Differential effects of two indigenous broilers exposed to cold stress and characters of follicle density and diameter

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Abstract

Indigenous chickens from various part of China, due to different feather characters, always performed differently when countered with cold stress. In this study, the effects of long term hypothermia on serum hormones (triiodothyronine, thyroxine and insulin) and activity of plasma enzymes (Alanine amino-transferase, aspartate aminotransferase, gamma-glutamyltransferase, creatine kinase, in laboratory animals (Weber and Bosworth, 2005; Obut et al., 2009) and human (Hong et al., 2008). When a significant change occurred, often indicate the body weak ability to cope with cold stimuli, which is always varied within and between breeds (Shinder et al., 2002; Venditti et al., 2006).

For avian, the major function of feather is to maintain body temperature or heat dissipations. One of the strongest characteristics of follicle is their variability, and it is evident that species from low environment always associated with a dense follicle distribution, although follicle characteristics do not differ in a clear-cut way between breeds (Hall et al., 1996). There is probably very considerable genetic variability for these characteristics; generally, in animals’ heritability of fibre diameter seems to be quite high, with estimates ranging between 0.2 and 0.6 (Iman et al., 1992). Thus, it would be much meaningful to measure follicle number and diameter among different breeds. Huainan partridge chicken for egg and meat purposes, as one of the qualified breed located mainly in the middle of China, showed high egg laying performance and egg quality, high breeding performance, fast growth rate and meat quality, and could be used to breed new type with strong resistance to outside stimuli (Chen. 2004).

Wenchang chicken, another well-known local breed located at south of China, ranking the first seat from the four famous dishes at Hainan province by its thin skin and bone, smooth and fragrant meat, and the small body type determined its relative large body surface area and high resistance to heat stress (Tang et al., 2009). In this study, the two breeds mentioned above were analyzed after long time cold stress. Blood biochemical parameters and enzymes, follicle density and diameter from different body parts were tested, which would provide powerful evidence for breeding new type of broiler with high stress resistance.

Introduction

Cold stress, a physical environmental stressor, has been shown to have variable modulatory effects on blood biochemical parameters and enzymes, such as T3, T4 and creatine kinase, in laboratory animals (Weber and Bosworth, 2005; Obut et al., 2009) and human (Hong et al., 2008). When a significant change occurred, often indicate the body weak ability to cope with cold stimuli, which is always varied within and between breeds (Shinder et al., 2002; Venditti et al., 2006).

Materials and methods

Bird management

The stocks used in the experiment were physically healthy and unrelated male Huainan partridge chicken (n=270) and Wenchang chicken (n=270) 4-week-old, obtained from the Old Hens Farming Co., Ltd (Feixi, Hainan Province, China). 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 National Research Council (1994) requirements were provided ad libitum.

Experimental design

Chickens were placed randomly in each of the 6 pens mentioned above (4 m² per pen), thus providing 0.044 m²/bird. This study was repeated synchronously in another house in the same period. Body weight was recorded individually and weekly after 12 h empty stomach. Each breed was randomly divided into three groups after body weight. The chickens were housed at a constant temperature of 20±2°C till end of the 5th week. In the sixth experimental week, the two breeds with...
three groups were individually housed under different temperature, group 1 (H 1 and W 1) housed at 20±2°C, group 2 (H 2 and W 2) housed at 15±2°C and group 3 (H 3 and W 3) housed at 10±2°C.

Blood collection and serum preparation

After one-week cold exposure under different temperature, 2 mL blood samples were collected from the brachial wing vein using sterilized syringes with 30 chickens in each group. All efforts were made to minimize pain or discomfort of the chickens and to minimize the total numbers of chickens in the study. Blood samples were centrifuged at 3500 rpm for 10 min at 4°C, after 20 min water bath at 37°C to collect serum for biochemical assays. The samples were all non-hemolytic serum.

