ab,A}$	$4.61^{a,B}$	$2.87^{a,C}$	$2.21^{b,D}$	$1.45^{b,E}$	$0.93^{a,F}$	0.27
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: < 0.001		
Number of lymphocytes	CK	$45.73^{a,A}$	42.82^{B}	$38.92^{a,C}$	$37.03^{a,D}$	$30.36^{a,E}$	$22.06^{a,F}$	1.35
	HS	$44.70^{ab,A}$	42.07^{B}	$32.88^{c,C}$	$29.76^{b,D}$	$24.77^{c,E}$	$16.89^{c,F}$	1.62
	GABA+HS	$43.43^{b,A}$	41.74^{B}	$36.04^{b,C}$	$35.93^{a,C}$	$27.76^{b,D}$	$18.71^{b,E}$	1.43
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: < 0.001		

CK = control group, HS = heat-stressed group

$^{a–c, A–F}$means within a column (lowercase superscripts) or a row (uppercase superscripts) and without a common superscript differ significantly ($P < 0.05$, $n = 6$)

had increased weight and index of thymus. There was a significant difference in the thymus weight in week 5 ($P < 0.05$), and in the index of thymus in weeks 1 and 5 ($P < 0.05$). The thickness of cortex, cortex/medulla ratio, and number of lymphocytes of CK group decreased significantly with growth, and reached minimum at 6 weeks of age ($P < 0.05$). Under heat stress, cortex thickness, cortex/medulla ratio, and the number of lymphocytes of HS group were significantly deceased ($P < 0.05$). Compared with HS group, GABA+HS group had significantly increased cortex thickness, cortex/medulla ratio, and the number of lymphocytes in weeks 3–6 ($P < 0.05$).

Effect of GABA on the antioxidant activity of thymus in chicks under heat stress. Table 2 showed that compared with CK group, the SOD activity in chick thymus of HS group was significantly decreased in weeks 3, 5, and 6 ($P < 0.05$). The GSH-Px activity of HS group was significantly decreased in all the weeks except for week 2 ($P < 0.05$). The T-AOC activity of HS group was significantly decreased in weeks 1 and 4–6 ($P < 0.05$). The MDA content of HS group was significantly increased in weeks 4–6 ($P < 0.05$). The activities of SOD, GSH-Px, and T-AOC in GABA+HS group were significantly increased in comparison with those in HS group ($P < 0.05$). The MDA content of GABA+HS group was significantly decreased in weeks 4 and 5 ($P < 0.05$).

Effect of GABA on thymocyte apoptosis and cell proliferation in chicks under heat stress. Table 3 shows that the apoptosis rate in chick thymocytes of CK group increased significantly with growth in weeks 5 and 6 ($P < 0.05$). Under heat stress, the apoptosis rate in chick thymocytes of HS group was significantly increased in comparison with that of CK group ($P < 0.05$). There was an interaction between age and treatment for apoptosis in chick thymocytes ($P < 0.05$). Compared with HS group, the apoptosis rate in chick thymocytes of GABA+HS group was decreased, and the difference was statistically significant in weeks 3, 5, and 6 ($P < 0.05$).

doi: 10.17221/67/2015-CJAS

Table 2. Effect of GABA on the activity of total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), and malondialdehyde (MDA) content in thymus of heat-stressed chicks

Parameter	Group	Age (week)						SEM
		1	2	3	4	5	6	
SOD (U/mg protein)	CK	123.38	108.01	131.23^{a}	112.55	123.16^{a}	121.17^{a}	2.72
	HS	111.88	103.15	109.56^{b}	115.31	104.36^{b}	102.26^{b}	2.01
	GABA+HS	115.09	110.89	112.43^{ab}	127.25	117.19^{a}	116.75^{a}	2.31
	P-value	age: ns		treatment: 0.001		age × treatment: ns		
GSH-Px (U/mg protein)	CK	142.84^{a}	135.72	125.99^{ab}	128.59^{a}	125.89^{a}	127.89^{a}	2.32
	HS	125.68^{b}	126.05	102.54^{b}	112.45^{b}	108.65^{b}	104.77^{b}	2.54
	GABA+HS	145.83^{a}	129.4	130.10^{a}	122.58^{ab}	118.07^{ab}	119.57^{a}	2.74
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: ns		
MDA (nmol/mg protein)	CK	6.49^{B}	6.93^{AB}	7.05^{A}	7.19^{b,A}	7.29^{b,A}	7.18^{b,A}	0.08
	HS	5.74^{C}	6.70^{B}	7.37^{AB}	7.74^{a,A}	8.13^{a,A}	7.85^{a,A}	0.17
	GABA+HS	5.95^{B}	7.03^{A}	7.00^{A}	7.23^{b,A}	7.36^{b,A}	7.70^{ab,A}	0.13
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: ns		
T-AOC (U/mg protein)	CK	2.94^{a}	2.91	3.04	3.31^{a}	3.64^{a}	3.21^{a}	0.13
	HS	2.08^{b}	2.52	2.39	2.21^{b}	2.03^{b}	2.37^{b}	0.09
	GABA+HS	2.66^{a}	2.68	2.42	3.26^{a}	2.88^{ab}	3.09^{a}	0.11
	P-value	age: ns		treatment: < 0.001		age × treatment: ns		

