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Yolk immunoreactive corticosterone in hierarchical follicles of Japanese quail (*Coturnix japonica*) exposed to heat challenge

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ABSTRACT

High temperature decreases the egg number, ovarian weight, and hierarchical follicle number. In the present study, we investigated the effect of high temperature on the quality of eggs of adult female quails. Laying quail were raised under a standard thermal condition of 25 °C until exposed to an elevated temperature of 34 °C (experimental) or maintained at 25 °C (control) from 12:00 to 16:00 for 10 consecutive days. Weight and number of eggs were measured; serum and the largest follicles were collected and used for hormone measurement. Ovaries and adrenals were collected for expression analysis of *3β-* and *17β-HSD*, genes encoding steroidogenic enzymes. Egg weight slightly decreased with an increase in the treatment time in the heat-challenged group; the egg weight significantly decreased in the heat treatment group than in the control group during the last 2 days of experiment ($P < 0.05$). The laying rate showed no difference during the experimental period but significantly decreased on the last day in the heat treatment group. In the experimental group the ovaries and oviducts were lighter ($P < 0.05$) and the hierarchical follicle number and ovarian weight decreased ($P < 0.05$) compared to the control group. Although serum corticosterone level significantly increased after heat challenge ($P < 0.05$) and immediately recovered to the normal level, yolk immunoreactive corticosterone in the hierarchical follicle (F1, F2, F3) significantly increased ($P < 0.05$). The expression level of *17β-HSD* showed no changes in the ovary but significantly increased in adrenals ($P < 0.05$). Our findings indicate that the effects of heat challenge on the maternal ovary in the quail are mediated through the adrenal function.

1. Introduction

Corticosterone is a dominant plasma glucocorticoid in birds. Animals show an increase in the production of corticosterone under stressed conditions (Kang et al., 2017). Heat stress may reduce the number of eggs and hierarchical follicles (Kirunda and Scheideler, 2001; Mashaly et al., 2004; Star et al., 2007) and decrease ovarian weight (Rozenboim et al., 2007) in domesticated birds. Avian egg provides abundant nutrition and is a rich source of maternal hormones such as steroids (Schwabl, 1993), derivatives of amino acids (Wilson and McNabb, 1997), and peptides (PABLO et al., 1982). Of these hormones, the steroid hormones have received considerable attention. Steroid hormones are highly lipid soluble. Avian yolks develop in proximity to steroidogenic ovarian tissues and contain large amounts of lipids. Quail exposed to long-term restraint stress shows an increase in the expression of corticosterone in hierarchical follicles and laid eggs (Okuliarová et al., 2010).

Heat stress is one of the main problems in modern aviculture. Rozenboim et al. (2007) found no significant change in plasma luteinizing hormone and follicle-stimulating hormone levels in chicken under heat stress; however, plasma 17β-estradiol and progesterone levels were significantly reduced and the mRNA expression of steroidogenic enzymes were decreased in response to heat stress. As 17β-estradiol and progesterone are mainly produced in the follicular granulosa cells and thecal cells, heat stress may exert direct effects on the ovarian function and affect the follicular development. Quail reared under hyperthermia can be used as a model to study the typical decrease in egg production during hot summer months.

The Japanese quail (*Coturnix japonica*) reach sexual maturity at 6–7 weeks of age. The female has a unique reproductive system, which can continuously complete follicular development, ovulation, and fertilization. The ovarian follicles may be categorized by follicle diameter

Abbreviations: SWF, small white follicle; LWF, large white follicle; SYF, small yellow follicle; RIA, radioimmunoassay; RT, reverse transcription; PCR, polymerase chain reaction

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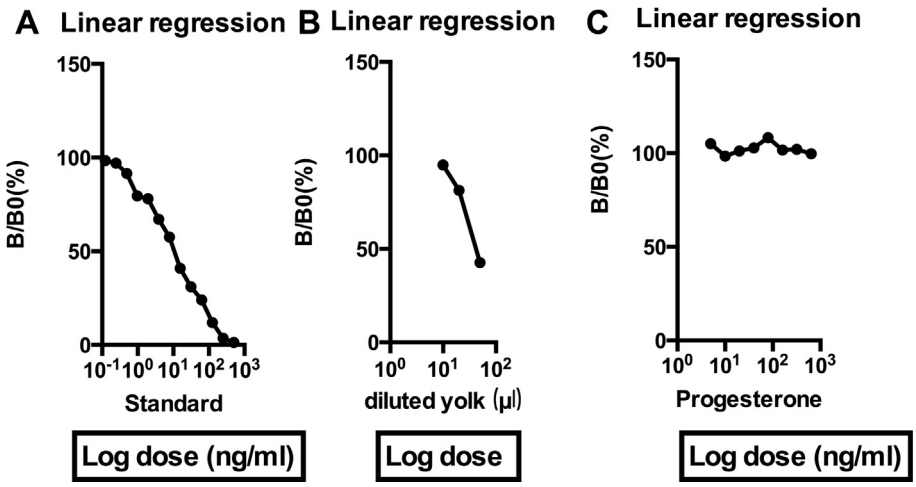

