PHYSIOLOGY AND REPRODUCTION

Effect of egg composition and oxidoreductase on adaptation of Tibetan chicken to high altitude

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ABSTRACT

Tibetan chickens have good adaptation to hypoxic conditions, which can be reflected by higher hatchability than lowland breeds when incubated at high altitude. The objective of this trial was to study changes in egg composition and metabolism with regards the adaptation of Tibetan chickens to high altitude. We measured the dry weight of chicken embryos, egg yolk, and egg albumen, and the activity of lactate dehydrogenase (LDH) and succinic dehydrogenase (SDH) in breast muscle, heart, and liver from embryos of Tibetan chicken and Dwarf chicken (lowland breed) incubated at high (2,900 m) and low (100 m) altitude. We found that growth of chicken embryos was restricted at high altitude, especially for Dwarf chicken embryos. In Tibetan chicken, the egg weight was lighter, but the dry weight of egg yolk was heavier than that of Dwarf chicken. The LDH activities of the three tissues from the high altitude groups were respectively higher than those of the lowland groups from d 15 to hatching, except for breast muscle of Tibetan chicken embryos on d 15. In addition, under the high altitude environment, the heart tissue from Tibetan chicken had lower LDH activity than that from Dwarf chicken at d 15 and 18. The lactic acid content of blood from Tibetan chicken embryos was lower than that of Dwarf chicken at d 12 and 15 of incubation at high altitude. There was no difference in SDH activity in the three tissues between the high altitude groups and the lowland groups except in three tissues of hatchlings and at d 15 of incubation in breast muscle, nor between the two breeds at high altitude except in the heart of hatchlings. Consequently, the adaptation of Tibetan chicken to high altitude may be associated with higher quantities of yolk in the egg and a low metabolic oxygen demand in tissue, which illuminate the reasons that the Tibetan chicken have higher hatchability with lower oxygen transport ability.

Key words: egg composition, high altitude adaptation, lactate dehydrogenase, succinic dehydrogenase, Tibetan chicken

INTRODUCTION

Hypoxia is the main physiological challenge that threatens individuals’ survival, development, and reproduction in high-altitude areas (Brown, 2012). Understanding the physiological responses and adaptation mechanisms to high altitude is of great interest to the fields of physiology and clinical medicine. Birds provide a useful model to study physiological adaptation to hypoxic stress. Because fertilized eggs are independent from the maternal system, embryonic development is directly affected by the environment. In the Andes Mountains in South America and the Himalaya Mountains in Asia, there are some permanent bird settlements located above an altitude of 3,000 m (Guillet et al., 1983). Native birds living at these high altitudes, such as the bar-headed goose (Anser indicus) and American coot (Fulica americana peruviana) are known to breed, and embryos to successfully hatch, even at altitudes between 4,000 and 6,500 m (Carey et al., 1993; Takekawa et al., 2013). Some domestic breeds, such as Tibetan chickens (Gallus gallus), are widely distributed at altitudes from 2,200 to 4,100 m in the Qinghai-Tibet Plateau and can maintain relatively higher hatchability and faster growth than lowland breeds when incubated under hypoxic conditions (Zhang et al., 2007).

The environment at high altitude is characterized by a decrease in the partial pressure of oxygen. In the avian embryo, oxygen transport relies on diffusion from the environment to the tissues through multiple steps (León-Velarde and Monge, 2004). In a previous study, we focused on adaptations in oxygen transport in embryos under hypoxic conditions and found that the conductance of Tibetan chicken eggshell was significantly lower than that of the lowland breeds (Wei and Wu, 2005). Moreover, the Tibetan chicken had lower arterialized oxygen partial pressure and higher venous carbon dioxide partial pressure than that of...
lowland chicken in d-18 embryos incubated at high altitude (Wei et al., 2007), which may indicate that the Tibetan chicken has no advantage in terms of oxygen transport.

