Arch. Anim. Breed., 58, 373–378, 2015
www.arch-anim-breed.net/58/373/2015/
doi:10.5194/aab-58-373-2015

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Archives Animal Breeding

Effect of heat stress on the pituitary and testicular development of Wenchang chicks

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Received: 25 April 2015 – Revised: 20 August 2015 – Accepted: 29 September 2015 – Published: 13 October 2015

Abstract. To study the effects of heat stress (HS) on the growth and reproductive performance of chicks, 1-day-old male Wenchang chicks were randomly selected and divided into control (CK) and HS groups. The two groups of birds were fed according to a routine. The chicks in the HS group were placed under HS for 2 h day^{-1} (temperature, $40 \pm 0.5°$; humidity, 63.0–80.0 %) until the sixth week. At the end of each week, six chicks were randomly selected from each group and dissected for pituitary and testicular tissues, which were then weighed and sectioned onto slides to observe the histological changes in pituitary and testis under a microscope. Our results indicated that compared with the CK group, with the increase in age, HS significantly reduced the feed conversion rate (FCR) and weight gain per week, and these changes were positively correlated. The pituitary and testicular weights and volumes of chicks in the HS group were significantly lower than those in the CK group ($P < 0.05$). For 3-week old chicks, the cross-sectional area of seminiferous tubule in chicks of the HS group was extremely significantly lower than that of the CK group ($P < 0.01$). Compared with the CK group, the seminiferous epithelium was thinner in the HS group, the arrangement of spermatogenic cells became loose and irregular, and the integrity of the histological structure of testicular tissues was also damaged. Therefore, the above results indicated that HS significantly impeded the growth and development of pituitary and testis in chicks.

1 Introduction

With the frequent occurrence of extreme weather globally (Barnett et al., 2012), the damage of heat stress (HS) to poultry has become increasingly evident, resulting in serious economic losses (St-Pierre et al., 2003). Because the skin of birds has no sweat glands and is coated with feathers, their heat generation, cooling, and body temperature regulation are more difficult compared to mammals. When birds are in an environment with a temperature higher than their appropriate temperature, their thermoregulatory mechanism cannot cope with the high temperature change, resulting in HS. HS can cause birds wheezing and can cause long-time alternating acid and base poisoning in the animal's body, lead to respiratory mucosal congestion, and increase heart rate. This results in an increased blood flow at the body surface and muscle but a reduced blood supply to the internal organs and gonads, affecting the uptake and utilization of nutrients in the body of poultry. Therefore, how to reasonably avoid HS during the process of intensive, large-scale, and high-density poultry production and how to reduce economic losses has become an important issue to solve in current poultry production. Studies have shown that HS-induced wheezing can lead to an increased respiratory rate; reduced blood volume in the gastrointestinal tract, liver, and kidney; a decline in feed conversion rate (FCR); and raised mortality (Quinteiro-Filho et al., 2010). Furthermore, HS significantly reduces the marker enzymes of intestinal absorption such as disaccharidases, alkaline phosphatase (AKPase), and adenosine triphosphatase (ATPase), causing dysfunction of the intestinal mucosal antioxidant system in poultry (Chen et al., 2013, 2014). By destroying the integrity of the tight junctions between the epithelial cells of the gastrointestinal tract, damaging the gastrointestinal mucosal barrier, and thereby triggering dysfunction of the barrier and increasing its permeability, HS ultimately causes an inflammatory response in

the body and greatly increases the rate of susceptibility to acute enteritis in poultry (Quinteiro-Filho et al., 2012). In addition, high temperatures can also induce endocrine disorders in poultry, resulting in phenomena such as instability of blood insulin levels; significantly reduced levels of total plasma protein, serum protein, and serum T_3/ T_4 (Liu et al., 2000); significantly increased Ca^{2+} concentration; and hypokalemia. (Du et al., 2000). Furthermore, under HS, the amounts of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) declined, and the progesterone (P4) level significantly reduced, too, after an initial elevation (Donoghue et al., 1989; Li and Cui, 2013). Short- and long-term heat exposure significantly reduces the egg-laying rate, egg weight, ovarian weight, and the number of large follicles in egg-laying birds, resulting in declined ovarian function, disordered follicular development and formation, significantly reduced reproductive performance, etc. (Rozenboim et al., 2007).

