Thermal Manipulation on Japanese quail embryo; An Embryological Study

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1. Abstract

Japanese quail embryo thermal manipulation was known as an effective protocol in improving post-hatching growth performance parameters and thermotolerance acquisition which alleviates heat stress during rearing. The current study aimed to evaluate the effects of high incubation temperature protocols (41°C for 3 hours / 3 successive days) during early and late embryogenesis on quail embryo performance. Two hundreds of quail eggs was incubated at 37.7°C and 55% RH as control group. Two thermally treated groups (two hundreds eggs for each) were incubated intermittently at 41°C and 65% RH intermittently (3 hours / day): The early embryonic group was thermally treated during embryonic days (ED 6 – 8), while the late embryonic group was thermally treated during ED 12 –14. These thermal manipulation protocols did not affect embryo development, yolk consumption, hatchability and hatched chick quality.

Key words: *Coturnix japonica*, High incubation temperature, Embryogenesis, Chick quality

2. Introduction

Several species of quail are present worldwide, the Japanese quail (*Coturnix japonica*) is the smallest avian species reared for egg and meat production also it has a global importance as a laboratory animal [1]. Japanese quail can give 3 to 4 generations yearly, therefore it is an important laboratory and economic animal model in biological and embryological researches [2]. Hamburger and Hamilton [3] elucidated that the entire process of chick embryogenesis can be sectioned into 3 major stages; The first two stages are organogenesis stages, the third stage start at embryonic day-ED13, during which growth and maturation occur. Several studies in chickens and turkey revealed that embryos are highly sensitive to elevated incubation temperature in early stages than late stages of incubation [4-6], and the greater tolerance of embryos to high incubation temperature in late stages of incubation depends on embryo adaptation to high metabolic energy produced in the second half of incubation [7]. When increasing the incubation temperature, consideration of three major aspects should be considered: timing, temperature degree, and length of exposure. Subsequently the period in which to apply thermal treatment protocols during embryogenesis should be selected to match the specific physiological system to be affected and also should be connected with the development of hypothalamic-hypophyseal-thyroid axis and hypothalamic-hypophyseal-adrenal axis [8-10]. It had been declared that exposing eggs to high incubation temperature may result in teratogenicity in embryos such as abnormal morphological development and malfunction of physiological systems such as heart and liver [11, 12], early timing of hatch [13], and poor chick quality [9].

Previous studies on quail embryos reported that thermal treatment (41°C / 3hs) resulted in improved final body weight at post hatch day 35 and improved thermotolerance of hatched chicks to alleviate heat stress during rearing [14, 15]. These studies didn't evaluate the possible effects of high incubation temperatures on quail embryo development, embryo yolk consumption, embryonic mortality rate and hatched chicks' quality. Therefore, the current study aimed to evaluate the effects of high incubation temperature protocols (41°C for 3 hours) during early and late embryogenesis on quail embryo development, growth, hatching rate and chick quality.

3. Materials and Methods

Egg incubation and hatching management.

The eggs were obtained from a quail maternal flock of 12 weeks old. The collected eggs were stored for 5 days at 15°C and relative humidity (RH) 75-80%. The eggs were inspected for breakage or any abnormalities, individually weighted, disinfected and a final number of 600 eggs were randomly divided into 3 groups (200 eggs per group, Table 1). The eggs were incubated in 3 incubators of continuous turning system, fully digital programmable temperature and humidity. Incubation conditions from embryonic day 0 to embryonic day 17 (ED0 – ED17) were 37.7°C and RH 55% (16) for control group [15, 17]. Two thermally treated groups: incubation temperature was increased to 41°C and 65% RH [18] intermittently (3h/day) during early embryogenesis (ED6 – ED8) group (A) and late embryogenesis ED12 – ED14 group (B). Immediately after the thermal treatment protocols were terminated, incubation conditions were restored to the normal levels (37.7°C and 55% RH). The eggs in all incubators were automatically turned through 270° every hour. The eggs were transferred to hatching trays at the 15th day of incubation. At hatch (the 17th day of incubation), the hatched quail chicks were counted, the unhatched eggs were removed from the incubator, carefully opened and visually examined to detect and calculate the unfertile eggs and

embryonic mortality rate. The hatchability percentage was calculated by dividing the number of hatched chicks by the total number of fertilized eggs. The chick quality was evaluated by navel inspection, feathering and congenital anomalies were recorded.