Thus, it appears that metabolic adaptations to hypoxia may play an important role in improving reproductive success in high-altitude-dwelling bird species. Changes in the activity of key metabolic enzymes regulate tissue metabolism according to oxygen availability (Sergina et al., 2015). Succinic dehydrogenase (SDH) is a mitochondrial enzyme involved in the Krebs cycle that acts in oxidative phosphorylation. Lactate dehydrogenase (LDH) is a cytoplasmic enzyme necessary for the anaerobic production of lactate (Harris, 1971). These enzymes may be associated with metabolic adaptations to high altitude (Bouverot, 2012). In chicken embryos, all nutrition for growth comes from the egg. Thus, egg size and composition will affect embryo development (Nangsuay et al., 2011). Different sizes of egg experience similar hypoxic effects on embryonic growth (Mortola and Al Awam, 2010). However, whether differences in the components of Tibetan chicken eggs compared to other breeds affect the response of embryos to hypoxic conditions has not been investigated. Differentiating with most previous reports about high altitude adaptation of bird embryos, which mainly focus on the air transport from the environment to the tissues (Carey et al., 1993; Carey et al., 1994; Gou et al., 2005), we compared dry weights in eggs, and SDH and LDH activities in tissues, between chicken embryos of Tibetan and Dwarf chicken to understand their effects on the adaptation of Tibetan chickens to high altitude.

**MATERIALS AND METHODS**

**Eggs and Incubation**

All the procedures for this study were approved by the China Agricultural University Animal Care and Use Committee. The study was done in Linzhi (altitude 2,900 m, “high”) and Beijing (altitude 100 m, “low”). Fertilized eggs from a flock of Tibetan hens were gathered and incubated on a poultry farm in the College of Agriculture and Animal Husbandry of Tibet University in Linzhi (TH). One-day-old chicks produced by this flock were transported to a farm in China Agricultural University in Beijing. After maturity, fertilized eggs from these chickens were collected and incubated on the farm in Beijing (TL). The control eggs were taken from a single flock of Dwarf chicken hens in the low altitude site, and randomly divided into 2 groups. These were incubated either at a high altitude (DH) together with TH or at a low altitude (DL) together with TL. Each group included 500 eggs. All eggs were incubated at 37.5°C, with a relative humidity of about 55%, and turned every 4 h.

**Egg Component**

Twenty fertile eggs per group were randomly selected to determine dry weights of the yolk and albumen at d 0. From d 6 of incubation, 20 embryos were killed by decapitation and the dry weights of the embryo, residual yolk, and albumen were weighed every 3 days. Twenty hatchlings were killed by spinal transection immediately after hatching to measure the dry weight of “true hatchlings” (hatchlings after their residual yolk is removed) and residual yolk mass. The dry weights were measured after being dried in an oven at 65°C for 2 d, until there was no further weight loss.

**Measurement of Lactic Acid Content**

To measure lactic acid (LA) content, 500 μL of venous blood was collected at d 12, 15, and 18 from embryonic chorioallantoic membrane blood vessels using heparin as an anticoagulant. The samples were deproteinized by adding 150 μL of cold protein precipitating agent in a 1.5-mL microcentrifuge tube. The tube was vortexed and placed on ice for 10 min, then centrifuged at 3,500 rpm for 8 min at 4°C. The supernatant was removed for assay. The LA level was determined via a chromogenic reaction according to the Lactic Acid Detection Kit (Jiancheng Co., Nanjing, China) protocol, using a spectrophotometer (UV2000, UNICO, Shanghai, China) at 530 nm.

**Activity Measurement of SDH and LDH**

Tissue samples, including breast muscle, heart, and liver, were collected from embryos decapitated at d 15 and d 18 of incubation, and from hatchlings killed by spinal transection. These tissues were individually ground into homogenate with 9× saline solution and centrifuged at 3,500 rpm for 10 min. Total enzyme activities of LDH and SDH were determined according to the manufacturer’s protocols (Jiancheng Co., Nanjing, China).

