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Effects of Heat Stress on Somatostatin and Some Related Immune Factors in the Small Intestine of Wenchang Chicks

Zhong Chen, Ying-Ya Jiang, Yong-Wei Zhou, Chen Liang, Li-Jin Xie*

College of Life Sciences, Hainan Normal University, Haikou, China

**Corresponding author: zh.chen@hainnu.edu.cn*

ABSTRACT

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To investigate the effects of heat stress (HS) on developmental changes in immune functions of chick intestinal mucosa, one-day-old broiler chicks were randomly assigned into control check (CK) and heat-stressed (HS) groups and raised under indoor temperature. The chicks in HS group were subjected to HS at 40 ± 0.5°C from 12:00 to14:00 h every day. Intestinal mucosa samples were collected weekly during 6 weeks, and the effects of HS on somatostatin and its related immune factors were examined using immunohistochemical, physiological, and biochemical methods. The results showed that HS obviously increased the amount and integral optical density of somatostatin positive cells, somatostatin content, as well as IFN-γ and IL-2 levels in the small intestine, and these increases reached statistical significance in some intestinal segments ($P < 0.05$). In addition, IgG, IgA, and IgM levels fluctuated in different intestinal segments and their levels in jejunum, duodenum, and ileum in 6-week-old chicks were significantly lower in HS group than in CK group ($P < 0.05$). The contents of immune-related enzymes also fluctuated, but the activities of acid phosphatase, lysozyme, and glutathione reductase in duodenum and jejunum were lower in 6-week-old chicks in HS group than in CK group, some reaching statistical significance ($P < 0.05$). Growth hormone (GH) and HSP70 contents in multiple intestinal segments in 6-week-old chicks were significantly higher in HS group than in CK group ($P < 0.05$). The results indicate that (1) HS could increase the expression and secretion of somatostatin and affect the normal development of immunoglobulins, cytokines, and immune-related enzymes in the small intestine, and thereby impact the chicks' intestine immune function; (2) GH and HSP70 in the small intestine were involved in self-protection mechanisms against HS-induced intestinal injury and somatostatin regulation might be one of the important components.

Keywords: hyperthermia; somatostatin; intestinal mucosa; cytokines; lysozyme activity

Heat stress (HS) has caused great economic losses to the poultry industry with global warming and the development of modern intensive breeding industrialization (St-Pierre et al. 2003). Chickens are warm-blooded animals with strong growth and metabolism. Because chickens are covered with feathers, but do not have sweat glands, they are more susceptible to HS-induced injury. Numerous studies have shown that HS has serious negative impacts on chicken production and immunity (Niu et al. 2009). Intestinal tissue is composed of a group of metabolically active cells and is one of the central stress responsive organs in the body, thus it is the first to suffer from injury during

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stress, and the last to recover. In addition, because it contains a large amount of immune cells and neuroendocrine cells (Liu et al. 2013), it is an ideal organ to study HS mechanisms.

Currently, a large number of studies have shown that HS could impair the structure of small intestine, reduce villus length, mucosa thickness, crypt depth, and wall thickness to different degrees (Santos et al. 2015; Chen et al. 2015a), induce necrosis and shedding of epithelial cells, nudity and edema of lamina propria, fracture and missing of villa, as well as other organic lesions (Quinteiro-Filho et al. 2010). Moreover, HS decreases the digestion and absorption capacity of small intestine by affecting the activities of key enzymes related to intestinal functions, which has been considered as an important cause for degradation of production performance (Chen et al. 2014). However, the effects of HS on the immune functions of small intestine mucosa, an important immune organ, have been barely reported. Somatostatin is a brain-gut peptide with complex physiological functions. It could reduce the nutrients absorption and utilization of the body by direct or indirect involving in regulating many physiological functions, thereby inhibit the growth of animals (Klein and Sheridan 2008). In small intestine, somatostatin may not only inhibit digestive juices secretion, intestinal muscle contraction, and villus motility by binding to its receptor, but also regulate the immune function of small intestine mucosa by affecting secretion and proliferation of immune cells (Ishihara et al. 1999) and participating in inflammatory reactions (Zhu et al. 2007). Therefore, in this paper, we studied the changes in the levels of somatostatin, related intestinal mucosa immune factors, and the activities of enzymes related with the development of chicken under HS on intestinal immune function with the hope to provide new evidences for studies on the physiological mechanisms underlying the effects of HS on chicken production.

MATERIAL AND METHODS

Experimental design. Totally 144 healthy one-day-old male Wenchang chicks (YongJi Live Stock Co. Ltd., Hainan, China) with no significant difference in body weight were randomly assigned into control check (CK) group and heat stressed (HS) group. They were subjected to conventional breeding management with free access to water and feed, meeting the nutrient standards (NRC 1994). During the experiment, chicks were hosted in large cages in the feeding house (7 × 3.5 × 3.5 m) and maintained at 30.6 ± 1.1°C and relative humidity of 65 ± 6%. At 12:00–14:00 h every day, chicks in the HS group were placed in a large-capacity artificial climate chamber with temperature of 40 ± 0.5°C and humidity of 70–80% for heat treatment and chicks in the CK group were placed in the same chamber without HS treatment for 2 h, and then returned to their rearing cages (Chen et al. 2015b). This experiment was conducted with ethics approval from the Hainan Normal University Animal Experimentation Ethics Committee.

