The Effects of Different Low Temperature Cold Stimulation on Tibias Fluctuating Asymmetry of China-Lindian Native Chickens

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Abstract. In this study, China-Lindian native chickens were given cold stimuli from the following three aspects: different interval days, different decrease of temperature and different stimulation time. The three levels of different interval days are interval of 0 day, interval of 1 day and interval of 2 days; The three levels of different decrease temperature are temperature decreasing 3\textdegree C, temperature decreasing 5\textdegree C and temperature decreasing 7\textdegree C; The three levels of different stimulation time are stimulating time of 1h, stimulating time of 3h and stimulating time of 6h. We identified the influence of cold stimuli on welfare of CLNC through detecting fluctuating asymmetry welfare indexes. The main fluctuating asymmetry welfare index of detection were asymmetry of tibias. It was concluded from the study that the stimulation time of cold stimuli had effect on asymmetry of tibias (P<0.05); the decrease of temperature of cold stimuli had no impact on asymmetry of tibias (P>0.05); and the interval days of cold stimuli had no effect on asymmetry of tibias (P>0.05).

Introduction

Cold stimulation is a most common stress factors for livestock and poultry. It has more harmful to the newborn livestock and poultry. Fluctuating asymmetry (FA) has recently been advocated as one such potential welfare indicator that might, even in an integrated way, reflect the ability of an animal to cope with the sum of challenges that have been affecting the individual during its growing life. Fluctuating asymmetry has been suggested to comprise a putative measure of animal welfare (Møller et al., 1999; Møller and Manning, 2003), because it reflects the ability of the individual to cope with the sum of challenges that it faces during its development (Swaddle and Witter, 1997; Kellner and Alford, 2003; Knierim et al., 2007). Our workgroup start to study the cold stimulation on China-Lindian native chickens, China-Lindian native chickens were given cold stimuli from the following three aspects: different interval days, different decrease of temperature and different stimulation time.

Materials and Methods

Animal Selection and Daily Feeding Management

We selected China-Lindian native chickens as test animals. They were bred in the cage from 1 to 3 weeks. 3-week-old China-Lindian Native Chickens, physically healthy and weighing evenly were selected. 448 chicken were divided into 28 groups according to the design, 16 chickens in one group, four repeat experiments in one group, four chickens for each repetition. The diet composition of test group and the control group of are same.1-3 weeks of age Commercial complete feed standards respectively are crude protein 21.00\% and energy 12.10MJ/kg. 4-6 weeks of age Commercial
complete feed standards respectively are crude protein 19.00% and energy 12.60 MJ/kg. We cleaned the sink every day, to ensure the water supply and clean. Chickens were observed daily to check if the water and faeces are normal. Other feeding management program is according to China-Lindian native chickens production routine.

**Artificial Climate Chamber**

Artificial climate chamber can automatically control the temperature of each chamber by the computer program. The humidity, light and other environmental conditions are also controlled by the computer program.

**Low Temperature Stimulation Test Design**

Randomly selected 448 birds were given the low temperature stimulation test from 22 age in days to 42 age in days. These stimulated CLNC were still reared in normal brooding conditions after stimulating test. In this study, China-Lindian native chickens were given cold stimuli from the following three aspects: different interval days, different decrease of temperature and different stimulation time. The three levels of different interval days are interval of 0 day, interval of 1 day and interval of 2 days; the three levels of different decrease temperature are temperature decreasing 3℃, temperature decreasing 5℃ and temperature decreasing 7℃; the three levels of different stimulation time are stimulating time of 1h, stimulating time of 3h and stimulating time of 6h.

In strict accordance with the China-Lindian Native Chickens breeding management, we control the temperature, humidity and light in the shed. At the aspect of temperature, the control group at first week with 32-35℃, second weeks with 29-32 ℃, third weeks with 26-29℃, fourth weeks with 23-26 ℃, fifth weeks with 20-23 ℃, the sixth weeks temperature has been controlled at 20℃.

**Slaughter and Sampling Method**

Four birds were randomly selected for slaughter from each repeat at one days before the end of the cold stimulus test. The fluctuating asymmetry were carried out after slaughter. We use vernier caliper to accurately measure the length of left and right sides of the legs (tarsometatarsus), flange width (tarsometatarsus and tibial articulation) and wings length (ulna) when we measured the fluctuating asymmetry and calculated the FA value. The formula is as follows: \( \text{FA} (%) = 2 \times \frac{|R-L|}{R+L} \times 100 \).

