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Xie, XZ; Liang, C; Li, MH; Chen, Z

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#### ■Author(s)

Xie XZ<sup>1</sup>  
Liang C<sup>1</sup>  
Li MH<sup>1</sup>  
Chen Z<sup>1</sup>

<sup>1</sup> College of Life Sciences, Hainan Normal University, Haikou 571158, China

#### ■Mail Address

Corresponding author e-mail address  
Z Chen  
Hainan Normal University, Long Kun Nan  
Road, Haikou City, 571158, P.R. China  
Tel: +86-898-65883521  
Email: zh.chen@hainnu.edu.cn

#### ■Keywords

Heat stress; GABA; cytokines; thymus; chicks.

## Effects of GABA on the Thymus Cytokines of Wenchang Chickens Submitted to Heat Stress

### ABSTRACT

Under routine feeding conditions, 1-day-old male chicks were randomly divided into control group (CK), heat-stress group (HS), and GABA + heat stress group (GABA+HS). The thymus contents of IL-1, IL-2, TGF- $\beta$ 1, IFN- $\gamma$ , GH and HSP70 were determined by enzyme-linked immunosorbent assays. The results showed that: (1) IL-1 and TGF- $\beta$ 1 contents of HS group were significantly lower than those of the CK group ( $p < 0.05$ ), and those of the GABA+HS group were significantly higher relative to the HS group ( $p < 0.05$ ); (2) IL-2 and IFN- $\gamma$  contents of the HS group were significantly higher than those of the CK group ( $p < 0.05$ ), and those of the GABA+HS group were significantly lower relative to the HS group ( $p < 0.05$ ); (3) the thymus GH content of all three groups first increased and then decreased. The expression levels GH of the HS and GABA+HS groups were significantly lower than CK group ( $p < 0.05$ ); and (4) HSP70 expression levels in the thymus were significantly higher in the HS and GABA+HS groups relative to the CK group ( $p < 0.05$ ). These results indicate that heat stress affected thymus development, immune functions, and overall growth of chickens. Furthermore, it was shown that feeding GABA may significantly improve the immune responses of heat-stressed chickens by increasing the expression levels of IL-1 and TGF- $\beta$ 1, and effectively alleviate the negative effects of heat stress on thymus development by changing HSP70 expression and GH secretion.

### INTRODUCTION

Animals are frequently submitted to stress in the modern livestock industry. As a result of the intensification of livestock production and the development of large-scale farming, the rearing environment has become one of the main aspects that limits animal productivity. Among the environmental factors, high temperature has a huge impact on livestock and poultry production and may cause serious economic losses (Rispoli *et al.*, 2011).

Most regions of southern China, especially Hainan Island, are characterized by hot temperatures, long days, high humidity, and heavy rainfall during about two-thirds of the year (Tang *et al.*, 2008; Zou *et al.*, 2015). Animals reared in these regions often suffer heat stress. Therefore, exploring methods to alleviate heat stress may significantly improve their productivity and reproductive performance.

When the environmental temperature is higher than the upper limit chickens are able to cope with, their physiological functions are disturbed, causing elevation of body temperature and respiratory rate, as well as accumulation of metabolites. These changes can cause intestinal tissue structure changes and affect feed digestion and absorption, leading appetite loss, diarrhea, and eventually significantly increasing morbidity and mortality (Quinteiro-Filho *et al.*, 2012; Chand



*et al.*, 2014; Chen *et al.*, 2014). Heat stress not only affects the physiology of chickens, but also has negative effects on their immune function. It has been shown that heat stress can disrupt chicken's immune system, cause lesions in immune organs, and reduce the numbers of lymphocytes and macrophages, leading to immunosuppression (Smith *et al.*, 2003; Khajavi *et al.*, 2003). Therefore, the mitigation of heat stress is of great significance for the chicken industry.