Sample collection. Twelve chicks from each group were sacrificed at 15:00 at the end of weeks 1 to 6 after their jugular blood had been taken in a tube containing anticoagulant heparin. The plasma was obtained by centrifugation and stored at –20°C and their duodenum, jejunum, and ileum were dissected. After washed with ice-cold saline, a segment of 0.5 cm was taken and fixed in Bouin's fixative for 15–20 h, washed with 70% ethanol, and prepared as paraffin sections. Another segment of 3–5 cm was cut longitudinally. After rinsed with cold saline and dried with filter paper, the intestinal mucosa was collected by scraping with a razor blade into a centrifuge tube and stored at –20°C for future use.

Immunohistochemical staining. The collected tissues were prepared as 5-μm serial paraffin sections using conventional methods and stained using the SABC method and the DAB kit (Beijing Boisynthesis Biotechnology Ltd., China) as described by the manufacturers to detect the target proteins in chicks. After staining, the sections were sealed with neutral balsam, mounted on slides, and examined under a microscope. Cells with particles stained in tawny were considered positive. In addition, the negative control experiment was also performed.

ELISA and enzyme activity. Intestinal mucosal samples stored at –20°C were weighed and prepared as 10% suspension by adding a 9-fold volume of PBS solution. After centrifugation at 3000 rpm/min for 20 min at 4°C, the supernatant was collected and used to determine the contents of plasma and mucosal somatostatin and its related immune factors (immunoglobulin, IL-2, IFN-γ, growth hormone (GH), and HSP70) using

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the sandwich ELISA method in strict accordance with the manufacturer's instructions (Chicken ELISA kits, Shanghai Yu Ping Biotechnology Ltd., China) and to measure the activities of relevant enzymes (acid phosphatase, lysozyme, and glutathione reductase (GR)) using commercial kits according to the corresponding manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Images and data processing. The immunohistochemically stained sections of each intestinal segment were observed using an Olympus BX50F-3 microscope (Olympus Optical Co. Ltd., Japan). Images of 8–15 different fields of three cross-sections were taken using a digital camera YD400C (Shangdong Yichuang Electronics Ltd., China), saved, and analyzed using the Image-Pro Plus 6.0 analysis software. The amount and integral optical density (IOD) of somatostatin-positive cells were counted and measured, respectively.

Differences between different groups were statistically analyzed using ANOVA analysis and differences among multiple datasets were compared using Duncan's method with SPSS software (Version 17.0, 2008). *P*-values < 0.05 were considered statistically significant.

RESULTS

Changes in somatostatin content. As shown in Figure 1, somatostatin immunoreactive cells had tan granular particles and were round. These cells were present in all segments of small intestine, showing the highest amount in duodenum, followed in turn by jejunum and ileum. Meanwhile, not tawny granules were seen in the negative control. The amount of somatostatin immunoreactive cells gradually lowered with the weeks of age increasing. Compared with that of CK group, the amount of

