Archiv Tierzucht 56 (2013) 78, 789-796

Original study

The effects of thermal manipulation during early and late embryogenesis on hatchability, hatching weight and body weight in Japanese quails *(Coturnix coturnix japonica)*

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Abstract

This study aimed to determine the effects of thermal manipulation during early embryogenesis (EE) and late embryogenesis (LE) on hatching weight, body weight at 5 weeks of age, hatchability and embryonic mortality rate in 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 early embryogenesis (EE6-EE8 days), incubation temperature was increased to 41 °C and relative humidity to 65 % for 3 h (12.00-15.00) at 3 consecutive days. Also, in the late embryogenesis stage (LE12-LE14 days), incubation temperature was increased to 41 °C and relative humidity to 65 % 3 h (12.00-15.00) at 3 consecutive days. At hatching in each trial, all chicks were wing-banded and individually weighed. Thermal manipulations had significant effects on hatching weight, and lowest hatching weights were found in LE group in terms of male and female animals. In addition, thermal manipulations and gender had significant effects on body weight at 5 weeks of age and lowest body weights at 5 weeks of age were detected in LE group for both genders.

Keywords: quail, thermal manipulation, hatching weight, body weight, hatchability

Abbreviations: C: control, EE: early embryogenesis, LE: late embryogenesis, TM: thermal manipulation

Archiv Tierzucht 56 (2013) 78, 789-796
doi: 10.7482/0003-9438-56-078

Received: 6 November 2013
Accepted: 18 June 2013
Online: 18 June 2013

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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. The Japanese quail, which has been widely used for biological and genetic studies (Narayan *et al.* 1998, Minvielle 1998) has a lot of advantages, because it has a small body size, it is easily handled and large number of birds can be kept in a limited space. Depending on the day length, some females start laying with 35 days of age (average 40 days) and are in full production with 50 days of age. Its short generation interval and consequently its ability to produce 3 to 4 generations per year, turn the Japanese quail into an interesting laboratory animal. That is why it is an economic animal model for research (Ozcelik & Ozbey 2004, Seker *et al.* 2005, Kul *et al.* 2006, Nowaczewski *et al.* 2010).

The optimum temperature for the chicken embryo is 37.8 °C and it should not vary more than 0.3 °C (Wilson 1991). Because the developing embryo is poikilothermic, any changes in incubation temperature may affect embryo size, organ growth, metabolic rate, physiological development and hatching success (Yalcin & Siegel 2003). It was previously reported that embryos, exposed to high or low temperatures during incubation, improved their capacity to adapt to hot or cold environments, respectively, in the post hatch phase. The timing of thermal manipulation (TM) has to be linked to the development of the hypothalamus-hypophysis-thyroid axis to change the heat production threshold response, and to the development of the hypothalamus-hypophysis-adrenal axis to avoid increase in stress response (Minne & Decuypere 1984, Janke *et al.* 2002, Yahav *et al.* 2004a, 2004b). In other words, temperature during incubation may influence thermoregulation of birds after hatching. Therefore, the peculiarity of epigenetic adaptation, which may occur during early pre- or postnatal ontogeny (Nichelmann & Tzschentke 2002), may contribute to alleviating problems associated with the control of thermoregulation during rearing. This reasoning suggests that modifying standard incubation temperatures by altering temperatures may be a way to enhance post-hatching performance of poultry (Yalcin & Siegel 2003).

The present study was conducted to determine the effects of thermal manipulations during EE and LE on hatchability, embryonic mortality rate, hatching weight and body weight at 5 weeks old quails (*Coturnix coturnix japonica*).

Material and methods

This research was conducted in Akdeniz University, Faculty of Agriculture, and Department of Animal Science in Turkey in summer season between June and July. Eggs used in this study were obtained from quail flock at 10 weeks of age. Eggs were stored for 7 days at 15-18 °C and 75-80 % relative humidity conditions. Eggs were randomly divided into 3 groups in this study. 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 early embryogenesis (EE6-EE8 days), incubation temperature was increased to 41 °C and relative humidity to 65 % for 3 h (12.00-15.00) at 3 consecutive days. In the same way, in the late embryogenesis stage (LE12-LE14 days), incubation temperature was increased to 41 °C and relative humidity to 65 % for 3 h (12.00-15.00) at 3 consecutive days (Bruzual *et al.* 2000).