Gamma-aminobutyric acid (GABA) is widely present in animals as an inhibitory neurotransmitter. GABA plays many roles in livestock. It mediates a stress reaction and participates in a variety of metabolic activities to promote amino acid balance in the body (Dai *et al.*, 2012). GABA is currently used in animal production as a functional amino acid feed additive to promote feed intake, enhance the immune status, and to increase productivity. It was also reported that GABA improves the feed conversion ratio and the growth performance of broilers under heat stress (Chen *et al.*, 2002) and maintains the antioxidant function of the intestinal mucosa of broilers submitted to heat stress (Chen *et al.*, 2013). In addition, GABA is considered an effective and safe feed additive (Takeshima *et al.*, 2014).

The thymus is an important lymphoid organ of the central immune system and the main site of T cell differentiation and development, and therefore, participates in cell-mediated immunity. Heat stress has been shown to negatively affect the thymus development and to impair the immune functions (Quinteiro-Filho *et al.*, 2012). Consequently, mitigating the negative impacts of heat stress on thymus development in order to maintain the immune functions is key to ensure adequate broiler growth performance. In a previous study, we showed that feeding chicks with a GABA solution alleviated the effects of heat stress, increased body weight and feed intake, and regulated the immune function of the intestinal tract of broilers (Chen *et al.*, 2015). However, the effects of heat stress on thymus immune factors in chickens are still unclear. Therefore, we aimed at investigating these effects and the roles of GABA under heat stress in order to provide experimental support for the use of GABA to reduce losses due to heat stress in the poultry industry.

## MATERIALS AND METHODS

### Birds

A total of 108 healthy one-day-old male Wenchang chicks (Hainan Yong Ji Live Stock Co., Ltd). Upon arrival at the experimental facilities located in Haikou, Hainan

province, China, chicks were individually weighed and numbered. Initial body weight was not significantly different among groups.

Birds were randomly designated to a control group (CK), a heat stress group (HS), and GABA + heat-stress group (GABA+HS), with six replicates of six birds each. The chicks of the GABA+HS group received 0.2mL of a GABA solution (50 mg/kg of body weight) daily by oral gavage. The GABA solution was purchased from Sigma, St Louis, MO, USA. The chicks of CK and HS groups received 0.2mL saline solution daily by oral gavage.

Birds were housed in a conventional broiler house with natural ventilation, and divided in three pens (one per experimental group). A 14:10 light to dark lighting program was applied. All chicks were provided *ad libitum* access to feed and distilled water (15-20 chicks/m<sup>2</sup> feeding density). The house was cleaned and disinfected during the five weeks of experimental period.

The diet was formulated according to the NRC standards (1994, Table1), and the birds were subjected to routine management practices. The experiment was conducted with the approval of the Animal Experimentation Ethics Committee of Hainan Normal University.

**Table 1** – Ingredients and chemical compositions of the basal diet (g/kg)

Ingredient	Content
Corn	630
Soya bean meal	200
Cottonseed meal	60
Rapeseed meal	50
Fish meal	43.3
Calcium bicarbonate	15
Vitamin and mineral mix	1.7
Nutrient levels	
Crude protein	155
Calcium	8
Lysine	7.3
Total phosphorus	6
Methionine	3.5

Contained per kg diet: vit. A 10,000 IU; vit. D 32,000 IU; vit. E 10 mg; vit. K<sub>3</sub> 1 mg; vit. B<sub>1</sub> 1 mg; vit. B<sub>2</sub> 5 mg; vit. B<sub>6</sub> 1.5 mg; biotin 0.05 mg; BHT 10 mg; pantothenic acid 10 mg; folic acid 1 mg; nicotinic acid 30 mg; Mn 60 mg; Zn 50 mg; Fe 30 mg; Cu 4 mg; I 3 mg; Se 0.1 mg; Co 0.1 mg.

### Heat stress treatment

The birds submitted to heat stress (HS and GABA+HS groups) were daily placed at 01:00 pm for 2 h in a LRH-800-GS artificial climate chamber (40±0.5 °C, 70%-80% relative humidity; MingTian Environmental Protection Instrument Co., LTD, China), while the CK



group birds were placed in an incubator for 2 h at room temperature (25-28 °C). After the heat-stress treatments, birds were returned to the conventional sheds.

