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Neuroendocrine Responses to Cold Stress in Chinese Indigenous Breeds from Different Latitude

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Abstract: It is well established that stress is related to neurochemical and hormonal changes including alterations in adrenal and thyroid hormone levels. In the present study, thyroid axis and hypothalamic-pituitary-adrenal axis related hormomal, Triiodothyronine (T3), Thyroxine (T4), Thyroid-Stimulating Hormone (TSH), Adreno-Cortico-Tropic-Hormone (ACTH) and Thyroid Releasing Hormone (TRH) mRNA, ACTH mRNA changes during cold stress with different intensities were recorded in two Chinese indigenous breeds from different latitude. The results showed that Huainan partridge chicken from central China, showed obvious conversion in T3 and T4 under severe cold stress. Plasma TSH and pituitary TRH mRNA highly expressed over 12-24 h long time severe cold exposure. However, mild cold did not obviously affect those hormones mentioned above. In the Wenchang chicken which from south of China, severe and mild cold stress showed the same affection on the thyroid axis but not obvious on the adrenal axis. The results would further help the revealing of the mechanism of injury caused by cold stress and provide better controls for chicken from loss of cold stress in different areas.

Key words: Adrenal axis, thyroid axis, Chinese indigenous breed, cold stress, China

INTRODUCTION

Ambient temperature below the thermoneutral zone, lead to elevated core temperature and consequently initiate a number of responses leading to the neutralization of the metabolic changes (Selamoglu and Yurekli, 2005; Colinet *et al*., 2010).

The diverse sets of behavioral, autonomic and neuroendocrine adaptations evoked by stressful stimuli serve to maintain homeostasis and enhance survival in the face of a threat or challenge, either real or perceived (Ma *et al*., 2008; Haman *et al*., 2002; Matteri *et al*., 2000; Tikuisis *et al*., 1999). However, prolonged or excessive activation of the stress response itself can become maladaptive, contributing to the negative health consequences associated with stress (Shinder *et al*., 2002; Hangalapura *et al*., 2006; Hirabayashi *et al*., 2005; Yuksel *et al*., 2008).

Chronic and acute cold exposures are among the stressing factors affecting the metabolism and causing affection on poultry development and production (Shinder *et al*., 2007). Activation of the neuroendocrine system represents the major neuroendocrine responses to acute cold stress. Many have focused on the changes of the neuroendocrine system (Hypothalamic-pituitary-thyroid axis and hypothalamic-pituitary-adrenal) which would induce a cascade reactions affected immune responses, metabolic changes, deranged electrolyte balance etc. (Hangalapura *et al*., 2004; Collin *et al*., 2005; Mujahid and Furuse, 2008, 2009).

Huainan partridge chicken (H) oriented from southeast of China normally bred under an average temperature of 12.3°C and regularly facing chilling at night. Wenchang chicken (W), oriented from South of China, bred under an average temperature of 21.2°C with relatively smaller day and night temperature difference. It is supposed that species from two localities of different latitudes, perform differentially under severe cold stress. If it is positive, the fluctuation of neuroendocrines synthesis and secretion might be higher in populations from relatively heat climates when face to cold stress. In this study, neuroendocrine responses to cold stress were compared in two chicken breeds from different latitudes. The results might provide meaningful information for breeders in coping with reversed environment.

MATERIALS AND METHODS

Bird management: The stocks used in the experiment were physically healthy and unrelated male Huainan partridge chickens (n = 400) and Wenchang chickens (n = 400), Chinese indigenous breeds with 6 weeks age

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Table 1: Serum T3 content of two breeds under different cold stress