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 all incubators were turned through 270° 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 at hatching and with 5 weeks of age. 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 1.5 °C per week until the quails were 5 weeks old, and then supplemental heating was disconnected. Quails were exposed to light for 23 hours and to darkness for 1 hour. Gender determination of quails was made at 3 weeks of age according to the feather colour. 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} *ad libitum* and unlimited water was supplied during the experiment. All eggs used in this study were collected in an incubation basket and randomly distributed to groups before incubation. After the hatching period, the unhatched eggs were removed from the incubator, cracked open and also visually examined to determine the fertility and embryonic mortality rate.

Statistical methods

Following model was used for statistical analyses:

$$Y_{ijk} = \mu + Gi + Cj + e_{ijk} \quad (1)$$

where Y_{ijk} represents the hatching weight or body weight at 5 weeks of age, G_i is the effect of the i-th manipulation, C_j is the effect of the *j*-th gender and e_{ijk} is the error term, ~N $(0,\sigma_e^2)$.

Results

In this study, hatchability rates were found at 83.96 %, 84.84 % and 84.46 % for C, EE and LE groups. Also, in the same order, the embryonic mortality rates were determined at 14.50 %, 15.15 % and 15.53 %.

Descriptive statistics of egg weights belonging to the groups were presented in Table 1. The effects of thermal manipulations and gender on hatching weights were given in Table 2. The effects of thermal manipulations and gender on body weight at 5 weeks of age were indicated in Table 3.

Table 1
Average egg weights

Groups	N	Egg weight, g
Late embryogenesis	137	11.87±0.051
Early embryogenesis	131	11.88±0.076
Control	174	11.87±0.070

Table 2
Effects of thermal manipulation and gender on hatching weight, g

Gender	Late embryogenesis*	Early embryogenesis*	Control*	Average±SE
Male	7.95±0.067[a]	8.15±0.085[a]	8.55±0.099[b]	8.21±0.052
Female	7.85±0.083[a]	8.19±0.078[b]	8.60±0.089[c]	8.21±0.050
Average	7.90±0.049[a]	8.17±0.058[a]	8.57±0.067[b]	

*mean±SE, [a,b,c]Means in rows, with different letters differ significantly at $P<0.01$.

Table 3
Effects of thermal manipulation and gender on body weight at 5 weeks of weight, g

Gender	Late embryogenesis*	Early embryogenesis*	Control*	Average±SE
Male	158.57±1.621[ax]	166.18±1.495[bx]	161.42±1.1489[ax]	160.05±0.914[x]
Female	182.11±1.813[ay]	192.63±2.665[by]	184.61±2.124[ay]	186.45±1.262[y]
Average	170.34±1.682[a]	179.40±1.852[b]	173.01±1.574[a]	

*mean±SE, [a,b]Means in rows, with different letters differ significantly at $P<0.01$. [x,y]Means in columns, with different letters differ significantly at $P<0.01$.

Discussion

There was no significant difference among the groups according to egg weights. The eggs weighed 11.87 g in the LE, 11.88 g in the EE and 11.87 g in the C group, respectively. Egg weight was similar to those reported in the literature (Vali *et al.* 2005, Mielenz *et al.* 2006). Collin *et al.* (2005) reported that the different durations of thermal manipulation during E16 to E18 did not affect the body weight of the hatched chicks. Similarly, thermal treatments during E16-E18 of the chick's embryogenesis, did not affect body weight of 1 day old chick. Also, at hatching, no differences in body weight were demonstrated as previously exhibited by Yahav *et al.* (2004a, 2004b). But gender did not significantly influence hatching weights. Previous results on body weight differences between males and females after hatching were conflicting. Some authors (Khan *et al.* 1975, Whiting & Pesti 1983) found male chicks to be heavier than females, but others (Burke 1992, Reis *et al.* 1997) found no gender differences in body weight of day-old chickens. In this study, thermal manipulation did affect significantly hatching weight. While highest hatching weights were found in control group for male (8.55±0.084 g) and female (8.60±0.082 g), lowest hatching weights resulted in LE group for both genders. But gender did not affect significantly hatching weight in this study. One of the major concerns in dealing with domestic animals is how to maintain or even improve performance when various manipulative treatments are applied (Yahav *et al.* 2004b).

