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Effect of cyclical cold stress during embryonic development on aspects of physiological responses and HSP70 gene expression of chicks

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ABSTRACT

The objective of the present study was to evaluate the effects of cyclical lower incubation temperature at different embryonic ages on the hatchability, body and organs weights, thyroid hormones, and liver HSP70 gene expression of newly hatched chicks. In a completely randomized design, fertile eggs of a broiler breeder (34 weeks of age) were assigned to three treatment groups with six replicates and 145 eggs per each. The treatment groups were as: control group (C) that eggs were incubated at 37.6 °C during the whole incubation period; incubation temperature was decreased to 36 °C for 3 h per day at embryonic age from 12 to 14 (T1); and incubation temperature was decreased to 36 °C for 3 h per day at embryonic age from 15 to 17 (T2). No significant difference was found among treatments for hatchability ($P > 0.05$). There were no differences ($P > 0.05$) among treatments for body weight and liver weight, while heart weight of chicks in T1 and T2 groups were significantly higher than the control group ($P < 0.05$). There were no differences ($P > 0.05$) among treatments for the levels of thyroid hormones, however, the levels of both hormones tended to increase in chicks exposed to cold stress (T1 and T2). Chicks in T2 group had higher liver HSP70 gene expression compared with those in T1 and the control group ($P < 0.05$). Cold stress in both incubation periods had no significant effect on the plasma levels of aspartate aminotransferase and alanine aminotransferase. Treatments had no effect on the plasma levels of glucose, cholesterol and triglyceride. The results of this study suggest that cyclical lower incubation temperatures (36 °C) at the embryonic age from day 15–17 could induce the liver HSP70 gene expression, without negative effects on the hatchability and body weight of hatched chicks.

1. Introduction

It is well known that any kinds of stressors during embryo incubation rapidly induce the synthesis of heat shock protein 70 (HSP70) (Lindquist and Craig, 1988), which involved in development of heat and cold tolerance (Parsell and Lindquist, 1994; Zhao et al., 2014). From epigenetic aspect, cold tolerance in broiler chicks has become increasingly important (Zhao et al., 2014). It is possible to enhance the resistance of chicks to low ambient temperatures through manipulation of incubation temperature during sensible phases of embryonic development (Tzschentke et al., 2001; Nichelmann, 2004). This manipulation is being used as a tool to induce temperature adaptation and thermo or cold-tolerance in chicks (Tzschentke, 2007; Zhao et al., 2014) and has a practical advantage for reducing the rate of ascites syndrome (Stolz et al., 1992; Baghbanzadeh and. Decuypere, 2008). During the first half of incubation (embryonic ages from 1 to 11) chicken embryos go through a differentiation phase involving organs formation, and at the embryonic age 12, major organs are already formed (Smit et al., 2008). Embryos are more sensitive to thermal changes in differentiation phase; therefore, any changes in the first half of incubation period may have detrimental consequences for embryonic organogenesis and viability (Bensaude et al., 1990).

Shinder et al. (2009) reduced incubation temperature to 15 °C for 30 or 60 min at the late of embryonic development and produce cold resistance chicks with higher body weight at hatch without affecting the hatchability. Nichelmann et al. (1994) reported that resistance of hatched ducklings to cold stress enhanced by reducing the incubation temperature. In contrast, Willemsen et al. (2010,2011) reported that 3 °C incubation temperature under optimum at the embryonic age 16–18 had no effect on the embryo growth and hatchability. In the previous cold stress studies (Suarez et al., 1996; Joseph et al., 2006; Shinder et al., 2009; Willemsen et al., 2010), constant lower temperatures were applied throughout the incubation and deviations from optimum

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incubation temperature were high. Information scarcity in this field is physiological response and gene expression of HSP70 of hatched chicks that daily cyclically incubated 1 °C under optimum incubation temperature for 3 h during embryonic age of 12–14 or 15–17.

It was hypothesized that cyclical lower incubation temperature at these periods could enhance physiological aspects and HSP70 gene expression in newly hatched chicks. Therefore, the purpose of this study was to assess the effect of lower incubation temperature from the embryonic age of 12–17 on the hatchability, body and organ weights, HSP70 gene expression, blood biochemical and hormonal levels in the post-hatch chicks. In the stressed cells, HSP70 binds to sensitive proteins and protects them from degradation (Bensaude et al., 1990) and the liver HSP70 gene expression was evaluated in the present study as it is an index of cold and heat tolerance (Givisiez et al., 2001). Plasma triiodothyronine and thyroxine levels of hatched chicks were evaluated as they are reference measurements for status of metabolism (McNabb, 2000) and have important role in the hatching process and thermoregulatory mechanisms (Decuypere et al., 2007). In addition, plasma cholesterol, triglyceride and glucose were determined to give an indication of the effect of cold stress on lipid and carbohydrate metabolism.

2. Material and methods

Two single-stage incubators (Petersime, B-9870 Model, Olsene, Belgium) were used for incubation of eggs in this study. The incubators were equipped with electronic temperature and humid controllers that maintain a consistent temperature and relative humidity. The controls of one incubator were adjusted to hold the incubation temperature, for control group at 37.6 °C and 60% relative humidity from setting to the end of embryonic age, and for treatment 1 and 2 at 37.6 °C and 60% relative humidity from setting to the embryonic age 12 and 15, respectively; while being turned once per hour. Another incubator controls were adjusted to low temperature for two different periods as stated below.