		Cold stress time (h)									
Breed	Tem	0	0.5	1	2	3	6	12	24	72	144
W	2°C	5.07±1.39	2.97±1.48	5.89±2.04	5.14±0.88	4.91±1.23	3.78±1.05	3.38±1.08	2.44±0.99*	5.23±1.48	5.14±1.56
	10°C	5.07±1.39	3.56±0.68	4.20±1.11	4.92±1.23	4.24±0.90	3.21±0.57	4.75±1.06	2.45±0.95*	5.00±1.37	5.14±1.55
H	2°C	3.74±1.45	5.50±2.35	5.60±2.40	6.49±1.61*	6.21±1.40*	4.26±1.50	5.37±1.62	1.89±0.78	4.08±1.27	5.44±1.84
	10°C	3.74±1.45	2.81±0.93	5.42±2.29	5.11±1.68	4.14±0.72	3.79±1.88	4.55±0.78	1.65±0.29	2.82±1.36	6.11±1.26

W: Wenchang chicken; H: Huainan partridge chicken

obtained from Feixi the Old Hens Farming Co., Ltd. This study was conducted on the commercial farm using 1 part of a house. This part was divided into 6 floor pens equipped with nipple drinkers, artificial temperature control system and rice husk was used as floor bedding. Water and an unmedicated corn-soy-based diet that met the NRC requirements were provided *ad libitum*.

Chickens were allowed to acclimatize to the animal facility for 3 days before used in any experimental procedures. On the 1st day of the experiment, six experimental pens (two replicates in each breed) temperature was suddenly decreased from 20±2 to 2±2°C (exposed chickens to 2±2°C directly) and the other two (control group in each breed) maintained unchanged in another coop. Three replicated groups in each breed were subjected to cold stress for one of the following time periods: 0.5, 1, 2, 3, 6, 12, 24, 72 and 144 h. To regulate the temperature, the thermometer was monitered at the centre and four sides in each pen. During cold stress, birds were provided access to a commercial broiler feed and water *ad libitum*.

When cold stress period was over, 6 birds were humanely euthanized by cervical dislocation in each time for sampling. Blood samples (3 mL) were obtained, transferred into blood collection tubes containing heparin anti-coagulant (50 IU mL^{-1}) and immediately chilled on ice. The plasma samples for subsequent protein determination were obtained following centrifugation of whole blood at 3500 rpm for 10 min at 4°C after 20 min water bath at 37°C and stored at -20°C. The pituitaries from broilers were quickly dissected and placed into 1.5 mL tubes with 0.5 mL RNAlater All the experimental procedures followed the guidelines of the regional Animal Ethics Committee.

Serum biochemical measurement: Triiodothyronine (T3), Thyroxine (T4) and Thyroid-Stimulating Hormone (TSH) were determined by ROCHE E2010/ABBOTT I2000 automatic biochemical analyzer and related testing kits. Adreno-Cortico-Tropic-Hormone (ACTH) was determined by Immulite DPC 2000 using chemiluminescence and related testing kit.

Total RNA extraction: Samples were dissolved in TRIZOL reagent (Invitrogen Corporation, Carlsbad, CA) for total RNA extraction and separated electrophoretically on agarose gels under denaturing conditions in order to confirm the integrity of ribosomal RNA bands.

RT-PCR analysis: Single-strand cDNA synthesis was carried out from 1 mg of total RNA by the Reverse Transcription (RT) reaction. After denaturation at 70°C for 10 min, RNA samples were incubated in 1×PCR buffer (10 mM Tris-HCl, pH 9.5, 50 mM KCl, 0.1% Triton X-100), 2.5 mM $MgCl_2$, 1 mM dNTPs mix, 500 ng oligo (dT) primer and 200 U SuperScriptII enzyme (Gibco/BRL, Gaithersburg, Inc.) in a final volume of 20 μL. This reaction was maintained at 42°C for 50 min and subsequently incubated with 1 U of RNase H for 20 min at 37°C for RNA template digestion. Amplification reactions were conducted using the sense and antisense primers shown in Table 1. PCRs were performed with 10% of total reverse transcription reaction volume, 0.5 mM specific primers (Table 1), 1×PCR buffer (10 mM Tris-HCl, 1.5-2.5 mM $MgCl_2$, 50 mM KCl) and 1 U Taq DNA polymerase (Takara). These reactions were denatured at 94°C for 30 sec followed by annealing for 40 sec and an extension at 72°C for 40 sec. Annealing temperatures of GAPDH and ACTH, Thyroid Releasing Hormone (TRH) genes was 61°C in a total of 25 cycles.

