Effects of egg position in artificial incubation of Japanese quail eggs (*Coturnix japonica*)

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**Abstract**

Incubation procedures are important for maintenance and improvement of quail egg production. Many factors can interfere with the success of incubation or the quality of hatched chicks. Thus, the objective of this study was to verify the effects of different positions and turning during artificial incubation of Japanese quail eggs on hatchability, egg weight loss, chick weight, and embryonic mortality. Eighty Japanese quail (*Coturnix japonica*) were used for egg collection. Six hundred eggs were divided into four experimental groups (n = 150), according to their position during incubation: vertical position with small end up, vertical position with small end down, horizontal position without turning, and horizontal position with turning. Incubation process was done by automatic hatcheries with temperature of 37.5°C and relative humidity of 60%. All eggs were weighed on their collection day and during incubation period to verify egg weight loss. All quail chicks were weighed at hatch. The eggs incubated in horizontal position with turning had the highest hatchability (77%), while the ones incubated in vertical position with small end up presented the lowest hatchability (8%). Egg position and turning influenced water loss during incubation. The groups with better hatchability presented lower egg weight loss and better hatch weight. The infertility-early embryo death was similar to the late embryo death in the eggs incubated in horizontal position with turning, while in the groups incubated in static position, the late embryo death was higher. The late embryo death was considered the main cause of mortality in the groups incubated in horizontal position without turning (31%), vertical position with small end down (24%) and vertical position with small end up (70%).

**Keywords:** egg position, egg turning, incubation, Japanese quail.

**Introduction**

Quail raising has become an important poultry business in Brazil. Japanese quail have been reared for both egg and meat production all over the country, mainly by small and medium breeders. The Japanese quail raising is increasing because this bird can be kept easily in relative large numbers in a small facility and be used for a wide variety of works (Minvielle, 2004). Incubation procedures are important for maintenance and improvement of quail egg production. Many factors can interfere with the success of incubation or the quality of hatched chicks, such as egg position and turning during artificial incubation. Most avian eggs need to be turned during incubation for normal embryonic development to take place (Yoshizaki and Saito, 2002). The static incubation of eggs has been reported to be detrimental for embryo development, while egg turning during artificial incubation has some benefits, such as the reduction in mal-positioning of the embryo (Robertson, 1961), prevention of abnormal adhesion of the embryo or embryonic membranes to the shell membrane (New, 1957) and the complete and timely closure of chorioallantois at the small end of the egg (Deeming, 1989a, b). The avian embryo progresses through a series of positions throughout incubation and ends in a normal position for hatching (Wilson et al., 2003). Egg turning and egg position during storage (Tiwari and Maeda, 2005) and incubation (Wilson et al., 2003) can interfere with embryo position, affecting hatchability and chick quality. Egg position changes the exposed surface area, changing the loss of water from the egg, affecting hatchability indirectly (Tiwari and Maeda, 2005). Information about the effects of egg position during incubation for Japanese quail eggs is lacking. Thus the objective of this research was to verify the effects of different positions and turning during artificial incubation of Japanese quail eggs on hatchability, egg weight loss, chick weight, and embryonic mortality.

**Materials and Methods**

**Birds**

Eighty Japanese quail (*Coturnix japonica*) were used for egg collection. They were housed in experimental battery cages in the Laboratório de Estudos Ornitológicos da Universidade Estadual do Ceará. Three females and one male were lodged in each cage. The birds were 11 weeks of age and averaged 90% egg production. Water and balanced feed were supplied *ad libitum* according to National Research Council (1994) guidelines. They were also exposed to 17 hours/day of light.
Incubation and hatching

Quail breeders were used for egg collections, which were performed daily. The eggs underwent selection, verifying egg shape, extreme sizes and eggshell integrity by candling. A total number of 600 eggs was divided into four experimental groups (n = 150), according to their position during incubation (Fig. 1). Each group consisted of 150 eggs which were divided in five repetitions that were incubated in the same incubator. Eggs were incubated in vertical position with the small end up without turning (position A), in vertical position with small end down without turning (position B), in horizontal position without turning (position C), and in horizontal position with turning every 2 hours (position D). Each group was incubated in an incubator and the four machines were identical. Incubation process was done by automatic hatcheries with temperature of 37.5ºC and relative humidity of 60%. At the day 15 of incubation (360 h) all eggs were transferred to the hatchers where they were placed in horizontal position without turning. Incubation temperature and humidity conditions were maintained until hatching.

Weight measurement

All eggs were identified individually and weighed on their collection day. They were also weighed during incubation period on days 1 and 15 of incubation. All quail chicks were weighed after hatching. Weight measurements were obtained with a precision balance (0.001 g).

Embryonic mortality

Eggs that failed to hatch were opened for macroscopic observation, thus they were classified according to time of embryonic mortality. They were staged as infertile-early embryo mortality, which were the eggs with true infertility, pre-incubation mortality or initial stage mortality. This classification was according to Pedroso et al. (2006) that classified the embryo mortality in quail chicks as early death embryos (1 up to 4 days), intermediate (5 up to 15 days) and late death embryo (16 up to 18 days). The embryos that had mortality in the intermediate development stage were classified as intermediate embryo mortality. Unhatched eggs classified as late embryo mortality were the ones with final stage mortality or pipped eggs with dead embryos.