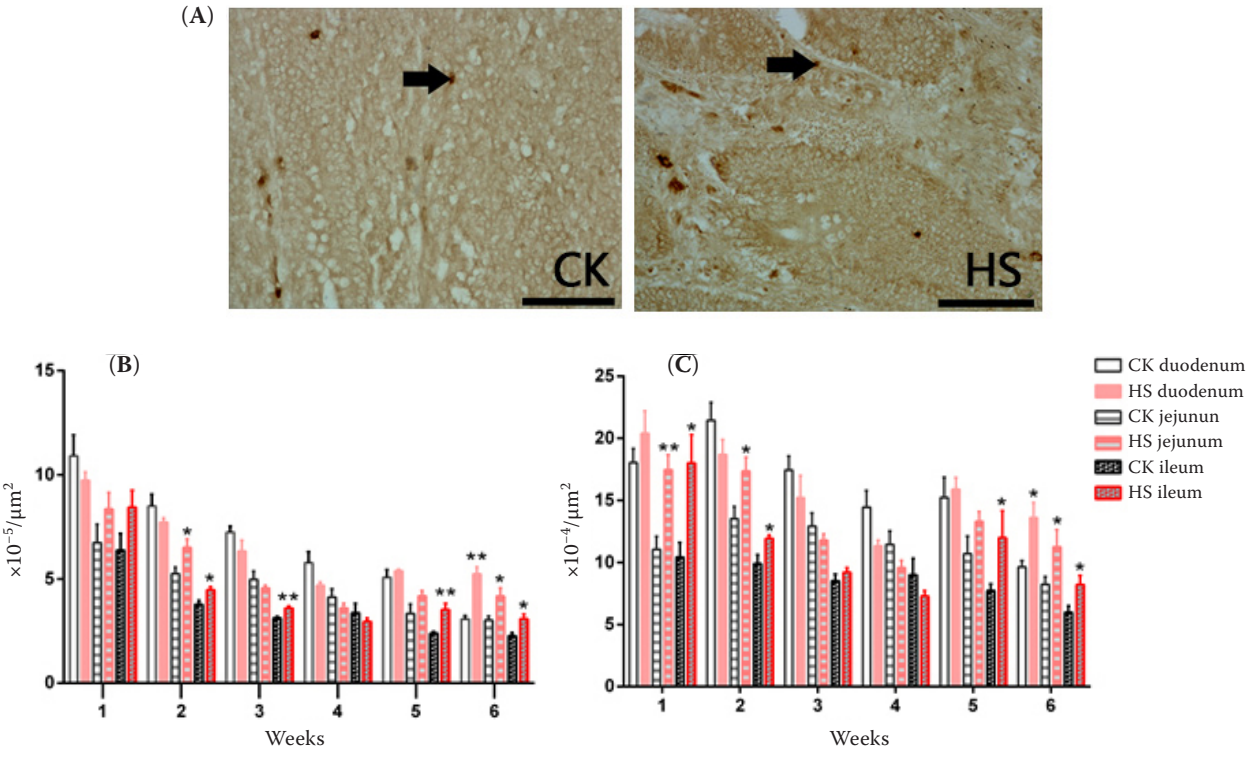

Figure 1. Effects of heat stress on somatostatin expression in the small intestine of heat stressed (HS) and control (CK) groups of chicks

(**A**) immunostaining images showing the distribution of somatostatin positive cells in the intestine of chicks, magnification 400×, bar = 50 μm; (**B**) distribution of somatostatin positive immune cells in different sections of chicks' small intestine after different time of treatment; (**C**) integral optical density of somatostatin positive immune cells in different sections of chicks' small intestine after different time of treatment, arrows indicate positive cells
significant difference between chicks at the same age in HS and CK groups (*$P < 0.05$, **$P < 0.01$)

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Table 1. Effects of heat stress on the somatostatin level in chicks (pg/ml, n = 6)

Items	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	SEM
Plasma	CK	156.38^{ab,B}	141.43^{b}	158.00^{ab}	183.81^{a}	154.13^{ab}	159.53^{ab}	10.56
	HS	191.29^{a,A}	130.83^{c}	167.17^{ab}	158.92^{b}	178.15^{ab}	178.39^{ab}	7.51
	P-value	groups: NS		weeks: 0.003		groups × weeks: 0.022		
Duodenum	CK	258.80^{a}	228.00^{ab}	230.58^{ab}	189.88^{b}	237.61^{ab}	230.11^{ab}	14.69
	HS	287.66^{a}	268.49^{ab}	240.49^{bc}	214.65^{c}	244.33^{bc}	240.19^{bc}	12.18
	P-value	groups: 0.017		weeks: < 0.001		groups × weeks: NS		
Jejunum	CK	81.74^{c,B}	82.31^{c}	90.10^{bc}	119.32^{a}	85.39^{c,B}	109.34^{ab}	6.83
	HS	198.70^{a,A}	112.50^{b}	105.28^{b}	125.16^{b}	111.37^{b,A}	128.01^{b}	9.11
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: < 0.001		
Ileum	CK	112.50^{c,B}	137.44^{bc,B}	124.80^{bc}	141.73^{bc,B}	152.69^{b}	229.39^{a,B}	10.99
	HS	127.60^{d,A}	164.71^{cd,A}	143.55^{cd}	226.47^{b,A}	176.43^{c}	286.00^{a,A}	12.73
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: 0.037		

CK = control group, HS = heat-stressed group, Week = week of age, NS = not significant
in the same row, data marked with the same superscripts or without lowercase superscripts are not significantly different ($P > 0.05$), while data marked with different lowercase superscripts are significantly different ($P < 0.05$); in the same column, data marked with A and B are significantly different ($P < 0.05$) in chicks at the same age between CK and HS groups, NS = $P > 0.05$

somatostatin immunoreactivity positive cells in HS group increased in 2-week-old jejunum and ileum, 3-week-old ileum, 5-week-old ileum as well as 6-week-old duodenum, jejunum, and ileum (Figure 1B). Their IOD values also increased in 1-week-old jejunum and ileum, 2-week-old jejunum and ileum, 5-week-old ileum, and 6-week-old duodenum, jejunum, and ileum of chicks in HS group (Figure 1C).