CK = control group, HS = heat-stressed group, ns = not significant

$^{a–b, A–C}$means within a column (lowercase superscripts) or a row (uppercase superscripts) and without a common superscript differ significantly ($P < 0.05$, $n = 6$)

Table 4 shows that the number of lymphocytes in the G0/G1 phases of HS group was decreased in week 1 as compared with that of CK group, but the difference was not statistically significant ($P > 0.05$). The number of lymphocytes in the G0/G1 phases of GABA+HS group was significantly increased in week 2 as compared with that of CK group ($P < 0.05$). No statistically significant difference was observed in the rest of weeks ($P > 0.05$). The number of lymphocytes in the S phase of each group showed irregular trend of change. The number of lymphocytes in the S phase of HS group increased since week 3 and reached the maximum in week 5, and was significantly different from that of GABA+HS group ($P < 0.05$). Compared with CK group, HS group had significantly increased number of lymphocytes in the G2/M phases in weeks 5 and 6 ($P < 0.05$). Compared with HS group, GABA+HS group had significantly different number of lymphocytes in week 6 ($P < 0.05$). There was a significant difference in PI between CK group and the other two groups in

Table 3. Effect of GABA on thymocyte apoptosis in chicks under heat stress (%)

Group	Age (week)						SEM
	1	2	3	4	5	6	
CK	9.80^{c,C}	12.91^{c,C}	21.36^{b,B}	21.51^{c,B}	32.59^{b,A}	34.04^{b,A}	1.61
HS	15.76^{a,E}	24.03^{a,D}	32.33^{a,C}	41.25^{a,B}	48.26^{a,A}	50.43^{a,A}	2.23
GABA+HS	13.07^{b,C}	19.41^{b,B}	23.22^{b,B}	36.58^{b,A}	37.27^{b,A}	38.73^{b,A}	1.86
P-value	age: < 0.001		treatment: < 0.001		age × treatment: 0.002		

CK = control group, HS = heat-stressed group

$^{a–c, A–E}$means within a column (lowercase superscripts) or a row (uppercase superscripts) and without a common superscript differ significantly ($P < 0.05$, $n = 6$)

doi: 10.17221/67/2015-CJAS

Table 4. Effects of GABA on the cell cycle and proliferation index (PI) in thymus of heat-stressed chicks (%)

Parameter	Group	Age (week)						SEM
		1	2	3	4	5	6	
GO/G1	CK	91.65^{A}	$83.69^{b,C}$	85.91^{BC}	85.13^{BC}	87.31^{B}	87.03^{B}	0.57
	HS	88.33^{A}	$85.81^{ab,AB}$	84.93^{AB}	83.29^{B}	83.44^{B}	83.45^{B}	0.63
	GABA+HS	87.83	86.36^{a}	86.1	84.71	87.39	86.41	0.55
	P-value	age: < 0.001		treatment: 0.027		age × treatment: ns		
S	CK	2.83	4.92	4.37	4.32	5.17	3.59	0.27
	HS	3.84	3.98	5.92	5.49	6.08	4.42	0.32
	GABA+HS	4.74	4.13	4.7	4.38	3.45	5.02	0.28
	P-value	age: ns		treatment: ns		age × treatment: ns		
G2/M	CK	$5.53^{b,C}$	10.97^{A}	9.27^{AB}	10.55^{A}	$7.52^{b,BC}$	$9.21^{b,AB}$	0.44
	HS	$7.93^{a,B}$	10.22^{AB}	9.15^{AB}	11.22^{AB}	$10.49^{a,AB}$	$12.14^{a,A}$	0.46
	GABA+HS	$7.43^{ab,B}$	9.57^{AB}	9.20^{AB}	10.91^{A}	$9.16^{ab,AB}$	$8.57^{b,AB}$	0.41
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: ns		
PI	CK	$0.084^{b,C}$	0.160^{A}	0.141^{AB}	0.149^{AB}	0.127^{B}	0.128^{AB}	0.006
	HS	$0.118^{a,B}$	0.142^{AB}	0.151^{AB}	0.167^{A}	0.166^{A}	0.166^{A}	0.006
	GABA+HS	0.122^{a}	0.137	0.139	0.126	0.126	0.136	0.005
	P-value	age: < 0.001		treatment: 0.021		age × treatment: ns		