### Sample collection and processing

At 1, 2, 3, 4, and 5 weeks of age, six birds per group were randomly selected and sacrificed after 2 h of heat stress for rapid and sterile thymus collection. The fat surrounding the thymus was removed, and thymuses were then weighed and stored at -20 °C until analyses.

The thymuses were rinsed in ice-cold PBS (pH 7.4) to remove the blood, and dried with filter paper. PBS was added (9:1 of thymus weight) for tissue homogenization on ice bath using a glass homogenizer until the tissue was completely broken down and presented uniform light color. The homogenates then were centrifuged at 3,000 r/min for 10 min at 4 °C, and the supernatant was stored at -20 °C until further use.

### Thymus immune factors

Thymus interleukin-1 (IL-1), interleukin-2 (IL-2), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), interferon- $\gamma$  (IFN- $\gamma$ ), growth hormone (GH), and HSP70 contents were determined using commercial ELISA kits purchased from Shanghai Yu Ping Biotechnology Ltd

(batch number 201407). The tests were performed following manufacturer's instructions. Absorbance was measured at 450 nm wave length, and the contents of each factor were calculated using standard curves. All data were analyzed using Elisa Reader (enzyme linked detector, DG5031)

### Statistical analyses

The data were analyzed by analysis of variance (ANOVA) using the software SPSS17.0 and are presented as mean  $\pm$  SEM. Differences with  $p < 0.05$  were considered statistically significant.

## RESULTS

### Effect of GABA on thymus IL-1 contents of broilers submitted to heat stress

As shown in Table 2, thymus IL-1 content was first increased and then decreased as broilers aged, and all three groups presented a similar pattern. At all evaluated ages, the thymus IL-1 contents of the HS group were significantly lower than those of the CK group ( $p < 0.05$ ), while the GABA+HS group presented higher thymus IL-1 contents compared with the HS group at 1 and 3 weeks of age ( $p < 0.05$ ).

**Table 2** – Effect of GABA on the thymus IL-1 content of chickens submitted to heat stress (ng/L, n=6)

Group	Weeks of age				
	1	2	3	4	5
CK	347.181 $\pm$ 12.278 <sup>b,B</sup>	526.544 $\pm$ 19.641 <sup>a,A</sup>	383.587 $\pm$ 16.753 <sup>a,B</sup>	206.260 $\pm$ 4.457 <sup>a,D</sup>	246.566 $\pm$ 6.364 <sup>a,C</sup>
HS	295.700 $\pm$ 16.211 <sup>c,AB</sup>	327.376 $\pm$ 28.971 <sup>b,A</sup>	269.587 $\pm$ 7.562 <sup>c,B</sup>	169.022 $\pm$ 6.211 <sup>b,C</sup>	192.241 $\pm$ 6.334 <sup>b,C</sup>
GABA+HS	372.495 $\pm$ 15.887 <sup>a,A</sup>	382.272 $\pm$ 8.272 <sup>b,A</sup>	314.135 $\pm$ 11.626 <sup>b,B</sup>	182.603 $\pm$ 4.898 <sup>b,C</sup>	208.451 $\pm$ 3.882 <sup>b,C</sup>

<sup>a-c</sup>Values in the same column with no common superscripts are significantly different ( $p < 0.05$ ).

<sup>A-D</sup>Values in the same row with no common superscripts are significantly different ( $p < 0.05$ ). CK: control, HS: heat stress, GABA+HS: GABA+heat stress.

### Effect of GABA on thymus IL-2 content of broilers submitted to heat stress

As shown in Table 3, thymus IL-2 contents of the HS and GABA+HS groups were significantly higher

compared with the CK group, except at 2 weeks of age for the HS group, and at 2 and 5 weeks of age for the GABA+HS group ( $p < 0.05$ ). Compared with HS group, GABA+HS group presented significantly higher IL-2 contents at 2, 3, and 4 weeks of age ( $p < 0.05$ ).

