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The effects of thermal manipulations during early and late embryogenesis on body temperatures of Japanese quails (*Coturnix coturnix japonica*)

Sezai Alkan[a]*, Taki Karsli[a], Kemal Karabağ[b], Aşkin Galiç[a] and Murat Soner Balcioğlu[a]

[a]*Department of Animal Science, Faculty of Agriculture, Akdeniz University, Antalya, Turkey;* [b]*Department of Beekeeping, Rize University, Vocational Academy of Pazar, Rize, Turkey*

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The aim of this study was to determine the effect of thermal manipulations during early embryogenesis (EE) and late embryogenesis (LE) on body temperature of Japanese quails (*Coturnix coturnix japonica*). Incubation conditions from day 0 to day 17 were 37.7°C and 55% relative humidity for control group. In the thermally treated eggs during EE (EE_6, EE_7 and EE_8 days), incubation temperature was increased to 41°C and relative humidity to 65% for 3 hours (12:00–15:00) on the sixth, seventh and eighth days of incubation. Also, in the LE stage (EL_{12}, EL_{13} and EL_{14} days), incubation temperature was increased to 41°C and relative humidity to 65% for 3 hours (12:00–15:00) on the 12th, 13th and 14th days of incubation. Average temperature, relative humidity and total heat of indoor air were changed from 28.71 to 30.44°C, from 45.69 to 57.15% and from 14.67 to 16.16 kcal kg^{-1} dry air, respectively. Higher total heat of indoor air in 10–11 weeks than that of other weeks was found. Significant differences between the control (41.52 ± 0.26°C) and manipulation groups were found, but no significant difference between the EE (41.23 ± 0.40°C) and LE (41.26 ± 0.37°C) groups with respect to body temperatures. Body temperatures of quails were measured as 41.55 ± 0.31°C, 41.56 ± 0.26°C; 41.17 ± 0.33°C and 41.07 ± 0.30°C at 10, 11, 12 and 13 weeks of age, respectively. In addition, differences among the weeks in point of body temperatures of quails were found to be significant ($p < 0.01$).

Keywords: Japanese quail; embryogenesis; thermal manipulation

Introduction

The Japanese quail is the smallest avian species farmed for egg and meat production and it has also assumed world-wide importance as a laboratory animal (Baumgartner 1994; Minvielle 1998;; Alkan et al. 2010). Heat tolerance of broilers can be improved by exposing eggs to higher temperatures during incubation. Tzschentke and Basta (2002) reported that incubation at temperatures lower or higher than standard resulted in changes in post-natal hypothalamic thermosensitivity. Thus, incubation temperatures may be of particular importance for improving the ability to adapt to an expected environment (Nichelman 2001). The development of the embryo in incubation processes (chicken, turkey, duck, etc.) is strongly dependent on the physical micro-environment around the egg (Swann and Brake 1990; Tullett 1990; Visschedijk 1991; Wilson 1991). The incubation temperature around the eggs is of vital importance for the development of the living organism, since the embryos are poikilothermal (they have a varying body temperature) during their ontogenesis (the development of the individual organism) and depend on the environment (mother animal or incubator air) to keep the body temperature at a constant level (Swann and Brake 1990; French 1997).

Acclimation during the animal's life span alters the thermoregulatory threshold response for heat production and heat dissipation, and this change may be coupled with expanded safety margins of the body core temperature (Horowitz 1998, 2002). Thermal manipulations (TMs) are very early in the chick's life, while its body temperature regulation and feedback mechanisms are still immature (Arjona et al. 1988; Modrey and Nichelmann 1992), also caused changes in the thermoregulatory threshold response (Yahav 2000). Thus, the application of TMs during incubation would likely lead to effective alterations in the thermoregulatory response thresholds of the embryo and the hatched chick, depending on the timing and severity of the manipulation. Moraes et al. (2003) indicated that heat treatment of 39°C on days 13–17 of incubation enhanced thermotolerance of broilers post-hatch. Yahav et al. (2004a) concluded that TM during incubation had a positive effect on thermoregulation without affecting body weight of chicks at hatch.

It was previously reported that exposing embryos to high or low temperatures during incubation

*Corresponding author. Email: sezaialkan@akdeniz.edu.tr

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improved their capacity to adapt to hot or cold environments, respectively, in the post-hatch phase (Decuypere 1984; Minne and Decuypere 1984; Janke et al. 2002; Yahav et al. 2004b). The timing of TM has to be linked to the development of the hypothalamus–hypophysis–thyroid axis to change the heat production threshold response (Yahav et al. 2004), and to the development of the hypothalamus–hypophysis–adrenal axis to avoid increase in stress response (Epple et al. 1997).

