

Effect of manipulating incubation temperature during late embryogenesis on sex reversal and post-hatch performance in Japanese quails (*Coturnix japonica*)

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Target audience: Hatchery operators, poultry farmers and the scientific community.

Abstract

Incubation temperature has been speculated to influence embryo sex and post-hatch performance hence, five different incubators were calibrated at 36°C, 37°C (control), 38°C, 39°C and 40°C. In each incubator, 123 eggs were set based on weight and each egg tray held 41 eggs. On incubation days 11, 12 and 13, the temperature was paused for 5hrs, to alter the embryo sex. Brooding temperature, feed intake, sex reversal, sexual maturity, carcass quality and organs weight of the birds were determined using standard operations. Mean brooding temperature was 34.81°C, 34.61°C and 34.74°C in weeks 1, 2 and 3. Feed intake in week 2 was highest (11.75 g) in chicks hatched at 40°C, followed by 10.84 g (38°C), 9.94 g (37°C), 8.82 g (39°C) and 7.67 g (36°C). The chicks hatched at 39°C and 40°C, started laying at 5 weeks of age, compared to those hatched at 36°C, 37°C and 38°C that laid at 6 weeks. There was sex reversal among the hatched chicks 24 hatched at 39°C. Carcass weight values varied between 68.12 g (37°C) and 97.44g (36°C). Survivability value varied between 2.27% (36°C) and 70.5% (40°C). Thus, manipulating incubation temperature during late embryogenesis may reverse sex and enhance post-hatch performance in Japanese quails.

Keywords: Artificial incubator, avian embryo, brooding, sexual maturity

Description of problem

The Japanese quail (*Coturnix japonica*) has been singled out due to its short generation interval. The hen begins to lay at about 40 days of age, producing about 250 – 320 eggs/year. The cock matures at about 30 – 60 days old hence, the possibility of four

generations annually. Other attributes include requirements of little starter-pack, land area, labour and medical attention as well as high resistance to diseases. Essentially, Japanese quail has been reported to be a veritable source of nutrients and the egg has been speculated to be rich in minerals

and vitamins but low in cholesterol. Japanese quail production is likely feasible in making the meat and eggs available at an affordable rate. Japanese quail hens in captivity hardly brood and incubate their eggs thus, artificial incubators are used to simulate the role of a broody hen, in providing optimum environmental conditions that will stimulate embryonic development and maintain the growth until hatching (1)

Incubation temperature was reported to directly influence hatchability, and post-hatch performance in broiler breeder chickens, Australian brush turkeys, domestic fowls and turkeys (2). In each case, sex reversal was reported but reproductive potentials of the sex-reversed chicks/poults were not provided. In some lower vertebrates (reptiles, amphibians and fish), incubation temperature has been reported to influence the sex of the hatchlings (3, 4). It was stated that in avian species, the primary sex ratio at fertilization was almost equal (1 male: 1 female), but when the environmental temperature was adjusted during embryogenesis, one sex was somewhat favoured. It has been observed in Australian brush turkey that more males hatched at 31°C, more females hatched at 36°C and the sex ratio was 1:1 at 34° C, which was the natural (control) mounds incubation temperature (2). This phenomenon may be adopted in avian species since it was reported that no convincing evidence of manipulating temperature to determine sex in avian species has been recorded (5).

For example, Japanese quails in domestication lack broody tendency hence, the adoption of artificial incubation for commercial production. However, inappropriate incubation temperature may affect hatchability and sex determination in

Japanese quails. Unfortunately, the conventional incubation temperature and sexing techniques used for other poultry species may cause inaccurate sexing and poor post-hatch performance. More essentially, information on optimum incubation temperature and *in ovo* sex predetermination in Japanese quails is inadequate. Therefore, manipulation of incubation temperature and its effect on feed intake, sex reversal, sexual maturity, carcass and organs quality of Japanese quails were assessed.

Materials and methods

Experimental site

The study was conducted at the National Veterinary Research Institute, Vom, Jos North, Plateau State, Nigeria located on latitude 9° 44' 0"N, longitude 8° 47' 0"E.