Sample collection and analysis.

Random daily collection of nearly 10 eggs per group from ED9 until ED15, then eggs were weighted and carefully opened, and the embryos were separated from the yolk sac, wiped. Yolk sac, free-yolk embryo weight and relative embryo weights were determined also. In addition, the embryos were examined for congenital anomalies.

Statistical analysis.

Analyses of covariance (ANCOVA) were used to test for differences in relative embryo weight and relative yolk weight among three treatment groups (A, B, and C). In these analyses, embryonic days were included as a covariate, and interaction terms between embryonic days and treatments were tested. Differences in hatchability percentages between sets were tested using Chi-square tests. Statistical significance was set at $p < 0.05$, and all data were presented as the mean ± standard error of the mean (*SEM*). Analyses were completed with SAS.

4. Results

Quail egg numbers and weights belonging to the treatments were presented in Table 1.

Influences of thermal treatment on quail embryogenesis and inspection of possible congenital anomalies.

Analysis of the results (Table 2) revealed that there were no differences ($p = 0.20$) in relative embryo weights between treatments within each day of incubation. Regardless treatment, there was a significant positive relationship between relative embryo weight and day of incubation ($p < 0.001$).

Observations of congenital anomalies. Congenital anomalies observed in embryos included bilateral absence of eye development (Anophthalmia) and head deformities, absence of frontal bone (Acalvaria) (Fig. 1). Only one observation for each congenital anomalies was recorded.

Influences of thermal treatment on quail embryo yolk consumption and absorption of yolk sac pigments.

Table 3 indicates that, Regardless treatment, there was a significant negative relationship between relative yolk sac weight and the day of incubation ($p < 0.001$). Yolk consumptions were nearly steady during ED9 - ED11 and slightly increased at ED12 through ED15. Within each day of incubation, no differences ($p < 0.89$) in relative yolk sac weight among treatments were found. In treatment groups only about 3 % of hatched healthy chicks were albino in color.

Influences of thermal treatment on hatchability and chick quality.

The hatching success is indicated in Table 4. The different protocols of thermal treatment during incubation did not affect hatchability rates ($p < 0.55$) as compared with control group. Chicks with unhealed navel in thermally treated groups were only 2%, no malformations in hatched chicks were detected.

5. Discussion

The embryo development of avian species can be described in terms of yolk-free embryo weight [19]. Subsequently, we randomly selected and gently opened 10 eggs/group/day from ED9-ED15, yolk sac weight and yolk-free embryo weight as well as their relative weights were recorded. The current study revealed that the intermittent (3h) early and late stage TM did not

negatively affect the daily embryonic development and yolk consumption if compared with control. Our results are in full agreement with Narinç et al., [20] who revealed that the long-term intermittent (6h) thermal treatment during early and late phases of incubation has no effects on the embryo morphology and development stability of broiler chickens. The continuous (24h) long term high incubation temperature resulted in reduced yolk consumption and regressed means of chicken embryo weights [21]. On the same way, Willemsen et al., [22] declared that relative embryo and yolk sac weights, as well as gases in the air cell, all these parameters caused a slower embryonic development of the chicken embryos exposed to a continuous high temperature during late incubation (ED 16 - ED 18.5). In turkey embryos and chicken embryos, the intermittent high incubation temperature had not negatively affected embryo growth, but it affected positively on relative embryo weights [8, 14, 23]. Piestun et al., [8] reported that high incubation temperature may lead to poor absorption of yolk sac pigment in turkey and broiler embryos which was manifested by albino color of hatched chicks. Concerning our study, the albino hatched chicks were only about 3 % of hatched chicks. In the light of the above mentioned studies and our study, it is clear that intermittent thermal treatment had not negatively affect the relative weight of both embryo and yolk sac but it can accelerate growth which was manifested by early onset of hatch, whereas the continuous thermal treatment distress the embryo growth and interferes the yolk consumption. Also, the current study elucidated that no negative effects of intermittent thermal treatment (3 hours / day) on general appearance were observed, indicating that intermittent thermal treatment allows embryos to dissipate excessive heat and to avoid teratogenic consequences.