**Statistical Analysis**

The differences in eggs from high and low altitudes, and from Tibetan or Dwarf chicken, were compared in a 2 × 2 factorial arrangement at different days of incubation. Embryos’ mass, LA content, and enzyme activity were analyzed with a generalized linear model using SAS v.8.0 software; initial egg mass was used as a covariate for embryo mass. The significance of differences between means was determined with the PDIF option of the LSMEANS statement and with a significance level of P < 0.05.
RESULTS

The dry weights of chicken embryo incubated at high altitude suggested restricted growth relative to chicken embryos incubated at low altitude ($P < 0.05$) from d 12 to the end of the incubation period, especially for Dwarf chicken embryos from d 12 to d 18 (Figure 1A). Eggs of Tibetan chickens were lighter (43.68 ± 0.70 g for TH; 45.54 ± 0.71 g for TL) than those of Dwarf chickens (48.21 ± 0.86 g for DH; 48.66 ± 0.74 g for DL), but the dry weights of Tibetan chicken egg yolks were greater (7.44 ± 0.21 g for TH; 7.79 ± 0.20 g for TL) than those of Dwarf chickens (6.02 ± 0.22 g for DH; 5.74 ± 0.18 g for DL). The dry weights of egg yolk showed similar changes over the whole incubation period for each group. After d 15 of incubation, the dry residual egg yolk weights dropped quickly in all groups, and the remaining yolk mass of the TH group was greater than that of TL at d 18 ($P < 0.05$) (Figure 1B). The DH and DL groups showed a similar relationship at d 12, 15, and 18 ($P < 0.05$), with DH having the greater dry residual egg yolk weight. Conversely, the dry residual egg albumen weights in the Dwarf chicken (3.89 ± 0.09 g for DH; 3.69 ± 0.07 g for DL) was greater than those of Tibetan chicken (2.98 ± 0.08 g for TL; 3.07 ± 0.08 g for TH) (Figure 1C). The mass of the remaining albumen in DH was higher than that of DL at d 15 and 18 ($P < 0.05$).

The results regarding LDH activity are summarized in Table 1. LDH activity of breast muscle, heart, and liver from the high altitude groups was greater than that of the lowland groups ($P < 0.05$), except for breast muscle in Tibetan chickens at d 15. Moreover, the level of LDH activity was lower in embryo hearts from the TH group than from the DH group at d 15 and 18 of incubation ($P < 0.05$); this was also the case for breast muscle at d 15 of incubation ($P < 0.05$).

Results of the analysis of SDH activity are shown in Table 2. The SDH activity within breast muscle, heart, and liver was lower in the hatchlings from the high altitude groups than those of the lowland groups ($P < 0.05$); a similar pattern was evident in breast muscle at d 15 of incubation ($P < 0.05$). In addition, SDH activity was lower in heart tissue from hatchlings in the DH group than in those from the TH group ($P < 0.05$).

Data concerning the LA content from whole blood are presented in Table 3. The LA content of TH embryos was lower than that in other groups at d 12 of incubation, and was also lower than that of Dwarf chicken groups at d 15 of incubation.

DISCUSSION

Low oxygen partial pressure at high altitude affected the metabolism, and nutrient utilization of eggs for growth may be influenced by environmental conditions. Not all egg nutrients were utilized during incubation, so the quantitative absorption could reflect the metabolic activities of tissues (Lourens et al., 2011). We found...
that the TH group always had a greater residual yolk weight than the DH group, but the magnitude of the difference decreased with incubation time (1.42 g before incubation, 1.05 g at day 18 of incubation, and 0.36 g in hatching). Conversely, a comparison of egg albumen weight found the opposite (Figure 1). Egg yolk is composed primarily of lipid, protein, and a small amount of carbohydrates, which act as an energy source and are the essential components for building embryonic tissue (Speake and Thompson, 1999). Egg albumen is the main reservoir of water and also provides proteins and other micronutrients. During chicken embryo development, more than 90% of the total energy requirement is derived from fatty acid oxidation of the yolk lipids (Noble and Cocchi, 1990). The relatively higher quantities of yolk in Tibetan chicken eggs may provide more energy material, enabling embryos to better survive in hypoxic environments.