Fig. 1. Parallelism between the standard (A) and the dose response curve of yolk samples (B) for corticosterone, progesterone dose response curve under corticosterone measurement assay (C).

size. Pre-hierarchical follicles comprise small white follicles (SWFs), large white follicles (LWFs), and small yellow follicles (SYFs). Hierarchical follicles are divided into F1 to F6 based on their volumes (Liu and Zhang, 2008; Onagbesan et al., 2009). For consistent ovulation, one pre-hierarchical follicle is selected to become hierarchical follicle every day. In sexually matured birds, the elevated level of circulating concentrations of estrogen indirectly regulates the rapid growth of oocytes by potentiating the synthesis of yolk precursor proteins in the liver (Schneider et al., 1998). Limited information is available on the effect of heat challenge on follicular development and hormones in Japanese quail. Rettenbacher et al found the antibodies for corticosterone measurement cross-react with progesterone, thus a cross-react detection has been conducted, and found corticosterone immunoassay in present test didn't bind to progesterone (Fig. 1C). Therefore, the present study investigated the association between high temperature and follicular immunoreactive yolk hormones.

2. Materials and methods

2.1. Animals and experimental design

Female Japanese quail of a laying strain (give the strain type or designation) were hatched and raised in a breeding colony at the Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology. Animals were provided a commercial quail food diet (Quail Cosmos, Aichi, Japan) and water *ad libitum*. Quail were housed in metal cages in a controlled environment (lights on, 05:00 to 19:00; temperature, 23 ± 2 °C; humidity, 50 ± 10%; air exchanged 20 times/h).

At the age of 20 weeks, 28 healthy females with normal laying rate were selected and divided into a control and a heat groups. The animals were placed into Bio Multi incubator (LP-80CCFL-6CTAR, Japan). Bio Multi incubator setting was same as the animal room except for heat group females that were exposed to 34 °C for 4 h per day (12:00 to 16:00) for 10 consecutive days. Egg laying was monitored every day and egg weight was measured. On day 10 of treatment, five quail from each group were selected to obtain blood samples immediately after heat challenge, while the other quail were killed by decapitation 2 h after laying eggs. The ovaries were dissected and the three largest follicles were separated for follicular hierarchy (F1 > F2 > F3). All follicles were weighed and stored at −20 °C. Body weight and ovary and oviduct weights were measured. The ovary and adrenal gland were stored at −80 °C.

The experimental protocol was performed in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Institutional Animal Care and Use Committee, Tokyo University of Agriculture and Technology.

2.2. Egg dissection

Eggs collected during the experimental period were weighted (W_E); the yolk and shell were separated and the attached albumen was removed using filter paper. Yolk weight (W_Y) and shell weight (W_S) were measured. The weight of albumen (W_A) was calculated.

2.3. Hormonal assay

2.3.1. Sample preparation

Blood was collected in plastic tubes, centrifuged at 3000 × *g* for 30 min at 4 °C, and the separated serum was stored at −20 °C until analysis.

Small amount of yolk (100 mg) was withdrawn from the inside of follicle. Yolk sample was diluted in 1 mL of deionized water and homogenized.

2.3.2. Radioimmunoassay (RIA)

Serum and yolk concentrations of corticosterone were determined by a double-antibody RIA system with ^{125}I-labeled radioligands, as previously described (Taya, 1985). The antiserum against 17β-estradiol was sheep anti-17β-estradiol (GDN244) kindly provided by Dr. GD Niswender, Animal Reproduction and Biotechnology Laboratory (Colorado State University, Fort Collins, CO, USA). The antiserum against corticosterone was goat anti-corticosterone produced in our laboratory (Kanesaka et al., 1992; Ahmed et al., 2015). The intra- and inter-assay coefficient of variation for corticosterone 4.5% and 6.5% respectively. The parallelism between dose response curve of samples and the standard for corticosterone is shown in Fig. 1A and B.