As shown in Table 1, the plasma somatostatin content oscillated around 155 pg/ml in CK group, reaching its maximum in 4-week-old chicks, and around 170 pg/ml in HS group. Moreover, compared with that of chicks in CK group, blood somatostatin content was higher in 1-week-old chicks in HS group ($P < 0.05$), but lower in 2- and 4-week-old chicks in HS group, then higher again in 5- and 6-week-old chicks in HS group. There was an interaction between groups and ages ($P < 0.05$). Among different segments of small intestine, somatostatin content was the highest in duodenum, followed by ileum and jejunum. Somatostatin content decreased in duodenum, but increased in jejunum and ileum with weeks of age increasing,

Table 2. Effects of heat stress on acidic phosphatase activity in the small intestine of chicks (U/g protein, n = 6)

Items	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	SEM
Duodenum	CK	138.05^{a}	159.21^{a}	152.46^{a,B}	132.59^{a}	90.62^{b}	127.91^{a,A}	10.56
	HS	132.99^{bc}	172.36^{ab}	215.09^{a,A}	159.49^{b}	106.11^{cd}	77.67^{d,B}	13.97
	P-value	groups: NS		weeks: < 0.001		groups × weeks: 0.004		
Jejunum	CK	126.38	122.93	145.42	139.79	153.22	136.40	15.92
	HS	89.61	98.63	125.60	125.94	130.09	120.20	11.62
	P-value	groups: 0.011		weeks: NS		groups × weeks: NS		
Ileum	CK	19.10^{b}	17.96^{b}	29.61^{b}	38.49^{b}	38.63^{b,B}	84.83^{a,B}	8.71
	HS	17.28^{c}	12.26^{c}	55.69^{b}	50.83^{b}	59.52^{b,A}	154.94^{a,A}	7.20
	P-value	groups: 0.001		weeks: < 0.001		groups × weeks: 0.003		

CK = control group, HS = heat-stressed group, Week = week of age, NS = not significant
in the same row, data marked with the same superscripts or without lowercase superscripts are not significantly different ($P > 0.05$), while data marked with different lowercase superscripts are significantly different ($P < 0.05$); in the same column, data marked with A and B are significantly different ($P < 0.05$) in chicks at the same age between CK and HS groups, NS = $P > 0.05$

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especially showing a dramatic increase in ileum. Somatostatin content in jejunum and ileum was higher in HS group chicks than in CK group chicks of the same age ($P < 0.05$). The interaction between groups and ages was significant in jejunum and ileum ($P < 0.05$).

Changes in immunoglobulin, IL-2, and IFN-γ contents. As shown in Figure 2, IgG content was higher in intestinal mucosa, while IgM content was the lowest. In addition, although IgG, IgA, and IgM contents fluctuated in different intestinal segments of chicks at different ages, they all similarly increased with weeks of age increasing. At the first week of age, IgG content in all intestinal segments was higher in HS group than in CK group, but especially in jejunum, IgG content in HS group was significantly lower than that in CK group from 2 weeks of age ($P < 0.05$). In addition, IgG content in ileum of 4-week-old chicks was also significantly lower in HS group than in CK group ($P < 0.05$).

Compared with that of CK group, IgA content in duodenum and jejunum was slightly higher in chicks at the early development stage in HS group. However, its content showed a downward trend with weeks of age increasing, reaching the same level to that in CK group in 6-weeks-old chicks, and was even significantly lower than that in CK group in duodenum ($P < 0.05$). HS decreased IgA content in ileum, and its level in 1-week-old chicks and 6-week-old chicks was significantly lower than that in CK group ($P < 0.05$). HS significantly