Statistical analyses

The experiment was performed in a completely randomized design with four experimental groups, with five replications of 30 eggs per group for hatchability and embryo mortality analyses. Each egg was considered a replication for egg weight loss and chick weight analyses. All data were analyzed using Statistix (1996). The results were analyzed using Analysis of Variance through general linear model, and the means of hatchability, egg weight loss and chick weight were compared using the test of Tukey. The means of embryo mortality were compared using Kruskal Wallis test. Statements of significance were based on P < 0.05.

Results

 Hatchability

Table 1 shows the hatchability of Japanese quail eggs incubated at different positions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hatchability ± SD (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal with turning</td>
<td>77.3 ± 7.2a</td>
<td>9.3</td>
</tr>
<tr>
<td>Horizontal without turning</td>
<td>59.3 ± 9.2b</td>
<td>15.6</td>
</tr>
<tr>
<td>Vertical small end down</td>
<td>65.3 ± 6.4b</td>
<td>10.0</td>
</tr>
<tr>
<td>Vertical small end up</td>
<td>8.0 ± 1.8c</td>
<td>22.8</td>
</tr>
</tbody>
</table>

Means in the column with different superscripts differ significantly (P < 0.05).

It was observed that the eggs incubated in any of the three static positions showed a lower hatchability compared to the eggs incubated with turning. Regarding the groups incubated in horizontal position, it was observed that the group without turning had a lower hatchability compared to the group with egg turning...

Every two hours. There was no statistical difference between the groups of eggs incubated in horizontal without turning and in vertical small end down (P > 0.05). The two groups of eggs incubated in vertical position presented a very different hatch rate. Thus, the eggs incubated in vertical small end down had an eight times higher hatchability compared to the ones incubated with the small end up.

**Egg weight loss**

Table 2 shows the egg weight loss during incubation of Japanese quail eggs at different positions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Egg weight loss ± SD (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal with turning</td>
<td>6.8 ± 1.2</td>
<td>15.5</td>
</tr>
<tr>
<td>Horizontal without turning</td>
<td>7.8 ± 1.2</td>
<td>15.1</td>
</tr>
<tr>
<td>Vertical small end down</td>
<td>8.6 ± 1.3</td>
<td>14.7</td>
</tr>
<tr>
<td>Vertical small end up</td>
<td>10.4 ± 2.2</td>
<td>20.8</td>
</tr>
</tbody>
</table>

"a-d"Means in the column with different superscripts differ significantly (P < 0.05).

The eggs incubated at vertical position with small end up presented the highest level of egg weight loss during incubation, while the eggs incubated in horizontal position with turning had the lowest egg weight loss. In a general way, the eggs incubated in horizontal position had lower weight loss compared to the eggs placed in vertical orientation. The egg weight loss was calculated just for eggs that hatched; this way, the eggs incubated in the vertical small end up position had a smaller number of hatched eggs compared to the other groups. This helped to increase its coefficient of variation for egg weight loss.

**Chick weight**

Table 3 shows the relation between chick weight at hatch and initial egg weight of Japanese quail eggs incubated at different positions.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Chick/egg weight ± SD (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal with turning</td>
<td>73.4 ± 3.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Horizontal without turning</td>
<td>69.5 ± 4.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Vertical small end down</td>
<td>69.9 ± 4.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Vertical small end up</td>
<td>65.8 ± 6.1</td>
<td>9.2</td>
</tr>
</tbody>
</table>

"a,b,c"Means in the column with different superscripts differ significantly (P < 0.05).

**Embryo mortality**

Embryo mortality

Table 4 shows the classification of unhatched Japanese quail eggs incubated at different positions. The causes of failure to hatch were infertile/early embryo mortality and late embryo mortality. Intermediate embryo mortality was not found in this study.

The late embryo death was higher than infertile/early embryo death in the three experimental groups that were incubated in a static position, while in eggs incubated with turning, the two embryo mortality classifications showed no statistical difference (P < 0.05). It was also observed that the infertile/early embryo death showed no statistical difference among treatments. The late embryo death was higher in the eggs incubated in the vertical small end up position when compared to the other three groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Infertile/early embryo death ± SD (%)</th>
<th>Coefficient of variation (%)</th>
<th>Late embryo death ± SD (%)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal with turning</td>
<td>14.7 ± 11.2 &quot;a&quot;</td>
<td>76.4</td>
<td>8.0 ± 8.7 &quot;a&quot;</td>
<td>108.6</td>
</tr>
<tr>
<td>Horizontal without turning</td>
<td>10.0 ± 5.3 &quot;a&quot;</td>
<td>52.7</td>
<td>30.7 ± 6.4 &quot;b&quot;</td>
<td>20.9</td>
</tr>
<tr>
<td>Vertical small end down</td>
<td>12.0 ± 4.5 &quot;a&quot;</td>
<td>37.3</td>
<td>24.0 ± 10.4 &quot;a&quot;</td>
<td>43.2</td>
</tr>
<tr>
<td>Vertical small end up</td>
<td>21.5 ± 23.6 &quot;a&quot;</td>
<td>110.8</td>
<td>70.5 ± 24.9 &quot;b&quot;</td>
<td>35.2</td>
</tr>
</tbody>
</table>

"a,b"Means in a column with different superscripts differ significantly (P < 0.05).