2.4. RNA extraction, reverse transcription (RT), and quantitative polymerase chain reaction (PCR)

Total RNA was extracted from the ovary and adrenal gland using an Isogen reagent kit (Nippon Gene, Tokyo, Japan) according to the manufacturer's procedure. The concentration and purity of the isolated total RNA were spectrophotometrically determined at 260 and 280 nm with a Nanodrop (Thermo Fisher Scientific, Wilmington, USA). Total RNA (1 µg) was reverse transcribed to cDNA with an Omniscript® Reverse Transcription kit (Takara) with Oligo-dT primers (Takara) according to the manufacturer's protocol. The primers used were as follows:

3β-HSD, *CTGGGGAAACAGCAACAGCAG* (forward) and *TTATTTTG GTTCTGGGGATGA* (reverse); *17β-HSD*, *TCTTGGTGTGGGAATG* (forward) and *CCGGAATAGAAGGAAC* (reverse); the housekeeping gene beta-actin (*β-actin*) *TGAACCCCAAAGCCAACAG* (forward) *CCACAGGA CTCCATACCCAAG* (reverse). Gene expression was quantified by real-time PCR using an ABI 7500 Fast and a commercial kit (SYBR Premix Ex Taq™ II, TaKaRa). The specificity of the PCR product was verified with melting curve and agarose gel electrophoresis. The relative concentration of mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Ct values from control samples were used as calibrators.

2.5. Statistical analysis

All data are presented as mean ± standard error of the mean (SEM) and analyzed by *t*-test. Statistical analysis was performed with GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Differences were considered statistically significant for the value of $P < 0.05$.

3. Results

3.1. Corticosterone concentration in serum

Blood sample was collected within 30 min (Fig. 2A) or 2 h (Fig. 2B) after heat challenge. Results indicated that the level of serum corticosterone significantly increased after 30 min of heat challenge (Fig. 2A, $P < 0.05$) but the difference disappeared after 2 h (Fig. 2B).

3.2. Body, ovary, and oviduct weights and hierarchical follicles

Body weight of quail from the heat challenge group decreased significantly ($P < 0.05$) (Fig. 3A). A similar effect was observed in ovary weight ($P < 0.01$) (Fig. 3B). Hierarchical follicle number (F1 and F2) significantly decreased ($P < 0.05$) (Fig. 3F), suggesting that the decrease in the ovary weight may be attributed to the decrease in the size of the hierarchical follicles [is this what you are meaning?]. Oviduct weight (Fig. 3C) and hierarchical follicle number (Fig. 3E) decreased after heat treatment ($P < 0.05$).

3.3. Egg production and weight of albumen, yolk, and shell

Egg laying rate was expressed as egg laying per day per quail. Egg weight slightly decreased with an increase in the treatment period in the heat-challenged group. During the last 2 days, a significant decrease in the egg weight was observed in the heat-challenged group than in the untreated group (Fig. 4A $P < 0.05$ or $P < 0.01$). The laying rate significantly decreased on the last day of heat treatment (Fig. 4B, $P < 0.05$). The decrease in the egg weight may be related to the decrease in the level of yolk ($P < 0.05$) and albumen ($P < 0.05$) (Fig. 4C).

3.4. Immunoreactive corticosterone content in yolk

Heat challenge had a significant effect on the content of yolk immunoreactive corticosterone with quail from the heat challenge group showing significantly higher immunoreactive corticosterone levels in hierarchical follicles (F1, F2, F3) ($P < 0.05$) (Fig. 5).

3.5. Gene expression of steroidogenic enzymes in the ovary and adrenal gland

The mRNA transcript levels of *17β-HSD* significantly increased ($P < 0.05$) in the adrenal glands from the quail of the heat-challenged group compared to those from in the control group (Fig. 6D); however, no difference was observed in the expression levels of *3β-HSD* and *17β-HSD* in the ovaries from the two groups (Fig. 6A and C) ($P > 0.05$). Adrenal *3β-HSD* level slightly increased, but no significant difference was reported (Fig. 6B) ($P > 0.05$).

4. Discussion

The present study demonstrates that heat challenge increased the serum corticosterone level during treatment period in the Japanese quail (Fig. 2A); however, these levels returned to near-normal values after treatment (Fig. 2B). Yolk corticosterone levels may reflect the level of plasma corticosterone in hens (Henriksen et al., 2011). Daily corticosterone surge may cause corticosterone accumulation in the yolk and influence the quality of egg.

High temperature decreases egg production. Egg production, quality, and hatchability are the most important economic traits in the avian industry. These variables are affected by environment factors such as temperature (Samara et al., 1996) and photoperiod (Renema et al., 2001). Both acute and chronic heat stress have been documented to have adverse influence on egg production and feed intake of laying hens (Mashaly et al., 2004). This observation was also found in the present study, wherein a decrease in the egg weight (Fig. 4A) after heat challenge. The reduction in egg mass with no interruption in laying rate is commonly observed in laying hens exposed to mild heat (Yahav et al., 2000). We also find a decrease in the weight of the yolk and ovary follicle after heat challenge (Figs. 4C and 3F, respectively), suggesting that the decrease in the follicular mass upon heat treatment may result in the reduction in the weight of the egg.

High ambient temperatures were reported to suppress nutrient digestibility in poultry. Quail body weight decreased after heat treatment (Fig. 3A), as reported by Sahin and Kucuk (2003). The decreased egg production in heat-challenged quail may be owing to the reduction in feed intake and impairment in the utilization of nutrients.