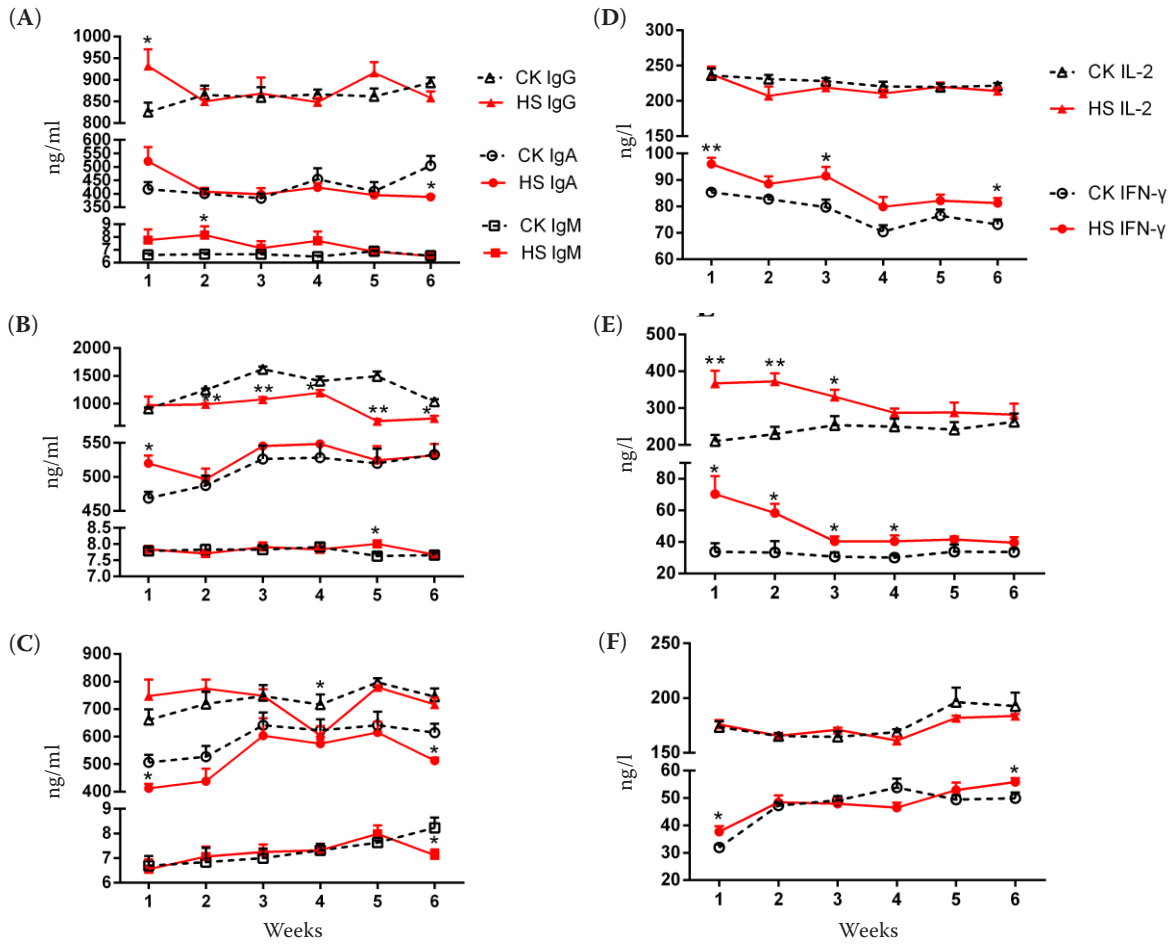

Figure 2. Effects of heat stress on the change of immunoglobulin and cytokines levels in the small intestine of heat stressed (HS) and control (CK) groups of chicks

(**A–C**) content of immunoglobulin in duodenum, jejunum, ileum, respectively; (**D–F**) content of IL-2 and IFN-γ in duodenum, jejunum, ileum, respectively. In accordance with the kit manufacturer's requirements, sample was prepared as 10% suspension

significant difference between chicks at the same age in HS and CK groups (*$P < 0.05$, **$P < 0.01$)

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Table 3. Effects of heat stress on lysozyme activity in the small intestine of chicks (U/mg protein, n = 6)

Items	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	SEM
Duodenum	CK	11.28^{c}	12.77^{c}	9.57^{c}	24.47^{a}	18.78^{b,A}	22.78^{ab,A}	1.24
	HS	9.58^{bc}	12.71^{bc}	7.81^{c}	19.41^{a}	13.75^{b,B}	12.05^{bc,B}	1.53
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: 0.021		
Jejunum	CK	30.72	29.75	27.69^{A}	24.13	27.57	29.52^{A}	1.80
	HS	28.48^{a}	27.33^{ab}	17.94^{c,B}	20.68^{bc}	25.81^{ab}	22.97^{abc,B}	2.13
	P-value	groups: 0.001		weeks: 0.004		groups × weeks: NS		
Ileum	CK	18.70	11.31	17.09	17.47^{B}	10.85	7.32	3.00
	HS	20.76^{ab}	18.03^{ab}	19.85^{ab}	25.64^{a,A}	12.36^{b}	12.02^{b}	3.01
	P-value	groups: 0.025		weeks: 0.001		groups × weeks: NS		

CK = control group, HS = heat-stressed group, Week = week of age, NS = not significant
in the same row, data marked with the same superscripts or without lowercase superscripts are not significantly different (P > 0.05), while data marked with different lowercase superscripts are significantly different (P < 0.05); in the same column, data marked with A and B are significantly different (P < 0.05) in chicks at the same age between CK and HS groups, NS = P > 0.05

increased IgM level in duodenum of 2-week-old chicks (P < 0.05) and jejunum of 5-week-old chicks (P < 0.05), but significantly reduced it in ileum of 6-week-old chicks (8.23 vs 7.12, P = 0.050). IL-2 content fluctuated slightly, and showed significant difference in jejunum among 1, 2, 3-week-old chicks in HS group (P < 0.05). Compared with that in CK group, IFN-γ content in all intestinal segments showed varying degrees of increase in HS group, being significant in multiple segments (P < 0.05).

Changes in enzyme activities. Acid phosphatase, lysozyme, and GR exhibited some spatial specificity in different intestinal segments. As shown in Table 2, compared with CK group, HS increased acid phosphatase activity in duodenum and ileum, which was significant in duodenum of 3-week-old chicks (P < 0.05) and in ileum of 5- and 6-week-old chicks (P < 0.05), but it gradually decreased in duodenum of chicks older than 3 weeks, reaching significance in 6-week-old chicks (P < 0.05); the interaction between groups and ages was significant in duodenum and ileum (P < 0.05).