Experimental design and procedure

Five different electric incubators representing the treatments: 36°C, 37°C (control), 38°C, 39°C and 40°C, were used. A total of six hundred and fifteen Japanese quail (*Coturnix japonica*) eggs, were collected from layers reared in a deep litter system, for 4 days and were cooled at room temperature before setting in the incubators on the 5th day. In each of the incubators, 123 eggs were randomly distributed based on weight such that each of the 3 egg trays in the incubators held 41 eggs. On incubation days 11, 12 and 13, the temperature was paused for 5 hours by switching off the main to alter the embryo sex. The hatched chicks were allowed to dry fluffy in the incubators, before transferring them to the brooding unit.

Brooding temperature and relative humidity determinations

The brooding unit was properly covered with thick cellophane to conserve heat that was generated with 200w electric bulbs placed in 4 strategic locations. The temperature and relative humidity in the brooding unit were monitored and recorded daily at 9:00 am, 12:00 noon and 3:00 pm with two different hygrometers hung in different strategic positions throughout the brooding periods.

Feed intake determination

The quantity of feed offered to the chicks was weighed using sensitive weighing scale and the weight of the remnant was deducted to obtain feed intake recorded daily. The feed consumed by the birds was divided by the number of birds per treatment to obtain feed intake per chick.

Assessment of sexual maturity, sex reversal, carcass and organs quality in Japanese quails

The first egg drop was recorded as “the point of lay” representing sexual maturity. A week allowance was given for the late-hatched chicks to equally attain sexual maturity. At six weeks old (sexual maturity), 8 birds (1:1 sex ratio except, the chicks

hatched at 36°C that had only a female), were randomly picked per treatment, weighed using a sensitive scale to obtain live weight value and tagged for sex identification. The sex was reaffirmed at slaughter by carefully observing the testes or ovaries in the eviscerated carcasses and nonconformity with the identified sex was considered reversed sex. After bleeding, the carcasses were left for an hour before weighing again to obtain the bled weight value. Thereafter, they were all immersed in hot water for two minutes to ease defeathering. Then, the carcasses were weighed without the heads and shanks to obtain defeathered weight. The carcasses were cut open from the breast region through the abdomen to collect the kidneys, gizzards, livers, hearts and testes. Also, the developing yolk and eggs were carefully removed and counted to obtain number of yolk and formed eggs. The organs were weighed to obtain organs weights (6).

Survivability determination

The number of dead chicks was recorded throughout the study period and at six weeks of age, survivability of the Japanese quails was determined using Equation 1.

$$\text{Survivability} = \frac{\text{Total number of hatchlings} - \text{Total number of dead birds}}{\text{Total number of hatchlings}} \times 100 \quad \text{Equation 1.}$$

Table 1: Brooding temperature and relative humidity of Japanese quail chicks hatched at 36 – 40°C

| Parameters | Brooding periods | | | | | | | | | | | |
|-----------------------|------------------|-------|-------|------|--------|-------|-------|------|--------|-------|-------|------|
| | Week 1 | | | | Week 2 | | | | Week 3 | | | |
| | Mean | Min | Max | SEM | Mean | Min | Max | SEM | Mean | Min | Max | SEM |
| Temperature (°C) | | | | | | | | | | | | |
| Brooding pen | 34.81 | 30.50 | 34.92 | 0.43 | 34.61 | 30.50 | 34.84 | 0.41 | 34.74 | 30.50 | 34.80 | 0.42 |
| Ambient | 27.12 | 24.00 | 30.80 | 0.35 | 27.16 | 24.00 | 29.40 | 0.35 | 26.69 | 24.00 | 29.40 | 0.33 |
| Relative humidity (%) | | | | | | | | | | | | |
| Brooding pen | 69.98 | 54.00 | 79.00 | 0.88 | 70.82 | 61.00 | 79.00 | 0.76 | 70.87 | 61.00 | 79.00 | 0.70 |
| Ambient | 67.97 | 50.00 | 79.00 | 1.19 | 68.55 | 50.00 | 79.00 | 1.17 | 69.44 | 50.00 | 79.00 | 1.18 |