High temperature influences ovary function. Heat stress induces

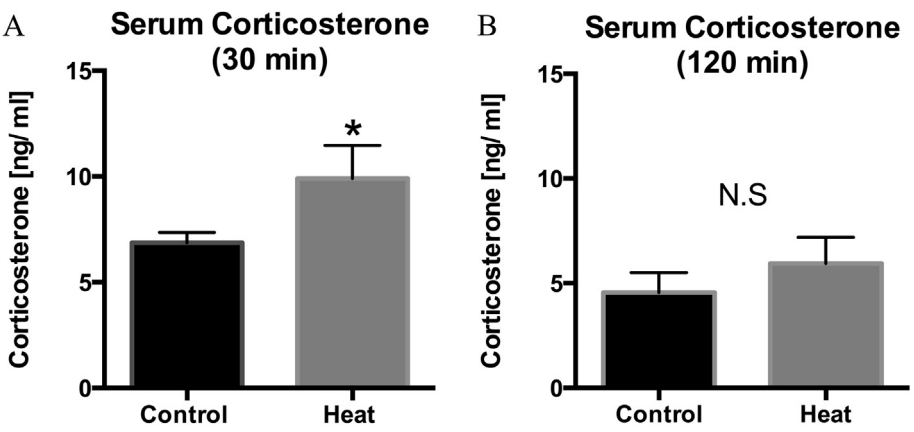

Fig. 2. Serum concentration of corticosterone evaluated from the blood obtained within 30 min of heat challenge (A) and 2 h after heat challenge (B) in the control and heat-challenged groups. Each bar represents the mean ± S.E.M. *Significantly different from control ($P < 0.05$, *t*-test). N.S means no significant difference ($P > 0.05$, *t*-test).