Current studies on the effect of HS in poultry mostly involve the aspects of nutrition, digestion, immune function, etc., of egg-laying and mature birds, but relatively few studies focus on the development of endocrine and reproductive systems in chicks. The present study used 1–6-week old male Wenchang chicks as subjects, aiming to shed light on the factors influencing the histological and structural development of the pituitary and testis in chicks of different weeks of age in order to provide basic information for a further exploration of the effect of HS on the development of pituitary and testis in Wenchang chicken.

2 Materials and methods

2.1 The animals

Eighty 1-day-old male Wenchang chicks provided by the Hainan Yongji Poultry Co., Ltd. (Hainan, China) were randomly divided into control (CK) and HS groups, with 40 chicks in each group. There was no significant difference in the body weight, feed intake, etc., between the two groups. All chicks had water and feed (Yilong Feed Factory, Zhanjiang, China) ad libitum. The basic daily diet met the NRC standards (1994). Chickens hosted in the large-capacity cages were placed in the feeding house (7 m × 3.5 m × 3.5 m), which had natural indoor ventilation and lighting (14 h of light and 10 h of dark). The house was cleaned and disinfected regularly. Temperature and humidity of the feeding house were 27.4 ± 0.9 °C and 72.1 ± 8.6 %, respectively, during the experiments.

2.2 HS treatment

Chicks in the HS group were placed in a heat chamber remodeled from a large-capacity artificial climate incubator at a temperature of 40 ± 0.5 °C and relative humidity of 73.6 ± 7.7 % for 2 h (13:00–15:00 UTC + 8) every day. The birds in the CK group were placed in another chamber under the same conditions but at room temperature. Chicks were returned to their cages for feeding at the end of treatment (Ramnath et al., 2008; Chen et al., 2014). The rectal temperatures of 10 chicks randomly selected from each group were measured before and after HS treatment. During the period of heat stress, chickens of the HS group and the CK group were provided with neither drinking water nor feed in the artificial climate incubator (Ramnath et al., 2008). The consumed feed and weight gain were recorded every week and the feed conversion ratio (FCR; feed-to-weight-gain ratio) was calculated.

2.3 Experimental method and data acquisition

At the end of each week, 6 chicks were randomly selected from each group ($n = 6$), weighed for fasting weight, and sacrificed by cervical dislocation. The pituitary and testis were completely dissected and weighed after removing excess tissue and washing with normal saline. The organs were then according to a particular routine sectioned and stained for hematoxylin and eosin (HE).

The histological structure of the pituitary and testis from chicks in both groups was observed under an Olympus BX50F-3 microscope, and the MiPrd3.1 microscopic image analysis software (Shangdong Yichuang Electronics Ltd., China) was used to measure and statistically analyze the diameter and cross-sectional area of 10 testicular seminiferous tubules randomly selected from each slide (two random slides for each testis). Digital images were taken of all sections using a YD400C digital camera (Shandong Yichuang Electronics Ltd., China) and saved.

2.4 Data processing and analysis

The experimental data were presented as mean ± standard deviation (SD) and analyzed using Excel 2003 and SPSS 16.0 statistical software. $P < 0.05$ and $P < 0.01$ were considered as significant and extremely significant differences respectively.

3 Results

3.1 The effect of HS on the body weight, FCR and rectal temperature

As shown in Fig. 1, with the increase in weeks of age, the mean weight of chicks in the HS group was significantly lower than that in the CK group, and there were extremely significant differences ($P < 0.01$) at 3, 4, and 6 weeks of age, indicating a significant effect of HS on the body weight of chicks. In addition, FCRs (feed intake / chick weight gain) in the HS group at 2, 4, and 6 weeks of age were significantly higher than those in the CK group ($P < 0.05$), further demonstrating the significant effect of HS on the feed con-

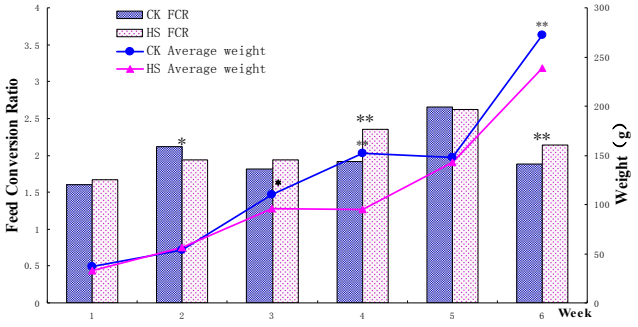

Figure 1. Effects of HS on FCR and weight of Wenchang chicks. Note: compared between CK and HS groups at the same age. * $P < 0.05$; ** $P < 0.01$.