CK = control group, HS = heat-stressed group, ns = not significant, GO = ambiguous phase, G1 = first gap, S = synthesis, G2 = second gap, M = mitosis

$^{a–b, A–C}$means within a column (lowercase superscripts) or a row (uppercase superscripts) and without a common superscript differ significantly ($P < 0.05$, $n = 6$)

Table 5. Effects of GABA on the plasma levels of interleukin-4 (IL-4), interferon-γ (INF-γ), IL-4/INF-γ, and tumor necrosis factor-α (TNF-α) in chicks under heat stress

Parameter	Group	Age (week)						SEM
		1	2	3	4	5	6	
IL-4 (ng/l)	CK	$69.22^{b,B}$	76.95^{AB}	$71.15^{b,B}$	$86.10^{b,A}$	$89.05^{b,A}$	$87.81^{b,A}$	2.09
	HS	$97.95^{a,AB}$	89.54^{B}	$100.49^{a,AB}$	$103.11^{a,AB}$	$106.14^{a,A}$	$112.82^{a,A}$	2.26
	GABA+HS	85.51^{a}	90.19	88.48^{a}	98.27^{ab}	92.40^{b}	86.03^{b}	2.19
	P-value	age: 0.002		treatment: < 0.001		age × treatment: ns		
IFN-γ (ng/l)	CK	70.21	72.32^{b}	64.63	75.78	70.37^{b}	69.33^{b}	1.83
	HS	72.30^{CD}	$95.71^{a,A}$	67.25^{D}	79.90^{BC}	$84.96^{a,AB}$	$87.15^{a,AB}$	2.13
	GABA+HS	69.18^{B}	$87.47^{ab,A}$	70.44^{B}	72.65^{B}	$70.72^{b,B}$	$76.84^{ab,AB}$	2.03
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: ns		
IL-4/IFN-γ	CK	1.00^{B}	1.07^{AB}	1.11^{AB}	1.17^{AB}	1.29^{AB}	1.31^{A}	0.04
	HS	1.38^{A}	0.95^{B}	1.56^{A}	1.30^{A}	1.27^{A}	1.31^{A}	0.05
	GABA+HS	1.24	1.07	1.29	1.36	1.35	1.12	0.04
	P-value	age: 0.010		treatment: ns		age × treatment: ns		
TNF-α (ng/l)	CK	88.96^{a}	88.10^{a}	100.36^{a}	77.37^{a}	87.70^{a}	73.3	2.32
	HS	65.31^{b}	76.80^{a}	75.11^{b}	64.03^{b}	55.26^{b}	82.33	2.39
	GABA+HS	78.11^{a}	60.31^{b}	55.86^{c}	65.54^{b}	73.03^{a}	71.35	2.08
	P-value	age: ns		treatment: < 0.001		age × treatment: < 0.001		

CK = control group, HS = heat-stressed group, ns = not significant

$^{a–c, A–D}$means within a column (lowercase superscripts) or a row (uppercase superscripts) and without a common superscript differ significantly ($P < 0.05$, $n = 6$)

doi: 10.17221/67/2015-CJAS

Table 6. Effects of GABA on the distribution density and integral optical density (IOD) of Toll-like receptor 2 positive cells in thymus tissue of chicks under heat stress