Thermal manipulations and gender had significant effect on body weight at the age of 5 weeks in this study. There were found significant differences among manipulations groups in respect to both genders. At the same time, the differences between genders were significant for all groups. Lowest body weights at 5 weeks of age were detected in LE group for both genders. Body weight at 5 weeks of age was similar to that reported by Balcioglu *et al.* (2005) and Alkan *et al.* (2008, 2010). Whereas highest body weights at 5 weeks of age were determined in EE group. Epigenetic adaptation which has been defined (Tzschentke & Basta 2002, Tzschentke *et al.* 2004) as a lifelong adaptation occurring during prenatal (embryogenesis) or

early postnatal ontogeny within critical developmental phases that affect gene expression, seems to be suitable to reach the goal of improving thermo tolerance acquisition in broilers. It was previously reported that exposing embryos to high or low temperatures during incubation improved their capacity to adapt to hot or cold environments, respectively, in the post-hatching phase (Tzschentke & Basta 2002, Yahav *et al.* 2004b). According to Hamburger & Hamilton (1992), the entire process of chick embryogenesis can be divided into 3 major phases: 2 early phases during which the organs and systems of the body are formed and the last phase, starting at E13, during which growth and maturation take place.

In late experiments (Yahav *et al.* 2004a, Collin *et al.* 2007) the application of thermal manipulations during embryogenesis to improve acquisition of thermo tolerance based on the hypothesis that the »set point, threshold response« of controlling systems can be altered most efficiently during the development maturation of the hypothalamus-hypophysis-thyroid axis (thermoregulation) and/or the hypothalamus-hypophysis-adrenal axis (stress). In another experiments, a chronic thermal manipulation (38.5 °C) in laid chicken embryos was applied from E18 up to the end of incubation. On the last day of incubation the thermal manipulated embryos showed a significantly higher level of heat production in comparison to the controls (Loh *et al.* 2004). Similar effects were found in Muscovy duck embryos that experienced thermal manipulation from E29 until hatching. Most of the studies demonstrated an improvement of thermo tolerance during the first 10 days post-hatching. However, chicks that were exposed to thermal manipulation during embryogenesis and raised up to marketing age did not exhibit a long-lasting improvement in thermo tolerance. Also, Collin *et al.* (2007) reported that thermal manipulation of chick embryos applied during early or late embryogenesis, or during both sides, did not improve acquisition of thermo tolerance tested at 6 weeks of age.

Although control group had highest hatching weight, this group was unable to survive in this position with increasing age. The EE group reached the highest body weight at end of the fifth week. Therefore it can be said that thermal manipulation in the EE period had positive effect on body weight at 5 weeks of age.

There was no significant difference among the groups in respect to hatchability and embryonic mortality in this study. However, previous results on hatchability and embryonic mortality rates are contradictory. Decuypere & Michels (1992) reported that incubation climate can significantly influence hatchability in poultry. Changes of only 1 °C from the optimum have a major impact on hatching results in turkey (French 1997). However, the strength of this influence depends on the time frame used and the duration of changes in incubation temperature during embryogenesis (French 2000). Collin *et al.* (2005) and Yahav *et al.* (2004) reported that the thermal manipulation significantly affected hatchability than control group. In contrast, Thompson *et al.* (1976), Lay & Wilson (2002), Yalcin & Siegel (2003) and Badran *et al.* (2012) found that increasing incubation temperature had no significantly effect on hatching rate. Also, Tzschentke & Halle (2009) incubated broiler eggs from day 1 to day 17 under normal incubation conditions (37.2 to 37.4 °C) and then divided them into three hatch incubators (control: 37.2 to 37.4 °C; chronic warm incubation: 38.2 to 38.4 °C, 24 h daily; short-term warm stimulation: 38.2 to 38.4 °C, 2 h daily). They reported that neither chronic nor short-term increase in incubation temperature had a negative effect on hatchability. The deleterious effect of heat stress on embryo survival was elaborated in turkey eggs by

French (1994), who demonstrated that turkey eggs incubated at 38.9 °C had poor embryo survival. Also, Ande & Wilson (1981) reported that high incubation temperature was inimical to embryo survival. These reports are in agreement with the findings of Lourens *et al.* (2005), who documented significant embryo mortality in chicken eggs when they were subjected to a high incubation temperature of 38.9 °C. The lack of consistency of our results with these studies may be probably due to different species, incubation temperature and humidity.

As a result, thermal manipulation of chick embryos applied during EE or LE did improve acquisition of thermo tolerance tested at 5 weeks of age. All the thermally treated chicks exhibited significantly lower hatching weight than control ones in this study. But early thermal manipulation significantly improved body weight at 5 weeks of age compared with LE and C chickens. The differences in studies may be probably due to the length of acclimation period, timing of acclimation, different species and incubation temperature and humidity, which may alter chronological time to complete development. This complex issue has to be intensively studied in order to shed light on epigenetic adaptation in different domestic poultry species.

Acknowledgements

This study was financially supported by the Scientific Research Projects Unit of Akdeniz University under the project number of 2009.01.0104.001.

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