Table 1. The Effects of HS on the rectal temperature of chicken ($n = 10$).

Week	Control	Heat stress
1	38.52 ± 0.197	40.13 ± 0.618
2	39.12 ± 0.310	40.87 ± 0.574
3	38.91 ± 0.314	41.43 ± 0.359^{b}
4	39.23 ± 0.216	41.52 ± 0.300^{a}
5	39.42 ± 0.482	41.61 ± 0.293^{a}
6	39.28 ± 0.425	41.82 ± 0.246^{a}

Compared between the CK group and the HS group at same age. [a] $P < 0.05$, [b] $P < 0.01$.

version efficiency in chicks. After heat stress, the rectal temperatures of chickens were significantly increased in the HS group, unlike in the CK group ($P < 0.05$; Table 1).

3.2 The effect of HS on the weight change in pituitary and testis in chicks

As shown in Fig. 2, the weight of chicks' pituitary gradually increased with the week of age; however, the weight of pituitary in chicks of the HS group was lower than that of chicks in the CK group, and the difference became extremely significant at 4–6 weeks of age ($P < 0.01$).

Similarly, Fig. 3 shows that with the increase in weeks of age, the weight of chicks' testes increased gradually; however, the mean testis weight of chickens in the HS group was significantly lower than that of the CK group, and the difference was extremely significant at 4–6 weeks of age ($P < 0.01$).

3.3 The effect of HS on the histological structure of pituitary and testis in chicks

Observation of the tissue sections revealed that the structure, outline, and cells in the pituitary of chicks in the CK group were clear; however, compared with the CK group, the pituitary in chicks of the HS group was smaller and had an

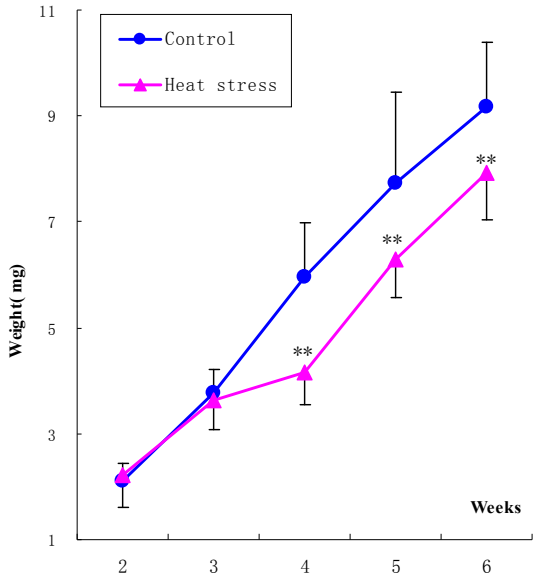

Figure 2. Effects of HS on the weight of pituitary in Wenchang chicks ($n = 6$). Note: compared between CK and HS groups at the same age. ** $P < 0.01$.

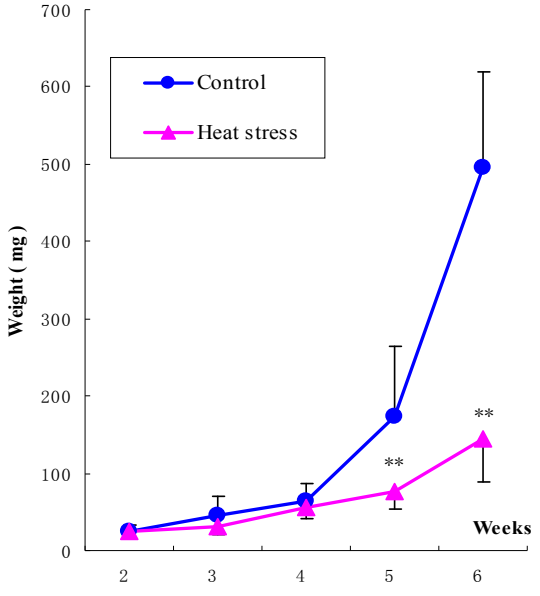

Figure 3. Effects of HS on the weight of testis in Wenchang chicks ($n = 6$). Note: compared between CK and HS groups at the same age. ** $P < 0.01$.

incomplete histological structure, displaying significant thermal damage (Fig. 4a and b).