Statistical analysis: Data was analysed using the Proc GLM procedure of SAS (SAS 9.1, 2001). The data on serum enzymatic and biochemical were grouped and expressed as mean pooled standard errors of mean. Data (mean±SD) were considered to be statistically significant when the value of $p<0.05$ or 0.01. Two-factor analysis of variance was used to compare the differences between different groups.

RESULTS AND DISCUSSION

Serum T3, T4, TSH and ACTH content of two breeds during cold stress: The T3 content rapidly rise to a high level after 2 h severe cold stress and maintained to 3 h in H and then, decreased to normal level. In the W, 24 h severe cold stress caused T3 decreasing to a significantly low level. When exposed to 10°C cold stress, there was no obvious T3 change in the H and a significant decrease of T3 after 24 h cold exposure in W (Table 1).

Table 2: Serum T4 content of two breeds under different cold stress

		Cold stress time (h)									
Breed	Tem.	0	0.5	1	2	3	6	12	24	72	144
W	2°C	20.39±4.54	22.35±2.36	16.39±1.93	13.98±2.86*	13.28±1.44*	12.05±1.31*	18.68±4.98	18.34±4.24	20.56±4.27	19.76±4.00
	10°C	20.39±4.54	20.84±3.04	18.84±2.97	16.46±1.31	15.30±2.77	17.57±6.13	15.03±4.32	17.52±3.53	18.12±2.97	22.97±4.28
H	2°C	19.19±4.15	32.62±4.85*	27.37±2.21	24.10±2.61	25.23±3.23	28.00±3.05	24.17±4.90	25.63±5.76	22.07±5.20	21.47±9.85
	10°C	19.19±4.15	22.81±4.47	19.12±2.61	15.87±1.28	19.51±2.51	20.68±3.88	17.85±6.66	20.76±2.07	21.09±3.54	21.94±4.23

W: Wenchang chicken; H: Huainan partridge chicken

Table 3: ACTH mRNA expression of the two breeds during cold stress

		Cold stress time (h)									
Breed	Tem.	0	0.5	1	2	3	6	12	24	72	144
W	2°C	1	0.49	0.42	1.24	1.08	0.29	0.90	0.76	0.65	1.47
	10°C	1	0.30	0.02	0.25	0.64	0.59	0.90	0.67	0.48	0.51
H	2°C	1	0.62	0.56	1.24	1.31	1.31	1.76	3.21	2.64	1.29
	10°C	1	2.01	0.43	1.25	1.43	1.25	2.57	2.12	1.02	1.77

W: Wenchang chicken; H: Huainan partridge chicken

Table 4: TRH mRNA expression of the two breeds during cold stress

		Cold stress time (h)									
Breed	Tem.	0	0.5	1	2	3	6	12	24	72	144
W	2°C	1	1.72	2.65	1.52	1.50	2.27	7.50	2.99	1.00	1.52
	10°C	1	1.03	1.11	1.43	3.08	1.29	1.00	0.91	0.83	1.78
H	2°C	1	0.87	0.72	0.81	1.07	0.79	2.31	1.80	0.58	0.49
	10°C	1	0.69	0.82	1.45	0.94	0.81	0.72	0.92	0.71	0.87

W: Wenchang chicken; H: Huainan partridge chicken

Severe cold stress caused rapid rise in 0.5 h of the serum T4 content and no obvious change under 10°C cold stress in H. In the W, severe cold stress caused a sustained decreasing content of T4 during 3-6 h and then rise to normal content (Table 2).

TRH and ACTH expression level from pituitary: ACTH mRNA expression was quantified during cold stress in the two breeds (Table 3). The ACTH mRNA expression level was not affected until 12-72 h long time severe cold stress and 12-24 h 10°C cold stress in H. In the W, rapid decreasing of ACTH mRNA expression was recorded during 0.5-1 and 6 h under severe cold stress. When the plasma level of ACTH and TSH decreased to a relatively low level, it could not be detected using the method so, the data does not shown in the text. However, Plasma ACTH and TSH secretion levels showed the same trend as to the mRNA expression level.