"A,B"Means within a row with different superscripts differ significantly (P < 0.05).
Discussion

According to Yoshizaki and Saito (2002), most avian embryonic eggs need to be turned during incubation for normal embryonic development to take place. Egg turning during incubation improves hatchability (Elibol et al., 2002). The increased mortality in unturned eggs occurs because embryos or extra embryonic membranes adhere prematurely to the shell membranes when they are not turned (New, 1957).

Yoshizaki and Saito (2002) found that quail eggs incubated at 39°C in vertical position with small end down and turned every two hours had a 85% hatchability, the eggs placed with their equatorial side down with turning had 63%, while the group of eggs placed with their equatorial side down without turning had only 24%. Compared to our results, it was observed that Japanese quail eggs presented a higher hatch rate for eggs placed in horizontal position with turning (77%) or without turning (59%). The hatch rate differences between these results were probably due to the use of different incubation temperatures.

The avian embryo progresses through a series of positions throughout incubation and ends in a normal position for hatching. There are variations of the normal position that are considered not to be detrimental to successful hatching. However, there are many positions which are associated with difficulty in hatching or are found in increased incidence in cases of poor hatchability. According to Wilson et al. (2003), a major cause of detrimental malposition, especially in broiler eggs, is setting eggs with the small end up; it can also explain the poor hatchability (8%) found in Japanese quail eggs incubated in this position.

It was observed in this work that the different egg positions have influenced the rate of egg weight loss. The static egg positions had a higher egg weight loss compared to eggs that were turned during incubation. Among the groups of static position, the eggs incubated in vertical small end up showed the highest weight loss.

The egg weight loss is an important parameter for incubation. It has been used to estimate vital gas exchange (Paganelli et al., 1978; Rahn et al., 1979) and has been correlated with the rate of embryonic metabolism and development (Rahn and Ar, 1980; Burton and Tullet, 1983). However, too low or too high water loss influences embryo development (Rahn and Ar, 1974) and, consequently, egg hatchability (Meir et al., 1984).

Tiwari and Maeda (2005) studied the effect of egg position on weight loss during pre-incubation storage and verified that egg weight loss did not differ between the normal (small end down) and opposite (small end up) position groups.

During the incubation, if the eggs remain in a static position there is a deficient absorption of albumen by the embryo (Deeming, 1989a) and this residual albumen can probably be an additional source for egg weight loss in eggs incubated in unturned positions.

It was observed that the egg position and turning interferes with the hatch weight of quail chicks. Vali et al. (2005) found that hatching weight of Japanese quail chicks was about 72% of the initial egg weight.

We found that the horizontal position with turning produced the heaviest chicks. However, the weight of chicks at hatch can also be affected by other factors, including species, breed, egg nutrient levels, egg environment, egg size (Wilson, 1991), weight loss during incubation period, weight of shell and other residues at hatch (Tullet and Burton, 1982), shell quality, and incubator conditions (Peebles and Brake, 1987).

Some studies reported that static incubation of the egg can promote a poor development of the area vasculosa which can produce a reduced rate of embryo growth (Deeming, 1989a) and also a lower weight at hatch, which is partially due to deficient absorption of the albumen.

Our results were similar to the ones in chickens, in which there are two phases of increased embryonic mortality during incubation: the first phase occurs during the first week of incubation and the second phase during the last week (Jassim et al., 1996).

Structural abnormalities account for a relatively high percentage of embryonic deaths. In addition to structural anomalies leading to the death of the embryo, there are also malpositions that may contribute to mortality. Malpositions become evident at or near the end of incubation and are of several types and several reasons (Romanoff, 1949). Elibol and Brake (2004) found an increase in malpositioned embryos with a complete absence of turning during the first week of incubation. Wilson et al. (2003), studying malpositioning in Bobwhite quail, found that setting quail eggs with small end up resulted in 75% of the embryos with head in the small end. We also observed, in eggs incubated in vertical small end up position, a greater number of quail chicks that failed to hatch because they were malpositioned and some were pipping eggshell in the small end of the egg.

In conclusion, this study found that different egg positions and turning can highly interfere with artificial incubation of Japanese quail eggs. It also elucidated that static incubation is detrimental to the reproductive performance of quail, but also that the different egg positions of static incubation showed quite different consequences for incubation parameters. However, this was just the first step, because it is also necessary to verify the critical periods during incubation for egg turning and positioning, as well as the post hatch performance of quail chicks.

Acknowledgments

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References