Observation of the histological structure on sections also showed that in comparison with the CK group, the testes of chicks in the HS group exhibited a higher degree of fragmentation, significantly reduced integrity, thinner seminiferous epithelium, occurrence of vacuolar changes, reduced number of spermatogenic cells, and a loose and disordered arrange-

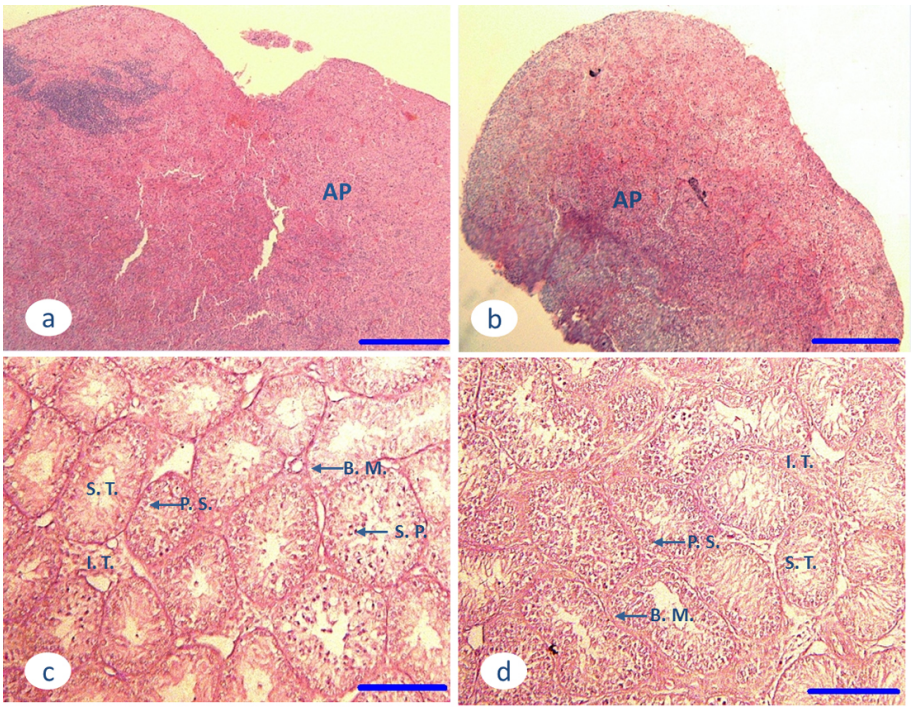

Figure 4. Effects of HS on the histological structure of pituitary and testis in 6-week-old chicks ($n = 6$). **(a)** The pituitary of a chick in the CK group; **(b)** the pituitary of a chick in the HS group; **(c)** the testis of a chick in the CK group; and **(d)** the testis of a chick in the HS group. A. P.: adenohypophysis; S. T.: seminiferous tubule; I. T.: interstitial tissue; P. S.: spermatogenic cell; S. P.: spermatoblast; and B. M.: basement membrane. Bar in **(a)** and **(b)**: 100 μm; in **(c)** and **(d)**: 50 μm.

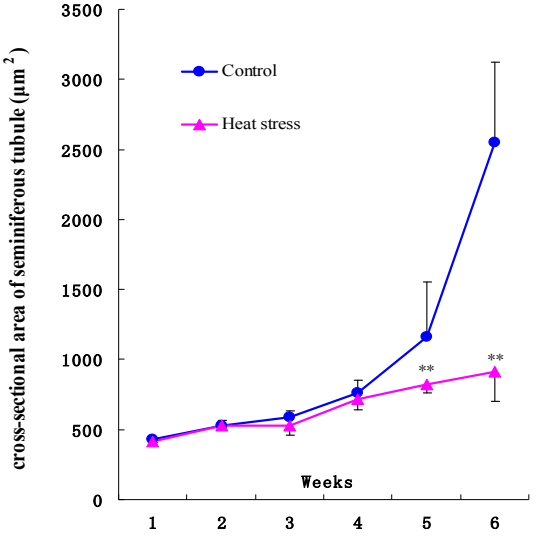

Figure 5. Effects of HS on the cross-sectional area of seminiferous tubule in chicks ($n = 6$). Note: compared between CK and HS groups at the same age. ** $P < 0.01$.