SEM: Standard error of means; Min: Minimum value; Max: Maximum value

Table 2: Daily feed intake and total mortality of Japanese quail chicks hatched at 36 – 40°C

| Week | Parameters | Treatments | | | | | SEM |
|------|-----------------------|--------------------|---------------------|---------------------|---------------------|---------------------|-------|
| | | 36°C | 37°C | 38°C | 39°C | 40°C | |
| | Parameters | 36°C | 37°C | 38°C | 39°C | 40°C | SEM |
| 1 | No. of chicks | 44 | 69 | 72 | 67 | 61 | - |
| | Feed intake/group (g) | ND | ND | ND | ND | ND | - |
| | Feed intake/chick (g) | ND | ND | ND | ND | ND | - |
| 2 | No. of chicks | 3 | 20 | 26 | 34 | 44 | - |
| | Feed intake/group (g) | 24.67 ^d | 209.86 ^c | 288.57 ^b | 283.43 ^b | 553.09 ^a | 8.65 |
| | Feed intake/chick (g) | 7.67 ^b | 9.94 ^a | 10.84 ^a | 8.82 ^{ab} | 11.75 ^a | 0.56 |
| 3 | No. of chicks | 3 | 18 | 26 | 34 | 44 | - |
| | Feed intake/group (g) | 29.14 ^c | 128.93 ^d | 380.07 ^c | 444.00 ^b | 632.07 ^a | 13.40 |
| | Feed intake/chick (g) | 9.71 ^b | 7.26 ^c | 13.93 ^a | 13.35 ^a | 13.95 ^a | 1.31 |
| 4 | No. of chicks | 2 | 16 | 26 | 33 | 44 | - |
| | Feed intake/group (g) | 25.28 ^d | 171.71 ^c | 443.36 ^b | 453.93 ^b | 540.28 ^a | 11.88 |
| | Feed intake/chick (g) | 12.84 ^b | 10.61 ^c | 16.83 ^a | 12.92 ^b | 12.30 ^b | 1.01 |
| 5 | No. of chicks | 2 | 16 | 26 | 33 | 44 | - |
| | Feed intake/group (g) | 20.14 ^c | 147.30 ^d | 369.00 ^c | 520.86 ^b | 623.21 ^a | 13.43 |
| | Feed intake/chick (g) | 9.78 ^b | 9.34 ^b | 14.20 ^a | 14.64 ^a | 13.87 ^a | 0.89 |
| 6 | No. of chicks | 1 | 16 | 26 | 33 | 43 | - |
| | Feed intake/group (g) | 19.30 ^c | 172.28 ^d | 386.70 ^c | 512.90 ^b | 597.00 ^a | 13.09 |
| | Feed intake/chick (g) | 19.30 ^a | 10.18 ^c | 14.85 ^b | 14.85 ^b | 13.68 ^b | 1.15 |
| | Total mortality | 43 ^b | 53 ^a | 46 ^b | 34 ^c | 18 ^d | 6.12 |

^{abcde}: Mean values on the same row with different superscript differ statistically at 5% probability test; SEM: Standard error of means, ND: Not determined due to irregular incubation periods; Group: Represent total number of birds per treatments.

Table 3: Sexual maturity, sex reversal, carcass weight, organs quality and survivability of Japanese quail chicks hatched at 36 – 40°C