The energy needed for embryo development is generated either by oxidative phosphorylation in the mitochondria or at a substrate level in the glycolytic pathway. The former process requires oxygen and the latter does not. Under normal oxygen conditions, glycolysis can be expected to provide only a minor proportion of high-energy phosphate bond energy. In the prolonged hypoxia that accompanies life at high altitude, glycolysis may play a role of increased importance (Harris, 1971). The LDH system is an important enzyme system that can cause a shift in metabolism toward the glycolytic or aerobic pathway (Sergina et al., 2015). LDH activity, an indicator of relative anaerobic metabolic capacity, shows a positive correlation with altitude in

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<table>
<thead>
<tr>
<th>Tissue</th>
<th>Days</th>
<th>DH</th>
<th>TH</th>
<th>DL</th>
<th>TL</th>
<th>Altitude</th>
<th>P-value</th>
<th>Breed</th>
<th>Altitude × Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast muscle</td>
<td>15</td>
<td>7.358± 0.021 475.87</td>
<td>6.474.88 ± 439.28</td>
<td>6.399.87 ± 501.61</td>
<td>6.330.28 ± 487.26</td>
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<td>18</td>
<td>6.297.26± 249.31</td>
<td>5.548.80± 211.02</td>
<td>4.861.99 ± 222.43</td>
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<td>7.132.26 ± 571.36</td>
<td>6.789.82 ± 478.03</td>
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<td>3.682.12 ± 482.18</td>
<td>2.861.02 ± 454.60</td>
<td>1.450.29 ± 431.27</td>
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<tr>
<td></td>
<td>18</td>
<td>3.244.22 ± 638.54</td>
<td>3.622.11 ± 643.33</td>
<td>10.023.17 ± 673.08</td>
<td>10.276.65 ± 673.08</td>
<td>&lt;0.001</td>
<td>0.19</td>
<td>0.29</td>
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<td>0.17</td>
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<td></td>
<td>18</td>
<td>3.244.22 ± 638.54</td>
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**Table 1.** Lactate dehydrogenase activity from three tissues of Dwarf chicken and Tibetan chicken embryos in high and low altitudes at d 15 and 18 of incubation and on hatching (IU/mg mass).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Days</th>
<th>DH</th>
<th>TH</th>
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<th>P-value</th>
<th>Breed</th>
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<tr>
<td>Breast muscle</td>
<td>15</td>
<td>34.19 ± 3.32</td>
<td>35.24 ± 2.39</td>
<td>52.73 ± 2.45</td>
<td>56.37 ± 2.53</td>
<td>&lt;0.001</td>
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<td>0.59</td>
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<td>18</td>
<td>29.38 ± 4.08</td>
<td>31.60 ± 4.42</td>
<td>26.49 ± 3.95</td>
<td>30.20 ± 4.31</td>
<td>0.66</td>
<td>0.78</td>
<td>0.32</td>
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<tr>
<td>Heart</td>
<td>15</td>
<td>46.85 ± 5.90</td>
<td>32.15 ± 4.63</td>
<td>94.76 ± 8.76</td>
<td>127.71 ± 9.84</td>
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<td>0.13</td>
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<td>18</td>
<td>15.96 ± 3.99</td>
<td>18.48 ± 4.15</td>
<td>15.39 ± 3.88</td>
<td>14.57 ± 3.72</td>
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<tr>
<td>Liver</td>
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<td>49.54 ± 4.11</td>
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<td>41.79 ± 4.33</td>
<td>38.51 ± 3.75</td>
<td>0.35</td>
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<td></td>
<td>18</td>
<td>19.06 ± 6.63</td>
<td>28.56 ± 6.82</td>
<td>31.54 ± 6.98</td>
<td>27.18 ± 6.76</td>
<td>0.41</td>
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<tr>
<td>Breeding</td>
<td>15</td>
<td>85.47 ± 9.48</td>
<td>98.58 ± 9.50</td>
<td>75.43 ± 9.28</td>
<td>81.47 ± 9.15</td>
<td>&lt;0.001</td>
<td>0.53</td>
<td>0.47</td>
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**Table 2.** Succinic dehydrogenase activity from three tissues of Dwarf chicken and Tibetan chicken embryos in high and low altitudes at d 15 and 18 of incubation and on hatching (IU/mg mass).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Days</th>
<th>DH</th>
<th>TH</th>
<th>DL</th>
<th>TL</th>
<th>Altitude</th>
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<td>2.32 ± 0.23</td>
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<td>15</td>
<td>2.63 ± 0.22</td>
<td>1.86 ± 0.20</td>
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<td>2.31 ± 0.20</td>
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<td>0.42</td>
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<td>18</td>
<td>1.51 ± 0.21</td>
<td>1.39 ± 0.20</td>
<td>1.61 ± 0.20</td>
<td>1.78 ± 0.20</td>
<td>0.04</td>
<td>0.16</td>
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</table>