Parameter	Group	Age (week)						SEM
		1	2	3	4	5	6	
Distribution density ($\times10^{-4}/\mu m^2$)	CK	$1.19^{b,D}$	$3.43^{a,B}$	$4.80^{a,A}$	$2.26^{c,C}$	$1.48^{b,D}$	$1.13^{b,D}$	0.93
	HS	$0.81^{c,E}$	$2.10^{b,C}$	$2.65^{b,B}$	$3.39^{a,A}$	$1.98^{a,C}$	$1.42^{a,D}$	0.32
	GABA+HS	$1.53^{a,B}$	$2.88^{a,A}$	$3.19^{b,A}$	$2.83^{b,A}$	$1.77^{a,B}$	$1.48^{a,B}$	0.53
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: < 0.001		
IOD ($\times10^{-3}/\mu m^2$)	CK	$2.91^{a,C}$	$10.79^{a,B}$	$15.22^{a,A}$	$12.23^{a,B}$	$2.72^{b,C}$	$1.91^{b,C}$	0.23
	HS	$1.59^{b,E}$	$5.96^{b,AB}$	$6.4^{8b,}A$	$4.86^{c,BC}$	$4.73^{a,C}$	$3.20^{a,D}$	0.15
	GABA+HS	$3.76^{a,B}$	$9.45^{a,A}$	$9.37^{b,A}$	$7.88^{b,A}$	$3.99^{a,B}$	$2.65^{a,B}$	0.13
	P-value	age: < 0.001		treatment: < 0.001		age × treatment: < 0.001		

CK = control group, HS = heat-stressed group, ns = not significant

$^{a–c, A–E}$means within a column (lowercase superscripts) or a row (uppercase superscripts) and without a common superscript differ significantly($P < 0.05$, $n = 6$)

week 1. PI of HS group was higher than that of CK and GABA+HS groups in weeks 3–6, but the difference was not statistically significant ($P > 0.05$).

Effects of GABA on the plasma levels of IL-4, IFN-γ, IL-4/IFN-γ, and TNF-α in chicks under heat stress. Table 5 shows that the plasma level of IL-4 of CK group increased significantly with age in weeks 4–6 ($P < 0.05$). The plasma levels of IFN-γ and TNF-α of CK group fluctuated with growth. There was no significant change in IFN-γ level ($P > 0.05$), but there was a significant increase in TNF-α level in week 3 ($P < 0.05$). Compared with CK group, HS group had significantly increased plasma levels of IL-4 and IFN-γ ($P < 0.05$). Compared with HS group, GABA+HS group had significantly decreased plasma levels of IL-4 and IFN-γ in weeks 5 and 6 ($P < 0.05$). The plasma level of TNF-α of HS group increased with growth. There was an interaction between age and treatment for plasma level of TNF-α in chicks ($P < 0.05$). Compared with CK group, HS group had significantly decreased plasma level of TNF-α ($P < 0.05$). Compared with HS group, GABA+HS group had significantly increased plasma level of TNF-α in weeks 1, 3, and 5 ($P < 0.05$).

Effect of GABA on TLR2 expression in thymus tissue of heat-stressed chicks. Figure 1B showed that TLR2 expression was stained in the cytoplasm and cell membrane as yellow or brown particles in round, ovoid or bunchy shape. The TLR2 positive cells were mainly distributed in the medulla, with a small portion observed at the junction between medulla and cortex.

Table 6 shows that with the increase of age, the TLR2 expression in chick thymus had a trend of increasing first followed by decreasing. The distribution density of TLR2 positive cells and IOD value of CK group both reached the maximum at 3 weeks of age, and decreased afterwards. There was an interaction between age and treatment ($P < 0.05$). Compared with CK group, HS group had significantly decreased IOD in weeks 1–4 ($P < 0.05$), and significantly increased IOD in weeks 5–6 ($P < 0.05$). The distribution density of TLR2 positive cells of HS group was significantly lower than that of CK group in weeks 1–3 ($P < 0.05$), but significantly higher in weeks 4–6 ($P < 0.05$). Compared with HS group, GABA+HS group had significantly increased IOD in weeks 1, 2, and 4 ($P < 0.05$), and significantly decreased IOD in weeks 5 and 6 ($P < 0.05$). The distribution density of TLR2 positive cells of GABA+HS group was significantly lower than that of HS group in weeks 1–3 ($P < 0.05$), but significantly higher in weeks 4–6 ($P < 0.05$).