The present study was conducted to determine the effects of TMs during early embryogenesis (EE) and late embryogenesis (LE) on body temperatures of Japanese quails (*Coturnix coturnix japonica*).

Materials and methods

Incubation conditions from day 0 to day 17 were 37.7°C and 55% relative humidity for control group. In the thermally treated eggs during EE (EE_6, EE_7 and EE_8 days), incubation temperature was increased to 41°C and relative humidity to 65% for 3 hours (12:00–15:00) on the sixth, seventh and eighth days of incubation. Also, in the LE stage (EL_{12}, EL_{13} and EL_{14} days), incubation temperature was increased to 41°C and relative humidity to 65% 3 hours (12:00–15:00) on the 12th, 13th and 14th days of incubation.

Eggs were divided into three groups in this study. Immediately after the thermal treatments were terminated, incubation conditions were restored to the regular levels (37.7°C and 55% relative humidity). The eggs in incubators were turned automatically every hour. At the 15th day of incubation, the eggs were transferred to hatching trays. In each trial, hatched chicks were wing-banded and individually weighed. Chicks were housed in controlled temperature battery brooders at a density of 130 cm^2 $quail^{-1}$. The temperature was 34°C in the first week of age and was reduced by 2°C per week until the quails were 4 weeks old, and then supplemental heating was disconnected. Then, quails were housed in individual cages in quail house with windows at both sides and exposed to 16 hours of light and 8 hours of darkness. During the experiment, the quails were fed with a diet consisting of 11.7 MJ kg^{-1} metabolic energy and 210 g crude protein kg^{-1} as ad libitum, and unlimited water was supplied during the experiment. The temperature and humidity of inside air were measured using data logger.

Rectal temperature, as a measure of body temperature, was obtained using digital thermometer (± 0.01°C) by insertion of approximately 3 cm into the cloak. The temperature was considered to be stable if it does not increase more than 0.06°C in 5 s. The temperatures of most of the quails were stable within 10 s, but occasionally as long as 30 s were required to reach a stable body temperature. In this study, rectal temperatures of quails were measured in the afternoon hours (13:00–14:00 pm) and in age at 10, 11, 12 and 13 weeks in August month.

Statistical methods

Following enthalpy of air equation was used as described by Mutaf (1990).

$$i_r = 0.24t_{db} + (595 + 0.47t_{db})x$$

where,

i_r = Enthalpy (kcal kg^{-1} dry air);
0.24 = Specific heat of dry air (kcal kg^{-1} deg);
t_{db} = Dry – bulb air temperature (°C);
595 = Latent heat of evaporation at 0 °C (kcal kg^{-1}); 0.47 = Specific heat of water vapour (kcal kg^{-1} deg); x = Specific humidity of dry air (kcal kg^{-1} dry air).

Data were analysed by using the General Linear Model Procedure of SAS (SAS, Statistical.... 1999). The following model was used for determination of the effect of manipulations and weeks on body temperatures.

$$Y_{ijk}=\mu+A_i+S_j+e_{ijk}$$

Y_{ijk} = represents the body temperature
A_i = effect of the i^{th} manipulation
S_j = effect of the j^{th} week
e_{ijk} = error term, $\sim N(0, \sigma_e^2)$

Results and discussion

Means and standard errors for weekly temperature, relative humidity and total heat values of indoor air are given in Table 1.

Means and standard errors for temperature, relative humidity and total heat of air are given in Table 1. As it was presented, average temperature, relative humidity and total heat of air were changed from 28.71 to 30.44°C, from 45.69 to 57.15% and from 14.67 to 16.16 kcal kg^{-1} dry air, respectively. There was found higher total heat of air in 10–11 weeks than that of other weeks.

Means and standard errors of body temperature of quails in respect to weeks and TMs are given in Table 2. As it was seen, there were found significant differences between the control and manipulation

Table 1. Temperature, relative humidity and total heat of indoor air.

Age (week)	Temperature (°C)	Minimum	Maximum	Relative humidity (%)	Minimum	Maximum	Total heat (kcal kg^{-1} dry air)
10	30.29 ± 0.31	24.30	38.20	53.02 ± 1.29	17.20	81.00	16.04
11	29.31 ± 0.28	23.10	36.90	57.15 ± 1.12	19.90	80.80	16.16
12	30.44 ± 0.35	23.10	40.00	45.69 ± 1.49	13.50	79.00	15.23
13	28.71 ± 0.32	21.10	36.80	51.01 ± 1.45	12.80	80.00	14.67
Average	29.68 ± 0.16			51.72 ± 0.69			15.56

groups, but no significant difference between the manipulation groups in respect to body temperatures. In addition, significant differences were found among the weeks in point of body temperatures.