**Table 3.** Lactic acid content of whole blood from Tibetan chicken and Dwarf chicken embryos incubated at high and low altitude (mmol/L).
birds (Berlet et al., 1981). According to our results (Table 1), the high altitude groups had higher LDH activity than the low altitude groups in breast muscle, heart, and liver from d 15 to hatching, except for d 15 in breast muscle between TH and TL. The LDH activity of heart tissue from the TH group was significantly lower than that of the DH group in the later period of incubation. Cardiac muscle adaptation is an important factor for performance at high altitude. Saksena et al. (1981) pointed out that depression of cardiac performance in lowlanders was related to reduced physical work capacity at high altitude. The capacity of the heart to pump blood is closely associated with myocardial energy metabolism and contractile activity (Cai et al., 2010). The lower LDH activity of the heart tissue in the TH group suggests that the myocardium of the TH group was maintaining a relatively aerobic condition. The LA content of blood from TH embryos (Table 3) was significantly lower than that of DH at d 12 and 15 of incubation, further supporting this conclusion.

The enzyme SDH is a membrane-bound dehydrogenase linked to the respiratory chain and a member of the tricarboxylic acid cycle (Hederstedt and Rutberg, 1981). We found no difference in SDH activity of breast muscle, heart, and liver between the high altitude and low altitude groups except in three tissues of hatchlings and at d 15 of incubation in breast muscle. This suggests that the oxidative phosphorylation pathway provides similar amounts of energy in the Tibetan chicken and the Dwarf chicken. The SDH activity of heart tissue from the DH group was lower than that from the TH group in hatchlings, which may mean that Tibetan chickens have a respiratory advantage over Dwarf chickens living at high altitudes.

Hypoxia increases anaerobic glycolysis, which partially compensates for hypoxic cellular energy demands (Kim et al., 2006). However, as in the DH, this pathway may not be sufficient for hypoxic adaptation. According to previous results, Tibetan chicken has no advantage with regards oxygen transport (Wei and Wu, 2005; Wei et al., 2007). But Tibetan chicken embryos had normal growth and relative aerobic conditions at high altitude, which suggests that Tibetan chicken has a low metabolic oxygen demand in tissue.

In summary, the relatively higher quantity of yolk in Tibetan chicken eggs may better facilitate growth and survival in a hypoxic environment. The lower LDH activity of heart tissue and LA content of blood from the TH group suggest that the myocardium of the TH birds maintains a relatively aerobic condition. There was little difference among SDH activities from the three tissues between the high altitude and low altitude group embryos, which associated with the results that Tibetan chicken has no advantage with regards oxygen transport. In conclusion, this study indicates that the adaptation of Tibetan chicken to high altitude may be associated with higher quantities of yolk in the eggs and low metabolic oxygen demand in tissue. Whether the hypoxic adaptation can be improved by selection egg component requires further study.

ACKNOWLEDGEMENTS

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REFERENCES


ADAPTATION OF TIBETAN CHICKEN TO HIGH ALTITUDE