DISCUSSION

Studies have indicated that heat stress affects the normal growth and development of thymus tissue in chicks, and causes reduction in both thymus weight and index (Quinteiro-Filho et al. 2010). Our study showed that the thymus weight of chicks in CK group reached maximum at 5 weeks of age, which represented a high level of immune function. As chickens are warm blooded animals,

doi: 10.17221/67/2015-CJAS

their adaptation range to the ambient temperature is different at different growth stages. In the early growth stage, they need higher temperature to maintain the normal growth and development. Therefore, the index of thymus was not much affected in the early stage of heat stress. The thymus weight and index were significantly decreased in the late stage of heat stress, which suggested that heat stress hindered the normal development of thymus tissues in chicks. Chicks treated with GABA had significantly higher thymus weight and index than the HS group, which suggested that GABA could promote the growth and development of thymus in chicks, and enhance the immune function.

Morphological study of thymus has revealed that lymphocytes in the cortex are active, while those in the medulla are sparse. In this study, we found that the thickness of cortex in CK group first increased and then decreased with growth, and the number of lymphocytes in the cortex and the cortex/medulla ratio gradually decreased. In contrast, all these parameters of HS group decreased with growth. This suggested that heat stress affected the integrity of thymus tissue structure in chicks and caused severe tissue damage. The chicks treated with GABA had higher integrity of thymus tissue structure than HS group, which suggested that GABA effectively alleviated the damage caused by heat stress on the thymus tissue structure in chicks, and maintained the structural development of thymus tissue close to the normal level.

An animal body clears the large number of free radicals produced in the body through the functioning of its own antioxidant enzymes such as GSH-Px and SOD, and maintains a balance between formation and removal of free radicals (Barciszewski et al.1997). We found that chicks in HS group had significantly decreased activity of SOD and GSH-Px in thymus. This suggested that heat stress induced excess formation of free radicals in the body, thus affecting the activity level of the enzyme system**.** Studies have demonstrated that 75 mg/kg GABA increases the activity levels of SOD and GSH-Px in blood serum of chicks under heat stress, therefore GABA can alleviate the adverse effect of heat stress by improving the antioxidant capacity of heat-stressed chicks (Xia et al. 2012). In the present study, we found that the chicks treated with GABA showed elevated activity of SOD and GSH-Px in thymus. This could be attributed to the large amount of glutamic acid produced from metabolism of GABA (Curley et al. 2013). Glutamic acid is the key element for synthesis of GSH-Px, which maintains the GSH-Px level *in vivo*, and therefore improves the activity of antioxidant enzymes.

MDA is a metabolic product of the free radicals in thymus tissue induced by heat stress, and changes in its content may reflect the damage of the tissues and cells. T-AOC is a measure of the total antioxidant capacity, which plays an important role in maintaining the oxidant–antioxidant balance. We found that the T-AOC activity of CK group increased with growth and achieved the highest level at 5 weeks of age with the highest total antioxidant capacity. In contrast, the decreased T-AOC activity and elevated MDA content of HS group broke the oxidant–antioxidant balance in the tissue, resulting in tissue damage. Chicks treated with GABA had increased T-AOC activity, suggesting increased total antioxidant capacity. The reduced MDA content suggested that GABA could regulate the activity of antioxidant enzymes in thymus tissue of chicks under heat stress, increase the antioxidant capacity to remove excess free radicals, and reduce the damage to the body.

In the development of thymus cells, apoptosis occurs in a small amount. Matsushita et al. (2015) reported that the number of apoptotic cells was significantly increased in the testis tissue of mice under acute heat stress at 43°C, which suggested that heat stress can induce apoptosis in large amount and cause tissue damage. Our study found that the number of apoptotic thymocytes in chicks gradually increased along with the heat stress treatment. In this study, we found that excess cell apoptosis induces degeneration of thymus function, causes damage in the thymus tissue structure, and eventually leads to lesion and atrophy of the thymus. This is consistent with our observation of tissue damage in thymus through H&E staining.