**Table 3** – Effect of GABA on the thymus IL-2 content of chickens submitted to heat stress (ng/L, n=6)

Group	Weeks of age				
	1	2	3	4	5
CK	292.178 $\pm$ 16.511 <sup>b,D</sup>	484.874 $\pm$ 17.133 <sup>a,A</sup>	372.986 $\pm$ 7.215 <sup>c,C</sup>	437.218 $\pm$ 15.932 <sup>b,B</sup>	361.590 $\pm$ 7.507 <sup>b,C</sup>
HS	429.793 $\pm$ 27.621 <sup>a,AB</sup>	411.663 $\pm$ 10.633 <sup>b,B</sup>	474.687 $\pm$ 13.057 <sup>b,B</sup>	446.197 $\pm$ 15.413 <sup>b,AB</sup>	423.750 $\pm$ 9.495 <sup>a,B</sup>
GABA+HS	481.593 $\pm$ 30.219 <sup>a,B</sup>	495.407 $\pm$ 15.943 <sup>a,B</sup>	547.207 $\pm$ 13.932 <sup>a,A</sup>	548.933 $\pm$ 14.461 <sup>a,A</sup>	365.043 $\pm$ 15.132 <sup>b,C</sup>

<sup>a-c</sup>Values in the same column with no common superscripts are significantly different ( $p < 0.05$ ).

<sup>A-D</sup>Values in the same row with no common superscripts are significantly different ( $p < 0.05$ ). CK: control, HS: heat stress, GABA+HS: GABA+heat stress.



### Effect of GABA on thymus IFN- $\gamma$ content of broilers submitted to heat stress

As shown in Table 4, compared with CK group, the HS group exhibited significantly higher thymus IFN- $\gamma$

contents at 1, 3, and 5 weeks of age ( $p < 0.05$ ) and the GABA+HS group at 1 and 4 weeks of age ( $p < 0.05$ ). Higher thymus IFN- $\gamma$  contents were determined in the GABA+HS compared with the HS group at 2 and 4 weeks of age ( $p < 0.05$ ).

**Table 4** – Effect of GABA on the thymus IFN- $\gamma$  content of chickens submitted to heat stress (ng/L, n=6)

Group	Weeks of age				
	1	2	3	4	5
CK	47.474 $\pm$ 13.730 <sup>c,C</sup>	414.854 $\pm$ 10.853 <sup>a,b,A</sup>	334.981 $\pm$ 22.410 <sup>b,B</sup>	306.791 $\pm$ 22.259 <sup>b,B</sup>	353.775 $\pm$ 17.609 <sup>b,B</sup>
HS	410.743 $\pm$ 7.879 <sup>a,B</sup>	409.275 $\pm$ 17.555 <sup>b,B</sup>	410.254 $\pm$ 11.401 <sup>a,B</sup>	373.058 $\pm$ 17.647 <sup>b,B</sup>	505.690 $\pm$ 24.746 <sup>a,A</sup>
GABA+HS	309.434 $\pm$ 18.789 <sup>b,B</sup>	471.431 $\pm$ 28.816 <sup>a,A</sup>	332.436 $\pm$ 16.625 <sup>b,B</sup>	485.624 $\pm$ 28.516 <sup>a,A</sup>	345.651 $\pm$ 11.670 <sup>b,B</sup>

<sup>a-c</sup> Values in the same column with no common superscripts are significantly different ( $p < 0.05$ ).

<sup>A-D</sup> Values in the same row with no common superscripts are significantly different ( $p < 0.05$ ). CK: control, HS: heat stress, GABA+HS: GABA+heat stress.

### Effect of GABA on thymus TGF- $\beta$ 1 content of broilers submitted to heat stress

Thymus TGF- $\beta$ 1 contents (Table 5) decreased as broilers aged in all groups. TGF- $\beta$ 1 contents of the HS group were significantly lower than those of the CK

group at all ages, except at 4 weeks of age ( $p < 0.05$ ), whereas those of the GABA+HS group were only significantly different ( $p < 0.05$ ) relative to the CK group at 5 weeks of age. TGF- $\beta$ 1 contents of the GABA+HS group were significantly higher than those of the HS group at 1, 2, and weeks of age ( $p < 0.05$ ).