ment. Moreover, part of the seminiferous tubules only had a single layer of cells, composed of a small number of spermatocytes and Sertoli cells, without sperm cells (Fig. 4c and d). In addition, with the increase in weeks of age, the cross-sectional area of testicular seminiferous tubule increased in both groups; however, starting from 3 weeks of age, the difference in cross-sectional area of seminiferous tubule between the two groups became evident, and the difference was extremely significant at 5 and 6 weeks of age, reaching its maximum level at 6 weeks of age ($P < 0.01$). Starting from 4 weeks of age, the diameter of seminiferous tubule in chicks of the HS group was extremely significantly lower than that of the CK group ($P < 0.01$). These results indicated that HS induced seminiferous tubule atrophy and seriously affected the growth of chicks' testes (Fig. 5).

4 Discussion

Early studies have shown that high temperature may reduce the ability of the animal's brain to dissipate heat to the external environment, which affects the neuronal activity and thereby induces abnormality in the secretion of relevant hypothalamic hormones, further causing abnormal secretion of anterior pituitary FSH and LH. Because normally secreted LH and FSH in male animals promote the development of the seminiferous epithelium and the proliferation of spermatogonia, abnormal levels of LH and FSH secretion ultimately impair the normal process of testicular development. In addition, HS also seriously affects the reproductive performance of male mammals, drastically damages spermato-

genesis, impacts sperm motility, acrosome integrity, and the fertilization process, thereby reducing the success rate of animal breeding (Hansen, 2009; Paul et al., 2009; Kim et al., 2013). Recent studies have shown that under long-term HS, adult chickens displayed a series of physiological anomalies such as reduced ovarian weight and number of follicles, deteriorated ovarian function, and blocked development and formation of follicles. The serum contents of both LH and FSH in 30-week-old white Leghorn chickens reduced on the second day of HS; meanwhile, the levels of testosterone, P4, and E2 all significantly decreased (Rozenboim et al., 2007). HS also hinders the ovarian development and slows down the increase in the total number of follicles in different varieties of chicken. The contents of FSH and LH significantly reduced in laying hens under HS. After 7 days of HS, E2 content reduced significantly, while the content of P4 also significantly decreased after an initial elevation; meanwhile, HS significantly reduced the mRNA expression of LH and FSH receptors too (Li and Cui, 2013). These results indicated that HS seriously affected the normal developmental process of animal's reproductive organs.

Sertoli cells in testicular stroma are directly affected by certain environmental conditions or toxins. In male reproductive injuries caused by chemical and physical factors, the damage to the structure and function of Sertoli cells are all earlier than those of other tissues (Bergh et al., 1983; Richburg and Boekelheide, 1996). The number and functional status of Sertoli cells determine the testicular size and the normal progress of spermatogenesis (Russell, 1980), playing a decisive role in male reproductive function. The present study indicated that compared with the CK group, in the HS group, the integrity of the testicular structure in chicks was damaged significantly, and the seminiferous epithelium became thinner with vacuolar-like changes; the spermatogonia also showed a decreased number and loose and disordered arrangement. These data suggested that HS might have damaged the structure and function of the stromal Sertoli cells in testes of chicks in the HS group and thereby caused the thickening of seminiferous tubular epithelium and the inhibition of the development of different levels of spermatogenic cells, resulting in the phenomena of dysplasia exemplified by various degrees of atrophy. Between 3 and 6 weeks of age, the diameter of the seminiferous tubule in chicks of the HS group was significantly smaller than that of the CK group; at 5–6 weeks of age, the sectional area of the seminiferous tubule in chicks of the HS group was extremely significantly smaller than that of the CK group, further suggesting that 3–6 weeks of age may be one of the important stages for gonadal development in male chicks. In this period, the testicular weight and the diameter and sectional area of the seminiferous tubule increased significantly; however, HS extremely significantly affected this developmental process.

5 Conclusions

HS causes injuries to the histological structure of pituitary and testis in Wenchang chicks and seriously impedes the growth and development of reproductive organs in chicks.

Acknowledgements. This work is supported by research grants from National Natural Science Foundation of China (NSFC31260555, 3156068). We would like to express our great appreciation to Jia Xie, Jia Tang, Liang-yan Chen and Bo Wang for the assistance in the experiment.

Edited by: K. Wimmers
Reviewed by: two anonymous referees

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