Apoptosis of thymocytes is closely related to cell cycle arrest. Studies have indicated that heat stress induces disturbance in the percentages of chicken thymocytes in G0/G1, S, and G2/M phases (Zhang et al. 2003), resulting in a large number of lymphocytes arrested in S and G2/M phases. Animals alleviate the harm of heat stress by increased cell proliferation (Kuhl et al. 2000). In this study, we found that the percentage of lymphocytes in the G2/M phase of HS group was consistent with the increase in the apoptosis rate of thymocytes in

doi: 10.17221/67/2015-CJAS

chicks after heat stress, and both of them reached the maximum at 6 weeks of age. At this point, the percentage of lymphocytes in G0/G1 phase of HS group was the lowest, which suggested that cell arrest in G2/M phase increased cell apoptosis. The increase in PI implied increased cell proliferation of the body to alleviate the effect of heat stress. Chen et al. (2011) reported that high concentration of fluorine induces disturbance of the cell cycle and PI value. It is possibly because high concentration of fluorine induces oxidative stress and results in the disturbance of thymocyte cell cycle. Here, we found that heat stress reduced the antioxidant capacity of thymus in chicks and induced oxidative stress, eventually leading to the cell arrest in G2/M phase and resulting in cell apoptosis. After the treatment of GABA, the apoptosis rate of thymocytes was significantly decreased, and the percentages of lymphocytes in G0/G1, S, and G2/M phases also gradually returned to the normal level. This suggested that GABA could regulate cell cycle progression by repairing DNA damage, and thus reduce the apoptosis of thymocytes.

Heat stress induced the activation of HPA axis and secretion of hormones acting on cytokines, resulting in change in the levels of inflammatory cytokines in the body (Baccan et al. 2004). In this study, we found that the plasma levels of IL-4, IFN-γ, and TNF-α in HS group were significantly increased at 6 weeks of age. It suggested that under long-term heat stress, high levels of inflammatory cytokines inhibited the growth and development of thymus tissue. Moreover, tissue damage of thymus inevitably leads to an impaired immune function (Chand et al. 2014).

The Th cell produced by thymus is an important T lymphocyte subtype in the immune system, which releases a variety of cytokines. IFN-γ is secreted by Th1 cells, and IL-4 is secreted by Th2 cells. They modulate humoral and cellular immunity, respectively. Th1/Th2 balance is essential to a balanced immune system, and it plays an important role in regulation of the internal body environment and immune system (Mosmann and Coffman 1989). Therefore, changes in the levels of IL-4 and IFN-γ reflect the immune function of thymus. We found high expression of IL-4 in HS group in week 1 of heat stress, which broke the immune balance. The increase in IFN-γ level in week 2 enhanced the immune function of the body temporarily. After week 4, the levels of both cytokines increased significantly, indicating that the immune balance was severely destroyed. Besides, changes in IL-4/IFN-γ ratio showed that heat stress induced disturbance in Th1/Th2 balance. This suggested that Th1/Th2 balance was regulated by the mutual regulation and restriction between the cytokines, and the high expression of IL-4 severely destroyed the immune balance. After the treatment with GABA, the immune balance was gradually restored. This was possibly because GABA is a neurotransmitter that can regulate the body's endocrine activity and affect the hormone secretion of endocrine system (Rivest 2010). Therefore, it could adjust the cytokine levels in plasma, protect and restore the immune function.

TNF-α at normal level participates in the regulation of immune response and promotes the repair of tissue damage. Under heat stress, we found that the plasma level of TNF-α of chicks in HS group increased with growth and the difference was significant in week 6. It implied that at this point heat stress caused serious damage in the thymus tissue of chicks, which initiated the inflammatory response. It is known that large production of TNF-α destroys the body's immune balance (Deng et al. 2012). HS group had significantly lower TNF-α level than CK group, possibly because HS group had a high expression of IL-4 in plasma, which has been reported to inhibit the secretion of TNF-α (Wolterink and Hendriks 2013). In addition, heat stress induces tissue cells to produce a large number of heat shock proteins (HSP), which can bind with TNF-α to form a compound and prevent large release of TNF-α, resulting in a decrease in plasma level of TNF-α (Wong and Goeddel 1988). After the treatment with GABA, the plasma level of TNF-α in GABA+HS group gradually reached a similar level as that in CK group. This suggested that GABA could regulate TNF-α parasecretion in plasma of heat-stressed chicks and restore it to the normal level, thus maintain the normal immune function of the body.

TLR2 is an important natural immune cell factor expressed in immune cells, which plays an important role in the inflammatory response and signal transduction of immune function (Okun et al. 2014). We found that the TLR2 expression decreased in the early stage of heat stress, which suggested a low expression of TLR2 in the early stage of the inflammatory response of thymus induced by heat stress. With the increase of age,

doi: 10.17221/67/2015-CJAS

the TLR2 expression of HS group was found to increase gradually and it was significantly different from that of CK group. It suggested that the degree of thymus tissue damage was correlated with the expression of TLR2.