**Table 5** – Effect of GABA on the thymus TGF- $\beta$ 1 content of chickens submitted to heat stress (ng/L, n=6)

Group	Weeks of age				
	1	2	3	4	5
CK	906.614 $\pm$ 57.165 <sup>a,AB</sup>	982.620 $\pm$ 21.895 <sup>a,A</sup>	839.838 $\pm$ 23.398 <sup>a,B</sup>	712.799 $\pm$ 41.589 <sup>c</sup>	706.827 $\pm$ 36.022 <sup>a,C</sup>
HS	727.638 $\pm$ 40.711 <sup>b,BC</sup>	899.557 $\pm$ 28.053 <sup>b,A</sup>	670.181 $\pm$ 21.301 <sup>b,C</sup>	751.164 $\pm$ 15.970 <sup>b</sup>	512.740 $\pm$ 11.962 <sup>b,D</sup>
GABA+HS	938.464 $\pm$ 52.404 <sup>a,AB</sup>	971.943 $\pm$ 13.641 <sup>a,A</sup>	863.363 $\pm$ 26.448 <sup>a,B</sup>	707.732 $\pm$ 27.933 <sup>c</sup>	541.695 $\pm$ 20.878 <sup>b,D</sup>

<sup>a-c</sup> Values in the same column with no common superscripts are significantly different ( $p < 0.05$ ).

<sup>A-D</sup> Values in the same row with no common superscripts are significantly different ( $p < 0.05$ ). CK: control, HS: heat stress, GABA+HS: GABA+heat stress.

### Effects of GABA on thymus GH content and HSP70 expression of broilers submitted to heat stress

The GH contents (Table 6) of the HS group were significantly lower than those of the CK group at 2, 3, and 5 weeks of age ( $p < 0.05$ ). The GABA+HS group

presented lower GH contents than both CK and HS groups at 2, 4, and 5 weeks of age. The HS group had significantly higher thymus HSP70 contents (Table 7) at 2, 3, 4, and 5 weeks of age compared with the CK group ( $p < 0.05$ ), and compared with the GABA+HS group at 2 weeks of age ( $p < 0.05$ ).

**Table 6** – Effect of GABA on the thymus GH content of chickens submitted to heat stress (ng/L, n=6)

Group	Weeks of age				
	1	2	3	4	5
CK	24.892 $\pm$ 2.139 <sup>B</sup>	29.069 $\pm$ 0.525 <sup>a,A</sup>	26.334 $\pm$ 0.717 <sup>a,AB</sup>	23.487 $\pm$ 0.848 <sup>a,B</sup>	24.630 $\pm$ 1.053 <sup>a,B</sup>
HS	22.504 $\pm$ 0.645 <sup>A</sup>	23.581 $\pm$ 1.101 <sup>b,A</sup>	21.879 $\pm$ 0.812 <sup>b,A</sup>	23.081 $\pm$ 0.343 <sup>a,A</sup>	19.755 $\pm$ 0.352 <sup>b,B</sup>
GABA+HS	23.628 $\pm$ 1.213 <sup>A</sup>	19.008 $\pm$ 0.338 <sup>c,C</sup>	21.957 $\pm$ 0.623 <sup>b,AB</sup>	20.069 $\pm$ 0.226 <sup>b,BC</sup>	19.335 $\pm$ 0.857 <sup>b,C</sup>

<sup>a-c</sup> Values in the same column with no common superscripts are significantly different ( $p < 0.05$ ).

<sup>A-C</sup> Values in the same row with no common superscripts are significantly different ( $p < 0.05$ ). CK: control, HS: heat stress, GABA+HS: GABA+heat stress.