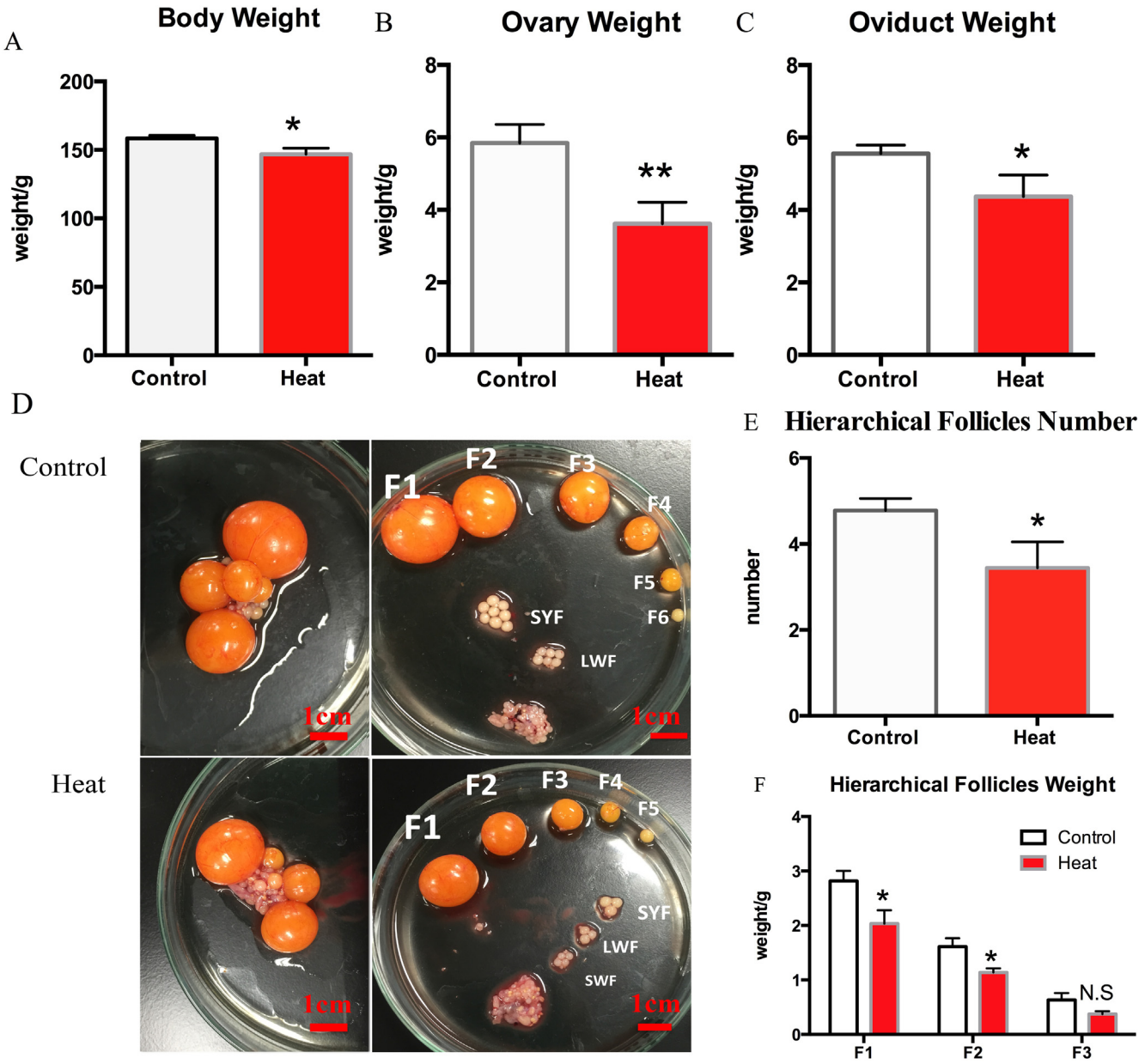

Fig. 3. Body weight (A), ovary weight (B), oviduct weight (C), hierarchical follicle number (E), and weight (F) in the control and heat-challenged groups. Ovaries from the control and heat-challenged groups (D left). F1 to F6 represent hierarchical follicles, prehierarchical follicle SYF (small yellow follicle), LWF (large white follicle), and SWF (small white follicle) (D right). Each bar represents the mean ± S.E.M. *Significantly different from control ($P < 0.05$, *t*-test). **Significantly different from control ($P < 0.01$, *t*-test). N.S means no significant difference ($P > 0.05$, *t*-test).

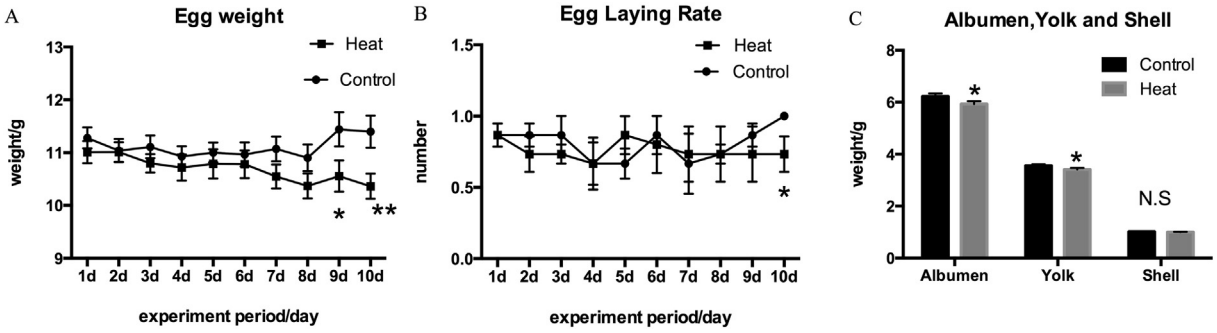

Fig. 4. Daily egg weight (A) and egg laying rate (B) during the experimental period in the control and heat-challenged quails. Weight of albumen, yolk, and shell (C) in the eggs collected from the control and heat-challenged groups. Each bar represents the mean ± S.E.M. *Significantly different from control ($P < 0.05$, *t*-test). **Significantly different from control ($P < 0.01$, *t*-test). N.S means no significant difference ($P > 0.05$, *t*-test).

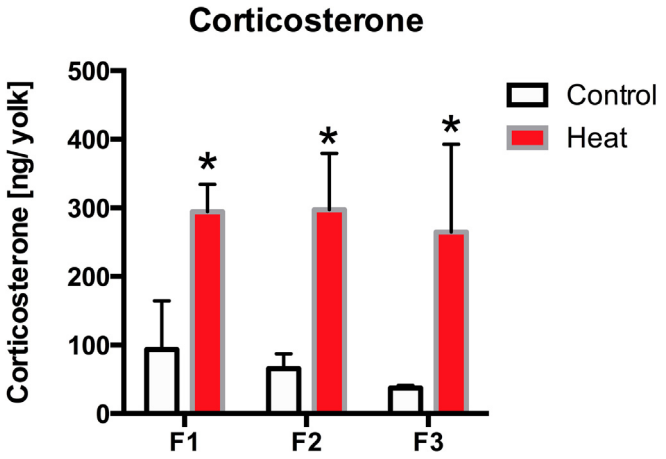

Fig. 5. Total corticesterone content in hierarchical follicles (F1, F2, and F3). Treatment effect indicates differences between the control quail (n = 9 for each follicle type) and heat-challenged quail (n = 9 for each follicle type). Each bar represents the mean ± S.E.M. *Significantly different from control (P < 0.05, *t*-test).