Fertile eggs with average weight of 58 ± 1 g (n=2700) were obtained from Arian broiler breeder flock with 34 weeks of age. The eggs were weighed and then divided into three groups as control and two cold stressed groups. On day 11 of incubation, eggs were candled to remove non-fertile eggs and early dead embryos from the trays.

In a completely randomized design, eggs with live embryo (n=2610) were assigned to three incubation temperature treatments with six replicates and 145 eggs per each. The treatment groups were as: control group (C) that eggs were incubated at 37.6 °C during the whole incubation period; incubation temperature was decreased to 36 °C for 3 h per day at the embryonic age from 12 to 14 (T1); and incubation temperature was decreased to 36 °C for 3 h per day at the embryonic age from 15 to 17 (T2). All eggs were arranged in tray racks equipped with wheels and incubated in an incubator at the same time. Eggs of treatments 1 (at the embryonic age 12–14) and 2 (at the embryonic age 15–17) were daily transferred to another incubator that adjusted for 36 °C and 60% relative humidity for 3 h, thereafter returned to the incubator with optimum temperature. After the embryonic age 14 or 17, eggs were incubated in the incubator with optimum temperature.

On day 18 of incubation, eggs were placed in suitable hatching trays and the same conditions of temperature (37.5 °C) and relative humidity (57%) were applied to eggs for three groups. The hatchability of fertile eggs was recorded for each treatment. Two hours after hatching and after the feathers dried; each chick was taken out of the hatcher tray for sex detection. All newly hatched males (n=1226) were weighed and then three males from each replicate (18 birds per treatment) were randomly selected for blood collection. The blood samples were taken from selected chicks in Heparin-gel containing vacuum tubes directly from heart. For separation of plasma, tubes were centrifuged ($2000 \times g$) for 15 min, and then supernatant removed. Plasma of three chicks per each replicate was pooled and stored at −20 °C for further analysis.

The plasma levels of triiodothyronine and thyroxine were determined using commercially ELISA kits (Biocheck Inc., Foster City, CA, USA). Plasma levels of glucose, cholesterol, triglyceride, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured photometrically by auto-analyzer (BS-120 Model, Minbray Co., USA) and the commercial kits (Pars Azmoon Co., Tehran, Iran).

After blood sampling, selected male chicks were anesthetized with diethyl ether, then abdominal cavity opened, liver and heart removed and weighed. Liver samples were obtained, pooled together (three samples of each replicate) and placed in a cryoprotectant tube, then frozen in the liquid nitrogen and stored in the freezer (at −70 °C) until analysis for HSP70 gene expression.

The relative abundance of HSP70 mRNA was determined using quantitative PCR. The frozen liver sample was crushed in a sterile mortar, and the powder was applied for total RNA extraction using Accuzol reagent (Cat. No. K-2102, Bioneer Co., Seoul, South Korea). The ratio of the absorbance readings at 260 and 280 nm was used for determining total RNA purity. In addition, the quality of RNA was evaluated by visualization of distinct 28 S and 18 S rRNA bands after gel electrophoresis with ethidium bromide staining. A quantity of 1 μg of each RNA sample was reverse transcribed to cDNA using the commercial kit (Bioneer Co., Seoul, South Korea). The 20 μL cDNA synthesis reaction contained in addition to the RNA template, 2 μL of 10 × RT buffer, 0.8 μL of 25X dNTP mix, 2 μL 10 × RT Random Primers and 1 μL MulitScribe Reverse Transcriptase. A volume of 10 μL of nuclease-free water was added bring the reaction up to final volume. The resulting cDNA was stored at −20 °C prior to use.

Quantitative PCR was performed with a specific primer pairs (Table 1, *Gallus gallus*, AY; 372 bp-763790) using Quanti Fast SYBER Green PCR kit (Cat. No: 204052, QIAGEN, Tehran, Iran). GAPDH (M-32599; 230 bp) was applied as a reference gene. Amplification of liver HSP70 gene was performed for 45 cycles, which consisted of an initial activations step (95 °C, 5 min), denaturation cycle (95 °C, 10 s) and combined annealing and extension (60 °C, 30 s). The GAPDH reference gene was amplified at 45 cycles under the same conditions in a different tube. After each run, preparation of standard curve was performed by serial dilution of pooled cDNA from samples. The relative expression ratio of HSP70 as a target gene was normalized to GAPDH gene based on the method of Livak and Schmittgen (2001). Quantification was performed in triplicates for each treatment group.

SAS software (version 9.1; SAS Institute, Cary, NC, USA) was used for statistical analysis. Analysis of variance was performed and differences among treatments were separated using the Turkey's test. The level of significance was $P < 0.05$.

3. Results

As presented in Table 2, no significant effect of cold stress found on the hatchability ($P > 0.05$). There were no differences ($P > 0.05$) among treatments for body weight and liver weight (Table 1), while heart weight of chicks in cold stress were significantly higher than the control group ($P < 0.05$).

The effects of cold stress on the plasma levels of triiodothyronine and thyroxine are presented in Table 3. Although there were