A sustained high expression of TRH mRNA was observed during the whole severe cold stress process and 10°C cold stress caused 3 h and 6 days high expression of TRH mRNA in W. In the H breed, severe cold stress caused high expression of TRH mRNA during 12-24 h and 10°C cold stresses did not affect the TRH mRNA expression (Table 4).

It is well established that stress is related to neurochemical and hormonal changes including alterations in adrenal and thyroid hormone levels (Hangalapura *et al*., 2004; Madden and Felten, 1995). In the present study, the effect of different durations of cold stress on neuroendocrine responses with plasma T3, T4, TSH and TRH levels and TRH mRNA and ACTH mRNA expression in two Chinese indigenous breeds from different latitude were studied.

There is a close interaction between the thyroid axis and the hypothalamic-pituitary-adrenal axis. It has been reported that T3 and T4 influence plasma TSH and ACTH levels (Lo *et al*., 1998). Moreover, chronic stress has been generally associated with a suppression of thyroid axis function. During stress, a suppressed secretion of thyroid-stimulating hormone and decreased conversion of the relatively inactive T4 to the more biologically active T3 has been described (Stratakis and Chrousos, 1995). Silberman *et al*. (2002) reported that exposure to chronic stress was able to induce a reduction of thyroid hormone levels in mice. Therefore, we determined T3 and T4 levels in the same samples in which TSH and ACTH were also determined. Levels of T3 were significantly decreased in W breed exposed to the 12 h duration of cold stress and an enhanced T4 levels at 12 h after 2-6 h duration of cold stress whereas significantly enhanced T3 levels were also detected in H breed when subjected to 2-3 h duration of severe cold stress with T4 levels decreased after an elevation when cold stress duration of 0.5 h and no effects when subjected to mild cold stress. The increasing and decreasing of the circulating T3 levels in chickens exposed to severe and mild cold period could be due to the conversion of T4-T3, a process that is known to be sensitive to ambient temperature. Apart from that the significant increase in plasma T3 levels after 2-3 h of cold stress in H breed suggests an increase in metabolic heat production. However, prolonged cold stress results in acclimation and restoration of heat balance as can be inferred from the normalized plasma T3 levels as well as progressively decreased plasma ACTH levels. The TRH mRNA and ACTH mRNA expression levels showed the same trend as to the plasma hormonal levels.

It has been demonstrated that the duration of cold stress had significant influence on the plasma hormonal levels and related gene expression (Altan *et al*., 2000; Sonna *et al*., 2002; Wang and Xu, 2008; Morimoto, 1993).

However, from the result of this study, the hormonal expression from hypothalamic-pituitary-adrenal axis and thyroid axis showed different rule between the H and W breed. Severe and mild cold stress highly affected the thyroid axis in the W breed and hypothalamic-pituitary-adrenal axis was much more influenced in the H breed during cold stress. Another key factor should be mentioned is the breed from different latitude. From the results of this study, 2°C severe cold stress caused relatively similar damage to the neuroendocrine of the two breed while under 10°C exposure, T3, T4, TRH mRNA and ACTH mRNA expression level obviously changed in W breed than in H breed.

Observation on the plasma level of hormonal and tissue mRNA expression during cold stress under different intensities in two Chinese indigenous breed from different latitude would provide meaningful information on the relationship of the change of neuroendocrine with stress intensity and geographic conditions. This would further help the revealing of the mechanism of injury caused by cold stress and better control chicken from loss of cold stress in different areas.

CONCLUSION

In the study, cold stress does have a modulating effect on thyroid axis and adrenal axis in a different regulatory pathway. The modulatory intensities caused by cold stress were also depended on the duration of the stress. In addition, this is the first study that reveals the correlations between hormonal regulation and latitude in the described selection lines. Further studies are needed however, to confirm the effect of the cold stress on hormone levels, species from different latitudes and their interactions.

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