In broiler chickens, ducks, turkey, and ostrich, intermittent thermal treatment protocols (≤ 12h) did not distress hatchability % whereas continuous thermal treatment (24h) significantly regressed hatchability [8, 23-26]. There were even other studies revealed that late-term (ED15 - ED18) intermittent thermal treatment increased hatchability [10, 27]. Piestun et al., [8] reported that thermal treatment protocol had a negative effect on chick quality and rough-navel rates in the thermally treated hatched turkey chicks were 6 and 11% higher than controls in 6H and 12H groups respectively. Concerning the current study, thermal treatment had the least effect on chick quality, and this may be attributed to a shorter duration and period of exposure (3 hours / day for 3 days).

6. Conclusion

Embryonic thermal treatment in quail had no deleterious effects on embryonic development, embryo growth, hatchability and hatched chick quality, so it can be used safely as an effective protocol in improving post hatch growth performance and thermotolerance acquisition in Japanese quail.

7. References

1. Huss, D., Poynter, G. and Lansford. R. (2008): Japanese quail (Coturnix japonica) as a laboratory animal model. Lab animal. 37(11), 513-9.
2. Minvielle, F. (2004): The future of Japanese quail for research and production. World's Poult. Sci. J. 60 (4), 500-507.
3. Hamburger, V. and Hamilton, H. L. (1992): A series of normal stages in the development of the chick embryo. Dev. Dyn. 195 (4), 231-272.
4. Romanoff, A. L., Smith, L. L. and Sullivan, R. A. (1938): Biochemistry and biophysics of the developing hen's egg.

5. Moreng, R. E. and Shaffner, C. (1951): Lethal internal temperatures for the chicken, from fertile egg to mature bird. Poult. Sci., 30 (2), 255-266.
6. Ande, T. and Wilson, H. (1981): Hatchability of chicken embryos exposed to acute high temperature stress at various ages. Poult. Sci. 60(7), 1561-1566.
7. French, N. (1997): Modeling incubation temperature: the effects of incubator design, embryonic development, and egg size. Poult. Sci. 76 (1), 124-133.
8. Piestun, Y., Zimmerman, I. and Yahav, S. (2015): Thermal manipulations of turkey embryos: The effect on thermoregulation and development during embryogenesis. Poult. Sci. 94 (2), 273-280.
9. Piestun, Y., Harel, M., Barak, M., Yahav, S. and Halevy, O. (2009): Thermal manipulations in late-term chick embryos have immediate and longer-term effects on myoblast proliferation and skeletal muscle hypertrophy. J. Appl. Physiol. 106 (1), 233-240.
10. Yahav, S., Collin, A., Shinder, D., and Picard, M. (2004): Thermal manipulations during broiler chick embryogenesis: effects of timing and temperature. Poult. Sci. 83 (12), 1959-1963.
11. Thinh, N., Cumming, R. and Thwaites, C. (1977): The effects of high incubating temperature on the growth of the chick embryo. Australian J. Agri. Res. 28(3):551-5.
12. Primmett, D., Stern, C. and Keynes, R. (1988): Heat shock causes repeated segmental anomalies in the chick embryo. Development. 104(2):331-9.
13. Leksrisompong, N., Romero-Sanchez, H., Plumstead, P., Brannan, K. and Brake, J. (2007): Broiler incubation. 1. Effect of elevated temperature during late incubation on body weight and organs of chicks. Poult. Sci. 86 (12), 2685-2691.
14. Alkan, S., Karslı, T., Karabağ, K., Galic, A. and Balcıoğlu, M. (2013): The effects of thermal manipulation during early and late embryogenesis on hatchability, hatching weight and body weight in Japanese quails (*Coturnix coturnix japonica*). Arc Tierz. 18, 789-796.
15. Alkan, S., Karsli, T., Karabağ, K., Galiç, A. and Balcioğlu, M. S. (2012): The effects of thermal manipulations during early and late embryogenesis on body temperatures of Japanese quails (*Coturnix coturnix japonica*). J. Applied Anim. Res. 40 (1), 13-16.
16. Bruzual, J., Peak, S., Brake, J. and Peebles, E. (2000): Effects of relative humidity during incubation on hatchability and body weight of broiler chicks from young breeder flocks. Poult. Sci. 79(6):827-30.
17. Alkan, S., Karslı, T., Karabağ, K., Galic, A. and Balcıoğlu, M. (2013): The effects of thermal manipulation during early and late embryogenesis on hatchability, hatching weight and body weight in Japanese quails (*Coturnix coturnix japonica*). Arc Tierz. 18, 789-796.
18. Yahav, S., Rath, R.S. and Shinder, D. (2004): The effect of thermal manipulations during embryogenesis of broiler chicks (Gallus domesticus) on hatchability, body weight and thermoregulation after hatch. J Thermal Biology. 29(4-5):245-50.
19. Hill, D. (2001): Chick length uniformity profiles as a field measurement of chick quality. Avian Poult. Biol. Rev. 12, 188.
20. Narinç, D., Erdoğan, S., Tahtabiçen, E. and Aksoy, T. (2016): Effects of thermal manipulations during embryogenesis of broiler chickens on developmental stability, hatchability and chick quality. Animal, 10 (8), 1328-1335.
21. Ozaydın, T. and Celik, I. (2014): Effects of high incubation temperature on the body weight and yolk