As shown in Table 3, HS decreased lysozyme activity in duodenum and jejunum, which reached significance in duodenum of 5- and 6-week-old chicks and in jejunum of 3- and 6-week-old chicks (P < 0.05). But HS increased lysozyme activity in ileum, reaching a significant level in 4-week-old

Table 4. Effects of heat stress on glutathione reductase activity in the small intestine of chicks (U/g protein, n = 6)

Items	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	SEM
Duodenum	CK	6.55^{ab}	4.24^{b}	8.16^{a,A}	8.06^{a,A}	6.19^{ab}	6.31^{ab}	0.76
	HS	4.27^{ab}	3.90^{b}	4.53^{ab,B}	5.18^{ab,B}	5.89^{a}	5.81^{a}	0.56
	P-value	groups: < 0.001		weeks: 0.006		groups × weeks: NS		
Jejunum	CK	12.90	12.33	9.77	9.05^{A}	10.10	10.58^{A}	1.14
	HS	9.92^{a}	8.80^{ab}	7.89^{ab}	6.56^{b,B}	8.38^{ab}	7.83^{ab,B}	0.87
	P-value	groups:< 0.001		weeks: 0.039		groups × weeks: NS		
Ileum	CK	11.93^{bcd}	7.65^{d}	16.34^{ab}	14.90^{abc}	10.37^{cd,A}	17.73^{a}	1.62
	HS	14.81^{a}	14.07^{a}	12.08^{ab}	11.47^{ab}	5.21^{b,B}	15.60^{a}	2.52
	P-value	groups: NS		weeks: 0.005		groups × weeks: NS		

CK = control group, HS = heat-stressed group, Week = week of age, NS = not significant
in the same row, data marked with the same superscripts or without lowercase superscripts are not significantly different (P > 0.05), while data marked with different lowercase superscripts are significantly different (P < 0.05); in the same column, data marked with A and B are significantly different (P < 0.05) in chicks at the same age between CK and HS groups, NS = P > 0.05

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Table 5. Effects of heat stress on growth hormone content in the small intestine of chicks (μg/l, n = 6)

Items	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	SEM
Duodenum	CK	$16.59^{b,B}$	$17.23^{b,B}$	17.05^{b}	16.16^{bB}	$16.94^{b,B}$	22.95 ± 0.97^{a}	0.91
	HS	$25.66^{ab,A}$	$27.39^{a,A}$	21.07^{ab}	$18.31^{b,A}$	$22.18^{ab,A}$	24.37 ± 1.39^{ab}	1.89
	P-value	groups: < 0.001		weeks: 0.009		groups × weeks: NS		
Jejunum	CK	11.64^{c}	$12.91^{bc,B}$	$15.60^{bc,B}$	$13.40^{bc,B}$	$18.79^{b,B}$	31.33 ± 3.51^{a}	2.12
	HS	12.12^{c}	$21.36^{b,A}$	$22.94^{b,A}$	$20.30^{b,A}$	$25.85^{b,A}$	46.84 ± 5.87^{a}	2.28
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: NS		
Ileum	CK	12.12^{c}	$12.38^{c,B}$	17.66^{bc}	18.98^{b}	$25.32^{a,B}$	29.54 ± 2.15^{a}	2.05
	HS	16.34^{c}	$20.04^{bc,A}$	22.15^{bc}	26.38^{b}	$33.77^{a,A}$	36.41 ± 2.27^{a}	2.22
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: NS		

CK = control group, HS = heat-stressed group, Week = week of age, NS = not significant

in the same row, data marked with the same superscripts or without lowercase superscripts are not significantly different ($P > 0.05$), while data marked with different lowercase superscripts are significantly different ($P < 0.05$); in the same column, data marked with A and B are significantly different ($P < 0.05$) in chicks at the same age between CK and HS groups, NS = $P > 0.05$

chicks ($P < 0.05$). There was an interaction between groups and ages only in duodenum ($P < 0.05$).

As shown in Table 4, HS reduced GR activity in duodenum and jejunum, reaching significance in duodenum of 3- and 4-week-old chicks and in jejunum of 4- and 6-week-old chicks ($P < 0.05$). By comparison, HS decreased GR activity in ileum of chicks older than 3 weeks, reaching significance in 5-week-old chicks ($P < 0.05$). There was no interaction between groups and ages ($P > 0.05$).

Changes in GH and HSP70 contents. As shown in Table 5, GH contents in different intestinal segments of chicks increased with their age increasing and were the highest in 6-week-olds. Compared with CK group, HS increased GH content, showing significant differences in different intestinal segments of chicks at different age ($P < 0.05$). There was no interaction between groups and ages ($P > 0.05$).