TLR activates the transcription factor NF-κB under external stimulation, resulting in the release of inflammatory cytokines such as TNF-α (Kawai and Akira 2007). The expression of TLR2 controls the secretion of inflammatory cytokines IFN-γ (Cottalorda et al. 2009). In this study, we found that the plasma level of IFN-γ of HS group increased significantly with the increasing TLR2 expression. In addition, TLR2 activator, *pam3cys*, activates TLR2 via ERK-MAPK signalling pathway and enhances the Th2 immune response (Netea et al. 2005). Therefore, the expression of TLR2 can regulate the Th1/Th2 balance and make it shift towards Th2. In the early stage of heat stress, the distribution density of TLR2 positive cells and IOD value of chicks treated with GABA were found close to those of CK group, but significantly different from those of HS group. In the late stage of heat stress, both parameters of GABA+HS group were decreased in comparison to HS group, but there were no significant differences. This suggested that GABA could maintain the normal immune function by regulating the expression of TLR2 in chick thymus.

CONCLUSION

Heat stress changed the structural development, cytokine levels, antioxidant activity, and cell apoptosis of thymus tissue in chicks, which severely hindered the development process of chick thymus. We demonstrated that GABA could be used as an additive to alleviate the negative effects on the development of tissue structure and immune function in chick thymus. The presented results provide a theoretical basis for improving the growth and development of chicks under the condition of heat stress.

REFERENCES

Baccan G.C., Oliveira R.D., Mantovani B. (2004): Stress and immunological phagocytosis: possible nongenomic action of corticosterone. Life Sciences, 75, 1357–1368.

Barciszewski J., Siboska G.E., Pedersen B.O., Clark B.F., Rattan S.I. (1997): Furfural, a precursor of the cytokinin hormone kinetin, and base propenals are formed by hydroxyl radical damage of DNA. Biochemical and Biophysical Research Communications, 238, 317–319.

Borges S.A., Fischer da Silva A.V., Majorka A., Hooge D.M., Cummings K.R. (2004): Physiological responses of broiler chickens to heat stress and dietary electrolyte balance (sodium plus potassium minus chloride, milliequivalents per kilogram). Poulty Science, 83, 1551–1558.

Chand N., Naz S., Khan A., Khan S., Khan R.U. (2014): Performance traits and immune response of broiler chicks treated with zinc and ascorbic acid supplementation during cyclic heat stress. International Journal of Biometeorology, 58, 2153–2157.

Chen T., Cui H., Cui Y., Bai C., Gong T., Peng X. (2011): Cell-cycle blockage associated with increased apoptotic cells in the thymus of chickens fed on diets high in fluorine. Human and Experimental Toxicology, 30, 685–692.

Chen Z., Wang T., Huang L.M., Fang D.N. (2002): Effects of GABA on the heat stress broilers. Zoological Research, 23, 341–344.

Chen Z., Tang J., Sun Y.Q., Xie J. (2013): Protective effect of γ-aminobutyric acid on antioxidation function in intestinal mucosa of Wenchang chicken induced by heat stress. Journal of Animal and Plant Sciences, 23, 1634–1641.

Chen Z., Xie J., Wang B., Tang J. (2014): Effect of γ-aminobutyric acid on digestive enzymes, absorption function, and immune function of intestinal mucosa in heat-stressed chicken. Poultry Science, 93, 2490–2500.

Chen Z., Xie J., Hu M.Y., Tang J., Shao Z.F., Li M.H. (2015): Protective effects of γ-aminobutyric acid (GABA) on the small intestinal mucosa in heat-stressed Wenchang chicken. Journal of Animal and Plant Sciences, 25, 78–87.

Cottalorda A., Mercier B.C., Mbitikon-Kobo F.M., Arpin C., Teoh D.Y., McMichael A., Marvel J., Bonnefoy-Berard N. (2009): TLR2 engagement on memory $CD8^+$ T cells improves their cytokine-mediated proliferation and IFN-γ secretion in the absence of Ag. European Journal of Immunology, 39, 2673–2681.

Curley A.A., Eggan S.M., Lazarus M.S., Huang Z.J., Volk D.W., Lewis D.A. (2013): Role of glutamic acid decarboxylase in regulating cortical parvalbumin and GABA membrane transporter 1 expression: implications for schizophrenia. Neurobiology of Disease, 50, 179–186.