**Results and Analysis**

**The Effects of Interval Days of Cold Stimulus on Tibias Fluctuating Asymmetry in China-Lindian Native Chickens**

<table>
<thead>
<tr>
<th>interval days</th>
<th>average</th>
<th>difference significant</th>
<th>α=0.05</th>
<th>α=0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>1.65±0.41</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>1 d</td>
<td>1.79±0.29</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>2 d</td>
<td>1.92±0.34</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>control group</td>
<td>2.07±0.21</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

As shown in table 1, tibias fluctuating asymmetry on all experimental group were lower than the control group, the difference was not significant \((P>0.05)\). Tibias fluctuating asymmetry on interval of 2 day was higher than interval of 0 day and 1 day in cold stimulus trial group, the difference was not significant \((P>0.05)\). Through the variance analysis, interval days had no significant effect on the tibias fluctuating asymmetry \((P>0.05)\).
The Effects of Decreased Temperature of Cold Stimulus on Tibias Fluctuating Asymmetry in China-Lindian Native Chickens

As shown in table 2, tibias fluctuating asymmetry on all experimental group were lower than the control group, the difference was not significant ($P>0.05$). Tibias fluctuating asymmetry on decreased temperature $3^{\circ}C$ was higher than decreased temperature $5^{\circ}C$ and $7^{\circ}C$ in cold stimulus trial group, the difference was not significant ($P>0.05$). Through the variance analysis, decreased temperature had no significant effect on the tibias fluctuating asymmetry ($P>0.05$).

<table>
<thead>
<tr>
<th>decreased temperature</th>
<th>average</th>
<th>difference significant</th>
<th>$\alpha=0.05$</th>
<th>$\alpha=0.01$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3$^{\circ}C$</td>
<td>2.03±0.32</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>5$^{\circ}C$</td>
<td>1.89±0.29</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>7$^{\circ}C$</td>
<td>1.74±0.41</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>control group</td>
<td>2.17±0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Effects of Cold Stimulation Time on Tibias Fluctuating Asymmetry in China-Lindian Native Chickens

As shown in table 3, tibias fluctuating asymmetry on all experimental group were lower than the control group, the difference was significant ($P<0.05$). Tibias fluctuating asymmetry on stimulation time 3h was higher than stimulation time 1h and 6h in cold stimulus trial group, the difference was not significant ($P>0.05$).

Through the variance analysis, cold stimulation time had significant effect on the tibias fluctuating asymmetry ($P<0.05$).

<table>
<thead>
<tr>
<th>stimulation time</th>
<th>average</th>
<th>difference significant</th>
<th>$\alpha=0.05$</th>
<th>$\alpha=0.01$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>1.56±0.19</td>
<td>b</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>2.04±0.26</td>
<td>ab</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>6 h</td>
<td>1.98±0.37</td>
<td>ab</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>control group</td>
<td>2.09±0.28</td>
<td>a</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Fluctuating asymmetry reflects that the growth condition is good or not under the condition of certain raising environment. It is a behavior parameters to general evaluate the environmental condition and genetic stress. It is also an index to evaluate animal welfare and comfort. The greater the value of FA, the more asymmetry of the traits of both sides of the poultry body. Heterophil-to-lymphocyte ratio, fluctuating asymmetry, and duration of tonic immobility may be used as criteria for measuring levels of stress, welfare, and fear in chickens (Gallup, 1979; Gross and Siegel, 1983; Parsons, 1990).

The tibias were selected in this experiment according to the characteristics of broiler bone development. The results of this experiment show that that the stimulation time of cold stimuli had effect on asymmetry of tibias ($P<0.05$); the decrease of temperature of cold stimuli had no impact on asymmetry of tibias ($P>0.05$); and the interval days of cold stimuli had no effect on asymmetry of
tibias ($P>0.05$). Yalcin and Siegel (2003) found that relative asymmetry of wing, shank, tibia, and femur lengths increased in broiler embryos exposed to cooling. Both genetic factors and environmental conditions may affect the ability of an organism to maintain symmetrical growth. However, the actual mechanisms involved in disruption or restoration of symmetrical growth are currently not well understood and different concepts are proposed by different authors (Klingenberg CP, 2003; Emlen JM, Freeman DC, Graham JH, 2003).

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References