Serum biochemical measurement

ALT (GP4040, Beijing Leadman Biochemistry Co., Ltd, Beijing, China), AST (G04050, Beijing Leadman Biochemistry Co., Ltd) and γ-GT (GG4060, Beijing Leadman Biochemistry Co., Ltd) were measured by kinetic assay, CK (CK9231, Beijing Leadman Biochemistry Co., Ltd, Beijing, China) and LDH (LH9260, Beijing Leadman Biochemistry Co., Ltd) was measured by lactic kinetic assay, T3 (1173130122, Roche Ltd., Shanghai, China), T4 (12017709122, Roche Ltd.) and insulin (8K41-25, Abbott S.A., Guangzhou, China) were measured by Scintillation analyzer I2000 automatic biochemical analyzer and related testing kits. Statistical analysis

Data were made by one-way ANOVA using the GLM procedure of the SAS commercial statistical program. Statistical analyses were carried out by standard analyses of variance or Student’s t-test as appropriate. Differences between experimental groups were considered significant at P<0.05 or as otherwise indicated. All data were expressed as mean ± standard error (SE).

Results

Differential chemical alterations in the two broiler breeds

After 7 d of hypothermia exposure (15°C or 10°C) of the two breeds, serum from each group was collected and serum enzymes ALT, AST, γ-GT, CK together with serum biochemical indices LDH, insulin, T3, T4 were tested (Table 1). When exposed to low temperature (15°C and 10°C) for 7d, serum enzymes and biochemical were obviously changed in the two breeds, such as the significant decrease of AST, LDH and the dramatic change of insulin (significantly decreased in H and increased in W). When comparing the changing of measured biochemicals in the two breeds, higher decrease in AST, LDH and CK was observed in W than in H. Therefore, it is obvious that the variation trend was significantly different compared in the two breeds and much higher infection on the biochemical indexes (INS, T4) and serum enzymes (ALT) from Wenchang chicken when exposed to long-term cold stress.

Table 1. Serum ALT, AST, γ-GT, CK, LDH, insulin, T3, T4 levels in Huainan partridge chickens and Wenchang chickens breeding at different temperatures for 7d. Values are means±SD.

| Breed | γ-GT (U/L) | ALT (U/L) | AST (U/L) | LDH (U/L) | CK (U/L) | INS (pmol/L) | T3 (nmol/L) | T4 (nmol/L) |
|-------|------------|-----------|-----------|-----------|----------|-------------|-------------|-------------|
| H 1   | 31±3.96b   | 409±152.93a | 31±3.96b  | 1122±496.01a | 2506±825.73a | 0.20±0.42b  | 7.18±2.02a  | 15.67±3.10b |
| H 2   | 31±3.29b   | 387±152.93a | 31±3.29b  | 780±184.43a  | 2163±735.35a | 0.1±0.06b   | 6.52±2.22a  | 15.54±3.59b |
| H 3   | 31±3.69b   | 362±154.36a | 31±3.69b  | 688±168.18a  | 2069±827.62a | 0.07±0.05b  | 7.15±1.40a  | 15.45±4.63b |
| W 1   | 31±3.06b   | 364±130.04b | 31±3.06b  | 1116±460.19b | 2119±776.54b | 0.05±0.03b  | 6.1±1.35b   | 16.82±3.05b |
| W 2   | 31±2.52a   | 297±125.15a | 31±2.52a  | 609±285.18a  | 1807±325.50a | 0.06±0.11a  | 7.12±1.80b  | 14.86±5.33b |
| W 3   | 31±2.69b   | 279±104.96a | 31±2.69b  | 597±89.16a   | 1474±317.23a | 0.18±0.16a  | 5.84±1.32a  | 12.76±2.95b |

H, Huainan partridge chicken; W, Wenchang chicken; 1, 2 and 3, different groups. a,b, A,B means with common letters do not differ significantly (P<0.05 or P<0.01 small or block letters, respectively).

Table 2. Weight body of Huainan partridge chicken and Wenchang chicken before and after one week low temperature exposure.

| Breed | Age      | Group 1       | Group 2       | Group 3       |
|-------|----------|---------------|---------------|---------------|
| H     | 4-5 week | 238±6.83±3.2a | 285±0.36±4.7a | 284.9±31.1a   |
|       | 6 week   | 368±7.47±3.3a | 346.7±51.6a   | 361.8±47.3a   |
| W     | 4-5 week | 327±8.50±3.4a | 349±7.49±5.4a | 324.8±43.2a   |
|       | 6 week   | 413±8.54±4.7a | 417±65.45±5.9a| 395.17±56.8a  |

H, Huainan partridge chicken; W, Wenchang chicken; a,bthe same breed means in the same column and with common letters do not differ significantly (P<0.05).
shoulder and latero-abdominal. Results from the comparison of follicle diameter between the two breeds among the three parts showed that, follicle diameter from back of Huainan partridge chicken was significantly smaller than that of Wenchang chicken (P<0.05), while much larger than the later at latero-abdominal part (P<0.05) and no difference at neck shoulder (Table 3).