| Parameters | Treatments | | | | | SEM |
|----------------------------|---------------------|--------------------|----------------------|----------------------|----------------------|------|
| | 36°C | 37°C | 38°C | 39°C | 40°C | |
| Sexual maturity | | | | | | |
| Age at point of lay (wks.) | 6 | 6 | 6 | 5 | 5 | - |
| Yolk | 6.00 ^a | 2.00 ^b | 3.67 ^b | 0.00 ^c | 2.00 ^b | 0.48 |
| Formed eggs | 0.00 ^{ab} | 0.00 ^b | 1.00 ^a | 0.00 ^b | 0.00 ^b | 0.00 |
| Sex ratio (M : F) | 0:1 | 7:9 | 6:7 | 1.1 | 7:16 | - |
| Reversed Sex M → F | 0 | 0 | 0 | 0 | 0 | - |
| F → M | 0 | 0 | 0 | 2 | 0 | - |
| Carcass quality (g) | | | | | | |
| No. of birds | 1 | 8 | 8 | 6 | 8 | - |
| Live weight | 134.18 ^a | 99.89 ^c | 117.12 ^{ab} | 110.89 ^{ab} | 115.78 ^{bc} | 6.79 |
| Bled weight | 128.67 ^a | 95.67 ^c | 111.67 ^{bc} | 105.13 ^{bc} | 110.50 ^{bc} | 2.86 |
| De-feathered weight | 113.20 ^a | 82.20 ^c | 97.67 ^{ab} | 90.78 ^{bc} | 95.62 ^{bc} | 2.65 |
| Carcass weight | 97.44 ^a | 68.12 ^c | 80.00 ^{bc} | 74.28 ^{bc} | 77.12 ^{bc} | 1.97 |
| Dressing percentage (%) | 72.52 ^a | 68.11 ^b | 68.42 ^b | 68.89 ^b | 66.78 ^b | 0.56 |
| Organs weight (g) | | | | | | |
| Gizzard weight | 4.24 ^a | 3.28 ^b | 3.23 ^b | 3.33 ^b | 3.64 ^{ab} | 0.12 |
| Liver weight | 3.12 ^{ab} | 2.55 ^{ab} | 2.09 ^{ab} | 1.88 ^b | 2.61 ^{ab} | 0.17 |
| Heart | 1.13 ^{ab} | 0.90 ^b | 1.05 ^{ab} | 1.30 ^a | 1.14 ^{ab} | 0.03 |
| Testes weight | | | | | | |
| Right | 0.00 ^b | 1.51 ^a | 1.22 ^a | 1.84 ^a | 1.56 ^a | 0.13 |
| Left | 0.00 ^b | 1.47 ^a | 1.43 ^a | 1.81 ^a | 1.58 ^a | 0.11 |
| Hatchling | 44 ^c | 69 ^a | 72 ^a | 67 ^a | 61 ^b | 3.26 |
| Mortality (%) | 97.7 ^a | 76.8 ^a | 63.9 ^b | 50.7 ^c | 29.5 ^c | 8.83 |
| Survivability (%) | 2.27 ^c | 23.2 ^d | 36.1 ^c | 49.3 ^b | 70.5 ^a | 5.55 |

^{abcd}: Mean values on the same row with different superscript differ statistically at 5% probability; SEM: Standard error of means.

Statistical analysis

All the data collected were subjected to analysis of variance procedure (7) software package and mean values were separated using Duncan Multiple Range Test of the same software package.

Results

Table 1 shows the brooding temperature and relative humidity of Japanese quail chicks hatched at 36 – 40°C. There were no statistical differences ($p < 0.05$) in all the parameters measured. Meanwhile, the mean brooding pen temperature was 34.81, 34.61 and 34.74°C in weeks 1, 2 and 3, respectively, compared to mean ambient temperature of 27.12°C, 27.16°C and 26.66°C. The brooding pen relative humidity ranged from a minimum of 54% to a maximum of 79% throughout the brooding period.

The daily feed intake and total mortality of Japanese quail chicks hatched at 36 – 40°C is presented in Table 2. There were statistical differences ($p < 0.05$) in all the parameters monitored. In week 1, feed intake was not determined due to irregular incubation periods of the hatchlings. In week 2, feed intake/chick was highest (11.75 g) in chicks hatched at 40°C, followed by 10.84 g (38°C), 9.94 g (37°C), 8.82 g (39°C) and 7.67g (36°C), in that order. In week 6, feed intake/chick was highest (19.30g) in chicks hatched at 36°C and lowest (10.18g) in chicks hatched at 37°C compared to a range of 13.68 – 14.85g recorded in chicks hatched at 38°C, 39°C and 40°C. At the end of 6 weeks, a total of 53 chicks were lost among those hatched at 37°C, followed by those hatched at 38°C (46), 36°C (43), 39°C (34) and 40°C (18).