As shown in Table 6, HSP70 content was significantly higher in duodenum than in other segments. With age increasing, HSP70 level decreased in duodenum, but increased in jejunum and especially in ileum, where it increased more dramatically. Compared with CK group, HS enhanced the HSP70 level in all intestinal segments, showing significant differences in different intestinal segments of chicks at different age ($P < 0.05$). There

Table 6. Effects of heat stress on HSP70 content in the small intestine of chicks (ng/l, n = 6)

Items	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	SEM
Duodenum	CK	366.22^{a}	$319.55^{bc,B}$	328.80^{b}	307.22^{bc}	$297.12^{c,B}$	$266.40^{d,B}$	8.28
	HS	382.63^{a}	$345.77^{b,A}$	361.29^{ab}	332.48^{b}	$338.34^{b,A}$	$288.23^{c,A}$	10.61
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: NS		
Jejunum	CK	$80.19^{c,B}$	$84.64^{c,B}$	$89.63^{bc,B}$	$89.99^{bc,B}$	121.69^{ab}	135.88^{a}	9.99
	HS	$112.25^{c,A}$	$111.36^{c,A}$	$137.71^{ab,A}$	$130.06^{bc,A}$	157.66^{a}	153.74^{ab}	7.75
	P-value	groups: < 0.001		weeks: < 0.001		groups × weeks: NS		
Ileum	CK	95.77^{d}	$186.68^{c,B}$	215.20^{bc}	255.27^{b}	319.38^{a}	$339.41^{a,B}$	16.18
	HS	106.46^{d}	$263.28^{c,A}$	231.23^{c}	267.29^{c}	355.44^{b}	$479.65^{a,A}$	18.64
	P-value	groups: 0.001		weeks: < 0.001		groups × weeks: 0.046		

CK = control group, HS = heat-stressed group, Week = week of age, NS = not significant

in the same row, data marked with the same superscripts or without lowercase superscripts are not significantly different ($P > 0.05$), while data marked with different lowercase superscripts are significantly different ($P < 0.05$); in the same column, data marked with A and B are significantly different ($P < 0.05$) in chicks at the same age between CK and HS groups, NS = $P > 0.05$

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was an interaction between groups and ages only in ileum ($P < 0.05$).

DISCUSSION

Many scholars have studied the shape and distribution of somatostatin positive cells within the avian gastrointestinal tract (Gulmez et al. 2003). However, because of differences in species or experimental methods, their results on the distribution and development of somatostatin positive cells in different segments of digestive tract vary. For example, in *Chrysolophus pictus,* the density of somatostatin immunoreactive cells is higher in small intestine than in duodenum and jejunum (Li and An 2009); in *Phasianus colchica*, its density is the highest in ileum, followed by jejunum and duodenum and is rare in rectum (Li 2011); in goose, the type and amount of somatostatin immunoreactive cells decreased gradually from the front segments to the back segments of intestine (Deng et al. 1996); in Chinese Yellow Quail, somatostatin positive cells were distributed mostly in proventriculus, followed in turn by duodenum, jejunum, ileum, and rectum (Li et al. 2013). Our results showed that the density of somatostatin positive cells was higher in duodenum than in jejunum and ileum, which is consistent with the digestion and absorption characteristics of poultry intestine, because duodenum is the main site for digestion and absorption while other segments are the main sites for water absorption and undigested substances discharge. The gradual decrease in abundance of somatostatin positive cells may facilitate the full digestion and absorption of food in different segments of intestinal tract, indicating that there are some similarities in the distribution of somatostatin positive cells in small intestine between chick and other poultry and differences among species. The reasons for these phenomena remain to be further studied. Integral optical density (IOD) is the sum of optical density of each somatostatin positive cell multiplying its area. It is proportional to the total amount of the target substance, and a relatively reliable and stable semi-quantitative indicator. Our results showed that the IODs of somatostatin positive cells in different intestinal segments were higher in chicks of HS group than of CK group, indicating that HS increased total intestine somatostatin in chicks. Somatostatin could inhibit the release of anterior pituitary GH, which is the key hormone promoting animal growth. HS could significantly increase the amount of somatostatin in intestine. In addition, one week of HS significantly increased the somatostatin level in HS chicks blood ($P < 0.01$ compared with CK), resulting in body's negative feedback regulation and subsequent fluctuation of somatostatin and GH contents in blood. But the overall somatostatin and GH contents were slightly higher in HS group than in CK group, indicating that HS increased the overall body somatostatin content in chicks.