infertility in farm animals and represents a major source of economic loss to the livestock sector. The decrease in animal fertility is caused by elevated body temperature that influences ovarian functions, estrous behavior, oocyte health, and embryonic development (Sahin et al., 2003). Rozenboim et al. (2007) [be specific as to species] found that heat exposure may induce significant hyperthermia and reduce egg production, egg weight, ovarian weight, and the number of large follicle, consistent with the results of the present study. The regulatory mechanism underlying the reduced reproductive efficiency in the heat-stressed animal may be modulated at the level of ovary. The hypothalamic-pituitaryadrenal axis is sensitive to environmental perturbations. In birds, the main outcome of this pathway is elevated plasma corticosterone level. The present study showed similar results. In the present study, the yolk immunoreactive corticosterone content (Fig. 5) and serum corticosterone level (Fig. 2A) increased after heat challenge.

Heat induces the expression of corticosterone in the target organ, the adrenal gland. There is no change in the serum level of 17β-estradiol (data not shown) and the expression of genes encoding the ovarian steroidogenic enzymes (Fig. 6A and C); however, *17β-HSD* expression increased in the adrenal gland, indicating that heat stimulated the adrenal gland to secrete corticosterone that accumulated in the yolk. Okuliarová et al. (2010) reported that restraint stress increases

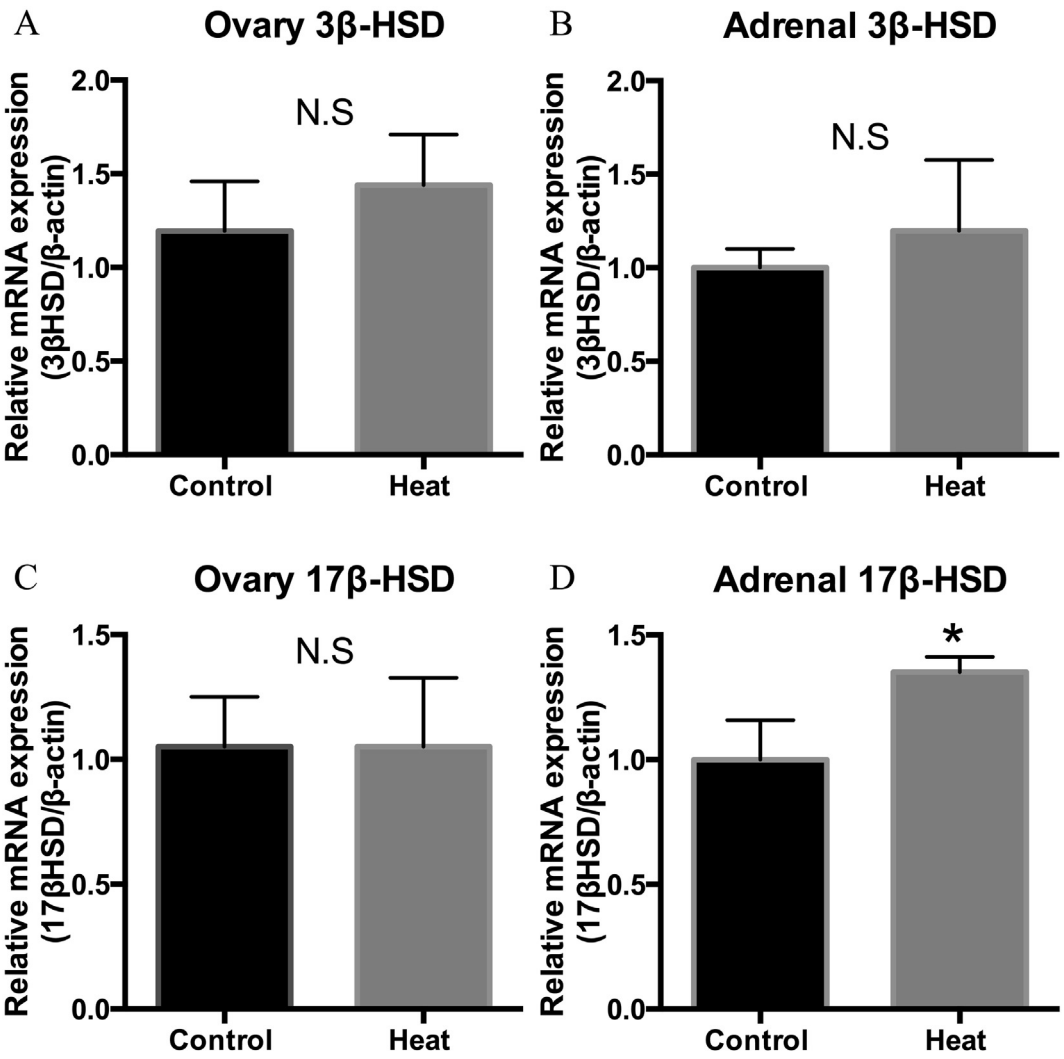

Fig. 6. Expression of genes encoding steroidogenic enzymes in ovaries and adrenal gland of control and experimental groups. Each mRNA level was normalized to β-actin mRNA level in the same preparation, and the mean of each experimental control was assigned a value of 1.0. A: ovary *3β-HSD* mRNA; B: adrenal *3β-HSD* mRNA; C: ovary *17β-HSD* mRNA; D: adrenal *17β-HSD* mRNA. The values shown are the mean ± SEM of 9 animals per group. *Significantly different from control (P < 0.05, *t*-test). N.S means no significant difference (P > 0.05, *t*-test).