consumption of two commercial broiler strain. Acta Scientiae Vet. 42 (1), 1-5.
22. Willemsen, H., Kamers, B., Dahlke, F., Han, H., Song, Z., Ansari Pirsaraei, Z., Tona, K., Decuypere, E. and Everaert, N. (2010): High-and low-temperature manipulation during late incubation: effects on embryonic development, the hatching process, and metabolism in broilers. Poult. Sci. 89 (12), 2678-2690.
23. Piestun, Y., Halevy, O. and Yahav, S. (2009): Thermal manipulations of broiler embryos: The effect on thermoregulation and development during embryogenesis. Poult. Sci. 88 (12), 2677-2688.
24. Massimino, W., Bernadet, M.-D., Pioche, T., Tavernier, A., Ricaud, K., Gontier, K., Bonnefont, C., Manse, H., Morisson, M. and Collin, A. (2019): Positive impact of thermal manipulation during embryogenesis on foie gras production in mule ducks. Front. Physiol. 10, 1495.
25. Elsayed, M. (2016): Effects of thermal manipulation during late incubation period on post-hatch thermotolerance in ostrich. Czech J. Anim. Sci. 61 (9), 421-431.
26. Piestun, Y., Shinder, D., Ruzal, M., Halevy, O., Brake, J. and Yahav, S., (2008): Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. Poult. Sci. 87 (8), 1516-1525.
27. Elsayed, N., Elkomy, A., El-Saadany, S. and Hassan, E. (2009). New suggested schemes for incubation temperature and their effect on embryonic development and hatching power. Asian J. Poult. Sci. 3 (1), 19-29.

Table 1: Quail egg number and average weights.

Treatment	N	Weight (g)	Sampling (Day 9 – 15)	Incubated eggs at Day 16 N
A	200	13.13 ± 0.08	70	130
B	200	13.11 ± 0.08	73	127
C	200	13.12 ± 0.08	68	132
Overall	600	13.11 ± 0.08	211	389

Table 2: Influences of thermal treatment on relative embryo weight in Japanese quail embryo. All data are presented as the mean ± standard error of mean (*SEM*). Means followed by different superscript letters are significantly different $p < 0.05$.