Thermal manipulation during the early life of the chick, when body temperature (T_b) regulation and feedback mechanisms are still immature, causes changes in the thermoregulatory threshold response (Yahav 2000; Nichelmann and Tzschentke 2002). Exposing embryos to high or low temperatures during incubation improves their capacity to adapt to hot or cold environments, respectively, in the post-hatch phase (Decuypere 1984; Minne and Decuypere 1984; Janke et al. 2002). Epple et al. (1997) suggested that the embryonic chicken is susceptible to stress. In a study to improve the broilers' thermotolerance, Yahav and Hurwitz (1996) showed that thermal conditioning at the age of 3 or 5 days improved acquisition of thermotolerance in broilers exposed to heat stress at 6 weeks of age. The improved thermotolerance was characterised by the development of relatively mild hyperthermia, suggesting better ability to maintain body temperature than that of the control chickens. Also, Yahav et al. (2004a) concluded that thermal treatments during LE (LE_{16}–LE_{18}) of the chicks did affect body temperature. The findings reinforced the evidence for a positive effect on thermoregulation, which most probably resulted in a reduced metabolic rate (Yahav et al. 2004a, 2004b). It was recently demonstrated that mild TM from day 16 to day 18 of incubation induced a reduction in body temperature at 3 days post-hatch. Such TM was shown to improve chick thermotolerance by limiting the increase in its body temperature during heat challenge at 3 days of age (Yahav et al. 2004b; Collin et al. 2005).

Collin et al. (2007) reported that all thermally treated (39.5°C and 65% humidity for 3 hours) chicks exhibited significantly lower body temperature than controls immediately post-hatch. At days 14 and 21, the body temperatures for all treated chickens were significantly lower than those of the control chickens, but from day 28 the body temperature for both EE and LE chickens no longer differed from those of control birds. At 35 days, only the body temperatures of EE chickens were significantly lower than those of control, both EE- LE and late (LE) embryogenesis chickens. There was no difference between groups at 41 days of age.

The body temperature of chicks that had been thermally treated (38.5°C and 65% humidity for 3 hours) during embryogenesis was significantly lower than that of the control chicks (Modrey and Nichelmann 1992). Yahav et al. (2004b) indicated that thermally (39.5°C and 65% humidity for 3 hours) treated chicks exhibited significantly lower body temperature (37.72°C) than that of the control chicks (38.12°C).

Thermal manipulation of quail chick embryos applied during EE or LE did improve acquisition of thermotolerance by limiting the increase in its body temperature tested at 10, 11, 12 and 13 weeks of ages. The amount of total heat in the air increases, birds are affected negatively. Birds produce more heat, and

Table 2. Body temperatures of quails in terms of weeks and thermal manipulations.

	Thermal manipulations			
Age (weeks)	Control (C)	Early embryonic (EE)	Late embryonic (LE)	Average
10	41.63 ± 0.29 S	41.52 ± 0.32 S	41.50 ± 0.33 S	41.55 ± 0.31 Y
11	41.64 ± 0.19 S	41.49 ± 0.34 S	41.55 ± 0.23 S	41.56 ± 0.26 Y
12	41.47 ± 0.28 S	40.95 ± 0.23 P	41.08 ± 0.23 PR	41.17 ± 0.33 X
13	41.36 ± 0.16 RS	40.95 ± 0.30 P	40.90 ± 0.16 P	41.07 ± 0.30 X
Average	41.52 ± 0.26 B	41.23 ± 0.40 A	41.26 ± 0.37 A	41.34 ± 0.37

Note: (A, B) means in a row with no common letters differ significantly for manipulations ($p < 0.01$).
(X, Y) means in a column with no common letters differ significantly for weeks ($p < 0.01$).
(P, R, S) means with no common letters differ significantly for subgroups ($p < 0.01$).

they have greater difficulty in dissipating heat in hot climates. Heat transmitting by radiation, convection and conduction depend on the temperature difference between the birds and their environment. When the heat of air increased, because of the low differences between the birds and their environment, heat loses from body surface to air decreased. Depending on this situation, body temperature of birds increases. So, body temperatures of quails at 10–12 weeks of ages were found significantly higher than those of other weeks. Because, the amount of total heat in the air was higher at 10–11 weeks of air than those of other weeks. As result, further researches are required to determine different effects of TM and improve the acquisition of thermotolerance.

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