Deng W., Dong X.F., Tong J.M., Zhang Q. (2012): The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens. Poultry Science, 91, 575–582.

Ghazi S., Habibian M., Moeini M.M., Abdolmohammadi A.R. (2012): Effects of different levels of organic and

doi: 10.17221/67/2015-CJAS

inorganic chromium on growth performance and immunocompetence of broilers under heat stress. Biological Trace Element Research, 146, 309–317.

Gu Z.T., Wang H., Li L., Liu Y.S., Deng X.B., Huo S.F., Yuan F.F., Tong H.S., Su L. (2014): Heat stress induces apoptosis through transcription-independent p53-mediated mitochondrial pathways in human umbilical vein endothelial cell. Scientific Reports, 4, 4469.

Han A.Y., Zhang M.H., Zuo X.L., Zheng S.S., Zhao C.F., Feng J.H., Cheng C. (2010): Effect of acute heat stress on calcium concentration, proliferation, cell cycle, and interleukin-2 production in splenic lymphocytes from broiler chickens. Poultry Science, 89, 2063–2070.

Kawai T., Akira S. (2007): Signaling to NF-κB by Toll-like receptors. Trends in Molecular Medicine, 13, 460–469.

Kuhl N.M., Rensing L. (2000): Heat shock effects on cell cycle progression. Cellular and Molecular Life Sciences, 57, 450–463.

Matsushita K., Miyake H., Chiba K., Fujisawa M. (2015): Clusterin produced by sertoli cells inhibits heat stress-induced apoptosis in the rat testis. Andrologia, 191, e743.

Mosmann T.R., Coffman R.L. (1989): TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, 145–173.

Netea M.G., Van der Meer J.W., Sutmuller R.P., Adema G.J., Kullberg B.J. (2005): From the Th1/Th2 paradigm towards a Toll-like receptor/T-helper bias. Antimicrobial Agents and Chemotherapy, 49, 3991–3996.

Okun E., Griffioen K.J., Rothman S., Wan R., Cong W.N., De Cabo R., Martin-Montalvo A., Levette A., Maudsley S., Martin B., Arumugam T.V., Mattson M.P. (2014): Toll-like receptors 2 and 4 modulate autonomic control of heart rate and energy metabolism. Brain, Behaviour, and Immunity, 36, 90–100.

Quinteiro-Filho W.M., Ribeiro A., Ferraz-de-Paula V., Pinheiro M.L., Sakai M., Sa L.R., Ferreira A.J., Palermo-Neto J. (2010): Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poultry Science, 89, 1905–1914.

Rivest S. (2010): Interactions between the immune and neuroendocrine systems. Progress in Brain Research, 181, 43–53.

Tang J., Chen Z. (2015): The protective effect of γ-aminobutyric acid on the development of immune function in chickens under heat stress. Journal of Animal Physiology and Animal Nutrition, 100, 768–777.

Wolterink R.G.K., Hendriks R.W. (2013): Type 2 innate lymphocytes in allergic airway inflammation. Current Allergy and Asthma Reports, 13, 271–280.

Wong G.H.W., Goeddel D.V. (1988): Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science, 242, 941–944.

Xia L., Zhan X.A., Zhu Q.M., Wang Y.X., Liu W.L., Ma Y.E. (2012): γ-Aminobutyric acid affects laying and reproductive performance of broiler breeders under heat stress. Chinese Journal of Animal Nutrition, 24, 137–144. (in Chinese)

Xiang H.J., Yan H.Y., Han J.X., Li L.A., Ying M.X. (2011): Impacts of heat stress on baseline immune measures and a subset of T cells in Bama miniature pigs. Livestock Science, 135, 289–292.

Zhang S.X., Huang H.R., Bao E.D. (2003): The effect and regulation of heat stress to apoptosis on chicken thymocytes. Journal of Nanjing Agricultural University, 26, 66–69.

Received: 2015–09–20
Accepted after corrections: 2016–07–12

Corresponding Author

Prof. Zhong Chen, Hainan Normal University, College of Life Sciences, Ministry of Education Key Laboratory for Tropical Animal and Plant Ecology, Long Kun Nan Road, Haikou, 571158, P.R. China
Phone: +86 898 65 883 521, e-mail: zh.chen@hainnu.edu.cn