### Table 3. Follicle density and diameter from Huainan partridge chicken and Wenchang chicken in three body parts.

| Breed | Follicle indices | Neck shoulder | Back | Latero-abdominal |
|-------|-----------------|---------------|------|-----------------|
| H     | Density (n/3×1.5 cm²) | 32.0±3.3b | 47.3±9.5a | 13.3±2.6b |
| W     | Density (n/3×1.5 cm²) | 43.3±12.7c | 35.2±7.8c | 14.5±1.4b |
| H     | Diameter (μm) | 427.3±96.2b | 428.2±17.9a | 551.9±48.8b |
| W     | Diameter (μm) | 508.3±93.9b | 564.3±35.9b | 369.6±29.8b |

H, Huainan partridge chicken; W, Wenchang chicken; “b” the same breed means in the same column and with common letters do not differ significantly (P<0.05).

### Discussion

Manifestations of disturbance of the temperature regulating mechanism of the body result from cold/heat environment, high humidity and inadequate ventilation. Ambient temperature exceeding the thermoneutral zone, lead to decreased core temperature and consequently initiate a number of responses leading to the neutralization of the metabolic changes on one hand and regulation of body temperature on the other hand (Bogin et al., 1997; Gabriel et al., 2003). Chronic and acute cold exposure is among the stressing factors affecting the metabolism, causing hypothermia, leads to a sequence of physiological and metabolic changes, such as changes of enzyme activities in almost all the tissues, resulting from the need to elevate the body temperature or a sequence of metabolic events originated from the hypothermia (Selman et al., 2000; Rajman et al., 2006). Researches have suggested that the variation of plasma enzymes is indicative of liver and skeletal muscle damage and is a consequence of the disruption in muscle cell membrane function and permeability (Mitchell and Sandercock, 1995; Yan et al., 2009).

Living cells, as well as whole animals, can adapt to changing living conditions, by modifying the biological systems to meet the new needs by enhancing or suppressing enzymes producing or excreting metabolites and creating new equilibrium (Bogin et al., 1996; Altan et al., 2000). Various metabolic abnormalities may decline the thermogenic capacity of low-temperature exposed chickens (Ahmad and Mitsuhiro, 2009).

In the present research, enzyme activities in the blood and plasma hormones affected by long-term low environmental temperature exposure was studied in two indigenous breeds from middle and south of China. As seen from the results, AST, LDH and CK decreased both in two breeds while insulin, which highly correlated with energy metabolism (Chiou et al., 2001), showed differential regulation, i.e. down-regulated in H and up-regulated in W. These might provide the information that, under cold stress condition, broiler from south showed little adaptation and initiate defense system to accelerate energy metabolism. At the other hand, the decreased activity of marker enzymes, like AST, LDH and CK, was due to the down-regulated skeletal muscle viability arise from low temperature (Yan et al., 2009). To be emphasized, the variation trends in each index showed significant difference in the two breeds with obviously higher alteration in W, which justified the decreased weight gain when exposed in low temperature. This may also indicated the high sensitivity of W from south of China when disposed to cold condition.

Feathers could have functioned in communication, defense, thermal insulation, or water repellency (Prum, 1999), and these are reflected in the follicle density and diameter. Follicle density and diameter was measured in the two breeds in this study and the results suggested that a relatively more dense of follicle at back from H, originated from the north of Anhui province, China, maybe just for the resistance of low temperature in winter. In the meantime, the wide follicle diameter at abdominal part might just in order to balance the body heat during summer with high temperature and humidity at Anhui Province. The W used in this experiment was introduced by the Old Hens Farming Co., Ltd, in the Hainan province, south of China, and bred for several generations. Environmental selection might cause the higher coefficient variation of follicle diameter.

### Conclusions

In conclusion, the pattern of serum biological change under cold stress and follicle characters of two natural broiler breeds from various areas was investigated. Different breeds, especially from different latitude, showed differential adaptation when exposed to cold stress. The analysis of serum enzymes and hormones together with follicle density and diameter will contribute to a better understanding of growth of local broiler breeds, thus provide guidance for breeding new breeds having cold resistance, high meat quality and good performance.

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