Sexual maturity, sex reversal,

carcass weight, organs quality and survivability of Japanese quail chicks hatched at 36 – 40°C are expressed in Table 3. The chicks hatched at 39°C and 40°C started laying at the age of 5 weeks, compared to the chicks hatched at 36°C, 37°C and 38°C that started laying at 6 weeks old. The male: female sex ratio was 0:1, 7:9, 6:7, 1:1 and 7:16 in chicks hatched at 36°C, 37°C, 38°C, 39°C and 40°C, respectively. There was a sex reversal from hens to cocks among the chicks hatched at 39°C. The live weight was highest (134.18g) in a chick hatched at 36°C, whereas, a range of 99.89 – 117.12g was recorded in chicks hatched at 37°C, 38°C, 39°C and 40°C. Carcass weight was 97.44g (36°C), 80.0g (38°C), 77.12 g (40°C), 74.28g (39°C) and 68.12g (37°C). The gizzard (4.24g) and liver (3.12g) weights were highest in a chick hatched at 36°C. While percentage mortality was highest (97.7%) among the birds hatched at 36°C, slightly followed by 76.8% (37°C), 63.9% (38°C), 50.7% (39°C) and 29.5% (40°C) survivability was best (70.5%) in birds incubated at 40°C, followed by those hatched at 39°C (49.3%).

Discussion

The brooding temperature was similar to 21 – 36°C described as normal hence recommended by Penn State Extension (8) for adoption in poultry chicks' production. Thus, the hatchlings were probably not exposed to adverse environmental conditions.

The observed feed intake was somewhat similar to 8 g (at 0 – 2 weeks old), 19.19g (at 2 – 4 weeks old) but less than 27.37g (at 4 – 6 weeks old) reported in Japanese quails that were administered water-soluble vitamins *in ovo* (9). The

differences may be largely due to the strain of Japanese quails used in the studies, feed quality and probably environmental or treatment effects on the birds. Therefore, feed intake of Japanese quails subjected to low or high incubation temperatures during domestication process, may not be compromised. It was shown that Japanese quails in the present study, probably built thermotolerant traits during embryogenesis hence the ability to resist, survive and thrive even at the ambient temperature both at pre- and post-hatch conditions. This observation ostensibly lent more credence to a report that avian species could acquire thermotolerant traits during embryogenesis (10). To buttress this, the hens hatched at 39°C and 40°C, started laying eggs at the same age of 5 weeks, signifying the attainment of sexual maturity. This probably implied that the Japanese quails developed coping strategy in microclimate temperature close to body temperature, in order to thrive well at high ambient temperature.

It was observed that the Japanese quails hatched at 36°C, 37°C and 38°C did not lay eggs until 6 weeks old unlike those that were hatched at 39°C and 40°C that started laying eggs at 5 weeks of age. Meanwhile, yolk and formed eggs were found in utero among the Japanese quails that were hatched at 36°C, 37°C and 38°C, which indicated sexual maturity. Thus, hatching Japanese quail eggs at 36–40°C during domestication, may not impede the sexual developmental processes.

Even though two cases of reversed sex (both from female to male) was seemingly recorded, it was only among the Japanese quail hens hatched at 39°C. More importantly, the sex ratio was uniformly 1

male: 1 female across the treatments except, in the birds that were hatched at 36°C, where there was no male at all and 40°C, where there was approximately 1 male: 2 females. In any case, the observed sex reversal may be coincidental thus, lending more credence to speculations that sex reversal normally occurs once in a while in a flock (11). Therefore, incubation temperature at 36–40°C, may play a vital role in sex reversal in Japanese quails.