**Table 7** – Effect of GABA on the thymus HSP70 content of chickens submitted to heat stress (ng/L, n=6)

Group	Weeks of age				
	1	2	3	4	5
CK	593.099 $\pm$ 32.672 <sup>B</sup>	872.252 $\pm$ 60.545 <sup>b,A</sup>	591.200 $\pm$ 39.720 <sup>b,B</sup>	653.867 $\pm$ 37.574 <sup>b,B</sup>	384.209 $\pm$ 29.018 <sup>b,C</sup>
HS	656.083 $\pm$ 40.321 <sup>C</sup>	1050.125 $\pm$ 44.751 <sup>a,A</sup>	771.605 $\pm$ 45.338 <sup>a,B</sup>	864.973 $\pm$ 39.871 <sup>a,B</sup>	500.998 $\pm$ 12.233 <sup>a,B</sup>
GABA+HS	679.820 $\pm$ 31.510 <sup>B</sup>	654.500 $\pm$ 31.852 <sup>c,B</sup>	687.733 $\pm$ 23.477 <sup>a,b,B</sup>	838.070 $\pm$ 36.429 <sup>a,A</sup>	469.348 $\pm$ 26.233 <sup>a,C</sup>

<sup>a-c</sup> Values in the same column with no common superscripts are significantly different ( $p < 0.05$ ).

<sup>A-C</sup> Values in the same row with no common superscripts are significantly different ( $p < 0.05$ ). CK: control, HS: heat stress, GABA+HS: GABA+heat stress.





## DISCUSSION

Immune factors, also known as cytokines, are soluble proteins secreted by immune cells that indirectly regulate cell activity and metabolism. The contents of immune factors represent the functionality of the immune organs in the body (Balkwill, 1988).

Stress and the immune system are linked through the neuroendocrine system. The hypothalamic-pituitary-adrenal (HPA axis) is an important component of the neuroendocrine system in stress responses. HPA activation by heat stress leads to the release of several hormones, which change cytokine production, affecting the immune functions (Baccan *et al.*, 2004). IL-1 is an important mediator between the immune system and the HPA axis (Dunn, 2008). It has been shown that heat stress causes the hypothalamus to produce large amounts of corticotrophin-releasing hormone (CRH), which stimulates the pituitary gland to secrete a large amount of corticotrophin (ACTH). ACTH stimulates the production and secretion of glucocorticoids by the adrenal cortex, inhibiting the production of IL-1 and IL-2 and other cytokines (Bateman *et al.*, 1989). Consistently, this study showed that heat stress caused significant reduction of the thymus IL-1 contents in broilers. When treated with GABA, broilers submitted to heat stress presented significantly lower IL-1 content. This suggests that GABA may regulate IL-1 levels in the thymus of heat-stressed broilers and restore them to the normal levels.

IL-2 is mainly secreted by type 1 T-helper cells (Th1) and stimulates the production of IFN- $\gamma$  and other cytokines by the T-helper cells, as well as natural-killer (NK) cell and B cell proliferation and antibody secretion. Aberrant secretion of IL-2 affects the HPA axis, which may lead to immune dysfunction (Tang and Chen, 2015). In this study, we found that, compared with the CK group, the thymus IL-2 contents of HS group were significantly higher when broilers were 1, 3, 4, and 5 weeks old, while the GABA+HS group presented higher IL-2 contents than both the CK and the HS groups. This indicates that GABA promoted IL-2 secretion. However, in a previous study by Regnier *et al.* (1981), heat stress increased HPA axis activity and corticosteroid levels, which may lead to immunosuppression, and as a result, to reduced IL-2 contents. IL-2 is mainly produced by T cells, including CD4<sup>+</sup> and CD8<sup>+</sup> subtypes. This discrepancy may be explained by the fact that heat stress increases the ratio of CD4<sup>+</sup> lymphoid cells to CD8<sup>+</sup> lymphoid cells (Trout and Mashaly, 1994), changing cytokine expression and

secretion, eventually increasing IL-2 expression. IL-2 production and CD4<sup>+</sup>/CD8<sup>+</sup> ratios are important indexes of cell-mediated immune function. The cytokine IL-2 is involved in the progression of T lymphocytes from the G1-phase to the S-phase of the cell cycle, and it is secreted by the activation of Th1 and CD4<sup>+</sup> cells. In this study, the high expression of IL-2 may be related to the increased in the CD4: CD8 ratio; however, the underlying mechanism needs to be elucidated. Although the thymus IL-2 contents of chickens fed GABA increased until week 4, they returned to normal in week 5, i.e., they were similar to those of broilers not submitted to heat stress, and were significantly lower compared with the HS group.