Like functional changes in the entire immune system, changes in immunoglobulin during HS showed a clear time-dependent trend: the immunoglobulin expression was enhanced at the beginning of HS, and then it was gradually suppressed. The levels of IgG, IgA, and IgM in the small intestine of 6-week-old chicks in the HS group were no longer higher than those in CK group. This may be related to the changes in hypothalamic–pituitary–adrenal axis (Chrousos 1992). At the initial stage of HS treatment, the increased level of immunoglobulins in the intestinal immune system of chicks is conducive to improve the chicks' immune system and resistance to HS. However, prolonged HS damages intestinal mucosa and subsequently leads to decreased intestinal immune function (Chen et al. 2014). The above results indicate that HS stimulates the expression and secretion of somatostatin in small intestine. Because somatostatin has broad immunosuppressive effects including suppressing immunoglobulin synthesis, changes in immunoglobulin levels in small intestine may be also related with a varying somatostatin content. IL-2 and IFN-γ belong to Th1 cytokines and are pyrogenic factors to increase body temperature through acting on hypothalamus (Bateman et al. 1989). IL-2 is a lymphokine secreted by antigen stimulated T-lymphocytes. It can maintain T/B lymphocyte proliferation and differentiation, and improve the activity of natural killer cells, thus it plays an important role in the regulation of immune response system (Boyman and Sprent 2012). IFN-γ could downregulate IgA secretion, a defensive substance that body produces to defend viral invasion, maintain organisms' and cells' self-stabilization (Schoenborn and Wilson 2007). Our results showed that HS increased the contents of IL-2 and IFN-γ, indicating that both IL-2 and IFN-γ were involved in the body's response to HS.

Acid phosphatase activity reached maximum in 3-week-old chicks after HS, then began to decrease, indicating that HS could increase acid phosphatase activity to a certain degree. However, this increase is not sustainable probably because HS activates the existing enzymes in the organism. Once the enzyme is activated, to protect itself, the body ini-

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tiates its feedback suppression system, leading to rapid decrease in the enzyme activity after reaching a certain level. As a biological feedback mechanism, it avoids excessive consumption of the body and stores enough enzymes to protect the body from next pathogen attack.

Lysozyme, as an important non-specific immune factor, is the material basis of phagocytic cells to kill bacteria. It can (1) reduce the release of bacterial endotoxin (Virnik et al. 1998) and decompose peptidoglycan of microbial cell wall, causing rupture of microbial cell wall and leading to the release of cell contents and cell dissolving and death (Vocadlo et al. 2001); (2) enhance phagocytic ability of macrophages and leukocytes (Krusteva et al. 1997). Therefore, lysozyme has antibacterial, anti-inflammatory, and anti-viral functions, and could improve immune function, so as to assume the responsibility of body's defenses. Our results showed that HS decreased lysozyme activity in duodenum and jejunum, indicating that HS suppressed the intestinal mucosal immune function in chicks.

Under HS, the metabolic process of the body is dysregulated and accelerated, leading to increased generation of free oxygen radicals, decreased antioxidant capacity, reduced efficiency of free radical scavenging by antioxidant enzymes, accumulation of free radical species, and occurrence of lipid peroxidation (Arnaud et al. 2002). GR as an important enzyme of glutathione antioxidant enzyme system may synergically function with NADPH and plays an important role in removing endogenous free radicals, maintaining normal metabolism of free radicals and cell membrane integrity, and improving antioxidant capacity of the body (Elia et al. 2006). Somatostatin could maintain the level of non-protein thiol group in small intestine mucosa through GR, thereby preventing the occurrence of lipid peroxidation and subsequently protecting cells and intestinal mucosa from damages due to external stimuli (Wang 1984). Our results showed that HS increased somatostatin level, while decreased the GR activity in small intestine. Therefore, somatostatin cannot effectively play its protective role in intestinal mucosal cells, leading to declined antioxidant capacity and immune ability of the body.

GH can promote the proliferation of intestinal epithelial cells, increase glutaminase activity in intestinal mucosal cells, and enhance glutamine uptake and utilization, therefore facilitate the repair of intestinal mucosa (Xie et al. 2001). Our results showed that HS increased the GH content in small intestine, indicating that a self-protection mechanism has been launched in the intestine of chicks to repair the damage of intestinal mucosa through GH. HSP70 is the most conservative heat shock protein family. Thus, its expression is an important indicator to evaluate HS. HSP70 can improve stress resistance, thermal endurance, and proliferation of cells, and its level is positively correlated with heat resistance capacity (Bemardini et al. 2004). In addition, studies have shown that HSP70 can increase antioxidant capacity and inhibit lipid peroxidation to protect the intestinal mucosa damage due to HS (Gu et al. 2012). Our results showed that HS increased the HSP70 content in small intestine, indicating that HS could induce the HSP70 expression and increase the body's heat resistance, and thus protect the chick intestinal tract from damages caused by HS.

CONCLUSION

HS significantly increased the expression and secretion of somatostatin in chick intestine and eventually led to the decreased content of immunoglobulins and decreased activity of acid phosphatase, lysozyme, and glutathione reductase in chick small intestine. In addition, HS evoked inflammation and improved the content of IFN-γ and IL-2 in chick small intestine, thus affecting normal development of the intestinal immune function. Moreover, GH and HSP70 of small intestine are involved in the self-protection mechanisms of intestine against HS-induced damages, where somatostatin regulation may be one of significant aspects.

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