corticosterone level in the hierarchical follicles and laid eggs of Japanese quail. High temperature in summer is a huge challenge to the avian industry, as yolk corticosterone level increases in response to heat. In addition, yolk accumulation during egg formation occurs over 7–12 days before ovulation (Johnson et al., 1986). During this period, serum corticosterone may accumulate in the yolk.

High corticosterone level in the yolk may cause multiple effects. Maternal stress during embryonic development affects the offspring phenotypes, physiology, and behavior (Del Giudice, 2012). Embryonic exposure to corticosterone results in the aggressive behavior of the chicken through alterations in the hypothalamic-pituitaryadrenal axis and serotonergic system, including modifications in DNA methylation or alteration in the 5-HT system (Ahmed et al., 2014). The high concentration of yolk corticosterone increases the pecking behavior of domestic chicks (Freire et al., 2006). Furthermore, prenatal corticosterone exposure is known to have both short- and long-term consequences (Love and Williams, 2008) such as decreased hatch weight (Janczak et al., 2006) and compromised immunity (Rubolini et al., 2005).

In the poultry industry, some strains are developed to be egg producers. Corticosteroids serve in part as anti-inflammatory agents and have been used to treat a variety of diseases for over seven decades. However, the long-term use of corticosteroids is generally avoided, given the risks of serious acute complications such as infection, venous thromboembolism, avascular necrosis, and fracture as well as chronic diseases such as diabetes mellitus, hypertension, osteoporosis, and other features of iatrogenic Cushing's syndrome (Waljee et al., 2017). Brassard et al. (2014) reported high risk for serious infections in patients with elderly onset inflammatory bowel disease on oral corticosteroid therapy. In the present study, yolk corticosterone level was three times higher in the heat-challenged quail than in the control quail (Fig. 5). The potential influence of egg corticosterone needs to be taken into consideration.

In conclusion, our study reports the elevated levels of immunoreactive corticosterone in hierarchical follicles. High temperature increases the level of serum corticosterone via regulation of adrenal steroidogenic enzymes, immunoreactive corticosterone in the yolk increased as well. Furthermore, the decrease in the follicular weight and number may be the reason underlying the decrease in the egg weight and number, suggesting that heat challenge may affect the maternal ovary by targeting the adrenal functions.

5. Declarations of interest

None.

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References

Ahmed, A.A., Ma, W., Ni, Y., Zhou, Q., Zhao, R., 2014. Embryonic exposure to corticosterone modifies aggressive behavior through alterations of the hypothalamic pituitary adrenal axis and the serotonergic system in the chicken. Horm. Behav. 65, 97–105.

Ahmed, E., Nagaoka, K., Fayez, M., Abdel-Daim, M.M., Samir, H., Watanabe, G., 2015. Suppressive effects of long-term exposure to P-nitrophenol on gonadal development, hormonal profile with disruption of tissue integrity, and activation of caspase-3 in male Japanese quail (Coturnix japonica). Environ. Sci. Pollut. R 22, 10930–10942.

Brassard, P., Bitton, A., Suissa, A., Sinyavskaya, L., Patenaude, V., Suissa, S., 2014. Oral corticosteroids and the risk of serious infections in patients with elderly-onset inflammatory bowel diseases. Am. J. Gastroenterol. 109, 1795–1802.

Del Giudice, M., 2012. Fetal programming by maternal stress: insights from a conflict perspective. Psychoneuroendocrinology 37, 1614–1629.

Freire, R., Van Dort, S., Rogers, L., 2006. Pre-and post-hatching effect of corticosterone treatment on behavior of domestic chick. Horm Behav. 49, 157–165.

Henriksen, R., Groothuis, T.G., Rettenbacher, S., 2011. Elevated plasma corticosterone decreases yolk testosterone and progesterone in chickens: linking maternal stress and hormone-mediated maternal effects. PLoS One 6, e23824.

Janczak, A., Braastad, B., Bakken, M., 2006. Behavioural effects of embryonic exposure to corticosterone in chickens. Appl. Anim. Behav. Sci. 96, 69–82.