Treatment	Mean ± *SEM*	Incubation day						
		9	10	11	12	13	14	15
A	24.08 ± 1.88^{c}	9.05 ± 1.30^{f}	13.88 ± 1.50^{e}	21.28 ± 1.15^{d}	27.84 ± 1.61^{c}	35.42 ± 1.19^{b}	35.65 ± 4.62^{a}	42.25 ± 7.18^{a}
B	29.39 ± 1.86^{ab}	9.65 ± 0.28^{f}	15.52 ± 0.78^{e}	21.04 ± 1.96^{d}	31.04 ± 0.55^{c}	39.81 ± 1.05^{b}	45.40 ± 2.84^{a}	40.19 ± 6.17^{a}
C	28.93 ± 2.27^{a}	10.50 ± 9.40^{f}	15.78 ± 0.53^{e}	21.78 ± 1.82^{d}	30.57 ± 1.26^{c}	37.34 ± 3.83^{b}	51.57 ± 3.09^{a}	54.51 ± 3.67^{a}

Table 3: Influences of thermal treatment on relative (yolk sac) weight in Japanese quail embryo. All data are presented as the mean ± standard error of mean (*SEM*). Means followed by different superscript letters are significantly different $p < 0.05$.

Treatment	Mean ± *SEM*	Incubation day						
		9	10	11	12	13	14	15
A	24.70 ± 1.03^{a}	26.44 ± 2.23^{a}	25.87 ± 1.57^{a}	30.72 ± 2.53^{a}	23.57 ± 1.11^{b}	21.61 ± 1.40^{b}	19.14 ± 2.71^{b}	19.21 ± 5.86^{b}
B	24.21 ± 0.92^{a}	29.42 ± 3.34^{a}	26.41 ± 1.86^{a}	31.38 ± 1.88^{a}	23.67 ± 1.23^{b}	21.50 ± 1.81^{b}	20.08 ± 1.65^{b}	17.20 ± 1.17^{b}
C	25.67 ± 0.91^{a}	32.05 ± 2.24^{a}	29.36 ± 1.68^{a}	26.81 ± 3.33^{a}	20.8 ± 1.63^{b}	23.89 ± 1.98^{b}	22.75 ± 1.41^{b}	22.07 ± 0.64^{b}

Table 4: Influences of thermal treatment on hatchability

Treatments	Fertilized incubated eggs at Day 16	Hatch	
		N	%
A	96	77	80.20[a]
B	104	85	81.73[a]
C	112	95	84.82[a]
Overall	312	257	82.32

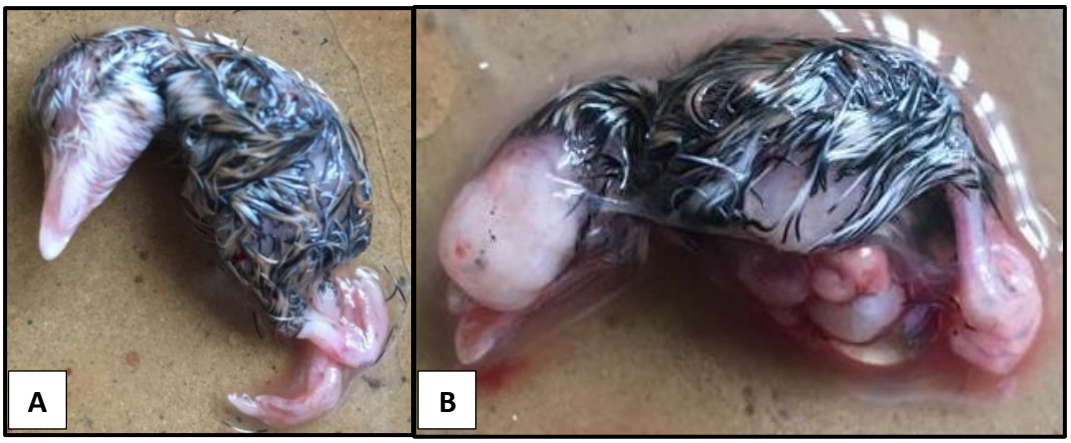

Fig .1.: Congenital anomalies in Japanese quail.
(**A**) Absence of eye development (Anophthalmia)
(**B**) Absence of frontal bone (Acalvaria).