Interestingly, the birds that were hatched at 36°C, had the best carcass quality, probably indicating a catch-up growth and compensatory growth reported in Japanese quails (12). This observation seemingly buttressed the possibility of using incubation temperature to improve breast meat, myofibre aggregation and growth rate in broilers (13). Meanwhile, the values of live weight, carcass weight and dressing percentage compared favourably well with those reported in healthy poultry species. More significantly, the heart, liver and testis weight were close to 0.95g, 2.29g and 18g but the gizzard was greater than 1.63g reported in Japanese quails (9). Therefore, Japanese quails hatched at between 36 and 40°C may not have organs dysfunction. It was observed that the percentage mortality was decreasing with rising incubation temperature. This seemingly buttressed the report (10) that thermal manipulations during broiler embryogenesis, resulted in acquisition of thermotolerance. Although, the mortality rate among the chicks hatched at 36°C was high, there were survivors across all the treatments. The observed upsurge in the mortality among the chicks hatched at 36°C, may be coincidental and since there were mortality in all the treatments, Japanese quails hatched at incubation temperature

between 36 and 40°C may survive.

Conclusions and applications

Alteration of incubation temperature during late embryogenesis in Japanese quails may reverse Japanese quail's sex. Japanese quail eggs incubated at 36°C, 37°C, 38°C, 39°C and 40°C and the hatchlings brooded at a range of 30.5 – 34.84°C, may develop thermotolerant traits which may improve post-hatch performance and survivability. Consequently, incubation temperature manipulation may be adopted to improve Japanese quail's productivity.

References

1. Fah, K.H. (2009). The nutrition and management of Japanese quail in the Tropics Pp. 1-3.
2. G.th, A. and Booth, D.T. (2005). Temperature-dependent sex ratio in a bird. *Biology Letters*, 1: 31-33.
3. Elmehdawi, A.S. (2013). The effects of altering incubation temperature on broiler chick hatchability, chick quality, sex ratio and subsequent performance under field conditions. Ph.D. Thesis, presented to the Graduate School of Clemson University, paper 1142, Pp. 82.
4. Krischek, C., Gerken, M. and Wicke, M. (2013). Effects of higher incubation temperature between embryonic day 9 and 12 on growth and meat quality characteristics of turkeys. *British Poultry Science*, 54: 5-11.
5. Pike, T.W. and Petrie, M. (2003). Potential mechanisms of avian sex manipulation. *Biology Review*, 78: 553-574.
6. Alamuoye, O.F. and Ojo, J.O. (2015). Comparison of carcass characteristics of sexed Japanese quails (*Coturnix japonica*). *Scholars Journal of Agriculture and Veterinary Science* 2(5):342-344.
7. SPSS, (2010). Statistical package for social sciences, SPSS Inc., 444. Michigan Avenue, Chicago, IL 60611.
8. Penn State Extension, (2016). Brooding of Domestic Fowl. College of Agricultural Sciences, The Pennsylvania State University. <https://extension.psu.edu/brooding-of-domestic-fowl>.
9. El-Kholy, M.S., Ibrahim, Z.A., El-Mekkawy, M.M. and Alagawany, M. (2019). Influence of *in ovo* administration of some water-soluble vitamins on hatchability traits, growth, carcass traits and blood chemistry of Japanese quails. *Annals of Animal Science*, 19(1): 97.111. DOI: 10.2478/aoas-2018-0041.
10. 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. *Poultry Science*, 87:1516-1525.
11. Jacob, J. and Mather, F.B. (2000). Sex reversal in chickens 1. FACTSHEET PS-53. Department of Animal Sciences, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. <http://edis.ifas.ufl.edu>.
12. Chin, E.H., Storm-Suke, A.L., Kelly, R.J. and Burness, G. (2013). Catch-

- up growth in Japanese quail (*Coturnix japonica*): relationships with food intake, metabolic rate and sex, *Journal of Comparative Physiology B.*, 183(6):821-831.
13. Piestun, Y., Halevy, O., Shinder, D., Ruzal, M., Druyan, S. and Yahav, S. (2011). Thermal manipulation during broiler embryogenesis improves post-hatch performance under hot conditions, *Journal of Thermal Biology*, 36:469-474.