Johnson, P., Dickerman, R.W., Bahr, J., 1986. Decreased granulosa cell luteinizing hormone sensitivity and altered thecal estradiol concentration in the aged hen, Gallus domesticus. Biol. Reprod. 35, 641–646.

Kanesaka, T., Taya, K., Sasamoto, S., 1992. Radioimmunoassay of corticosterone using 125I-labeled radio-ligand. J. Reprod. Dev. 38, j85–j89.

Kang, S.W., Madkour, M., Kuenzel, W.J., 2017. Tissue-specific expression of DNA methyltransferases involved in early-life nutritional stress of chicken, gallus gallus. Front. Genet. 8, 204.

Kirunda, D.F., Scheideler, S.E., 2001. The efficacy of vitamin E (DL-α-tocopheryl acetate) supplementation in hen diets to alleviate egg quality deterioration associated with high temperature exposure. Poultry Sci. 80, 1378–1383.

Liu, H., Zhang, C., 2008. Effects of daidzein on messenger ribonucleic acid expression of gonadotropin receptors in chicken ovarian follicles. Poultry Sci. 87, 541–545.

Love, O.P., Williams, T.D., 2008. The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. Am. Nat. 172, E135–E149.

Mashaly, M.M., Hendricks, G.L., Kalama, M.A., Gehad, A.E., Abbas, A.O., Patterson, P.H., 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Sci. 83, 889–894.

Okuliarová, M., Šárniková, B., Rettenbacher, S., Škrobánek, P., Zeman, M., 2010. Yolk testosterone and corticosterone in hierarchical follicles and laid eggs of Japanese quail exposed to long-term restraint stress. Gen. Comp. Endocr. 165, 91–96.

Onagbesan, O., Bruggeman, V., Decuypere, E., 2009. Intra-ovarian growth factors regulating ovarian function in avian species: a review. Anim. Reprod. Sci. 111, 121–140.

Pablo, F.D., Roth, J., Hernandez, E., Pruss, R.M., 1982. Insulin is present in chicken eggs and early chick embryos. Endocrinology 111, 1909–1916.

Renema, R., Robinson, F., Feddes, J., Fasenko, G., Zuidhoft, M., 2001. Effects of light intensity from photostimulation in four strains of commercial egg layers: 2 egg production parameters. Poultry Sci. 80, 1121–1131.

Rozenboim, I., Tako, E., Gal-Garber, O., Proudman, J., Uni, Z., 2007. The effect of heat stress on ovarian function of laying hens. Poultry Sci. 86, 1760–1765.

Rubolini, D., Romano, M., Boncoraglio, G., Ferrari, R.P., Martinelli, R., Galeotti, P., Fasola, M., Saino, N., 2005. Effects of elevated egg corticosterone levels on behavior, growth, and immunity of yellow-legged gull (Larus michahellis) chicks. Horm. Behav. 47, 592–605.

Sahin, K., Kucuk, O., 2003. Zinc supplementation alleviates heat stress in laying Japanese quail. J. Nutr. 133, 2808–2811.

Sahin, K., Onderci, M., Sahin, N., Gursu, M., Kucuk, O., 2003. Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. J. Nutr. 133, 1882–1886.

Samara, M.H., Robbins, K., Smith, M., 1996. Interaction of feeding time and temperature and their relationship to performance of the broiler breeder hen. Poultry Sci. 75, 34–41.

Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C T method. Nat. Protoc. 3, 1101–1108.

Schneider, W., Osanger, A., Waclawek, M., Nimpf, J., 1998. Oocyte growth in the chicken: receptors and more. Biol. Chem. 379, 965–971.

Schwabl, H., 1993. Yolk is a source of maternal testosterone for developing birds. Proc. Natl. Acad. Sci. U.S.A. 90, 11446–11450.

Star, L., Nieuwland, M., Kemp, B., Parmentier, H., 2007. Effect of single or combined climatic and hygienic stress on natural and specific humoral immune competence in four layer lines. Poultry Sci. 86, 1894–1903.

Taya, K., 1985. Radioimmunoassay for progesterone, testosterone and estradiol-17β using^ < 125 > I-iodohistamine radioligands. Jpn. J. Anim. Reprod. 31, 186–197.

Waljee, A.K., Rogers, M.A.M., Lin, P., Singal, A.G., Stein, J.D., Marks, R.M., Ayanian, J.Z., Nallamothu, B.K., 2017. Short term use of oral corticosteroids and related harms among adults in the United States: population based cohort study. BMJ 357, j1415.

Wilson, C.M., McNabb, F.A., 1997. Maternal thyroid hormones in Japanese quail eggs and their influence on embryonic development. Gen. Comp. Endocr. 107, 153–165.

Yahav, S., Shinder, D., Razpakovski, V., Rusal, M., Bar, A., 2000. Lack of response of laying hens to relative humidity at high ambient temperature. Brit. Poultry Sci. 41, 660–663.