Th1 cells also secrete IFN- $\gamma$ , an important immune cell and inflammatory factor, and indicate the degree of inflammatory infiltration. Under normal circumstances, Th cell subtypes Th1 and Th2 mediate cellular and humoral immunity, respectively, and they maintain the immune equilibrium by tightly controlling their respective cytokines. The results of this study indicate that heat stress significantly increased thymus IFN- $\gamma$  and IL-2 contents, which may have undermined the immune equilibrium and disrupted immune functions. The equilibrium may have recovered after GABA feeding, as indicated by the reduction of IFN- $\gamma$  content to normal levels in week 5. On the other hand, heat stress led to reduced TGF- $\beta$ 1 contents, but GABA supplementation effectively restored TGF- $\beta$ 1 levels. These results suggest that GABA mitigates heat stress by promoting or inhibiting the secretion of specific cytokines by the chicken thymus.

Heat stress affects HPA axis and causes the release of somatostatin by the hypothalamus, which inhibits the secretion of GH by the pituitary. The synthesis and release of GH is regulated by SS release by hypothalamus. According to Rhoads *et al.* (2010), cows under heat stress presented reduced content of insulin-like growth factor-I (IGF-I), suggesting GH reduction and inhibited growth and development that may be caused by a joint effect of growth hormones and insulin-like factors. In the present study, heat stress reduced thymus GH levels, as shown by the significantly lower GH contents in the HS group relative to the CK group in weeks 2, 3, and 5, while those fed GABA presented even lower GH contents. These results do not agree with the affirmation that GABA may stimulate brain dopamine and promote GH release, indicating that GABA concentration affects GH secretion (Vijayan *et al.* 1978).



It was also reported that immune factors are involved in GH regulation in the neuroendocrine system, as IL-1 injection into brain ventricle caused the release of GH from anterior pituitary cells, while IFN- $\gamma$  injection inhibited GH secretion (Tang and Qi, 1993). In the current study, heat stress reduced thymus IL-1 contents, as well as GH secretion; at the same time, IFN- $\gamma$  contents significantly increased, which may be one of the main reasons for the reduction of GH levels.

Heat shock proteins (HSPs) are a group of non-specific proteins synthesized under stressful conditions (Vidal *et al.*, 2012). Heat stress has been reported to stimulate the expression of large amounts of HSPs. HSP70 is an important member of HSP family, it is activated under heat stress to protect cells, and its expression levels are closely related with heat stress-induced tissue damages, especially in chickens. Therefore, HSP70 is considered as a marker for heat stress-induced damages in the chicken thymus. Our results show that heat stress activated the expression of a large amount of the HSP70 protein in the thymus, leading to significant tissue damages. The feeding of GABA, on the other hand, was able to protect the thymus from heat stress-induced damages. A previous study showed that HSPs can also help to maintain the physiological balance by regulating cytokines, especially pro-inflammatory cytokines, to protect tissues from inflammatory damages (Van *et al.*, 2005). The cytokine IFN- $\gamma$  investigated in the present study is an inflammatory cytokine, and its strong expression under heat stress indicates that the thymus suffered inflammatory infiltration. However, after week 4, IFN- $\gamma$  levels were significantly reduced, indicating that HSPs blocked the expression of pro-inflammatory factors. The high expression of IL-2 induced by heat stress may also be related to HSP70, as IL-2 promotes the activity of cytotoxic T (Tc) cells, while the high expression of HSP70 may stimulate Tc cells to increase the expression of IL-2. As the feeding of GABA mitigated heat stress, the body's physiological status gradually returned to normal, reducing HSP70 expression and IL-2 content in the thymus. Therefore, HSP70 expression can be used as an indicator of changes in the immune function.

## CONCLUSION

Under heat stress, the IL-1, TGF- $\beta$ 1, and GH contents of the thymus of chickens decreased, while IL-2, IFN- $\gamma$ , and HSP70 content increased. Heat stress changed the thymus contents of immune factors, with potential negative effects on the immune functions and

performance of the chickens. GABA can be used as an additive to mitigate the negative effects of heat stress on the immune function and growth performance of chickens, and may have extensive applications in animal production.

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