Effect of pre-incubation long-term storage and warming on hatchability of Japanese quail eggs (Coturnix coturnix japonica)

Einfluss einer langen Lagerdauer und des Anwärmens von Wachteleiern (Coturnix coturnix japonica) vor der Brut auf den Bruterfolg

I. Seker, M. Bayraktar and S. Kul


Introduction

Optimizations of storage period and storage conditions of hatching eggs play an essential role in poultry husbandry for economical reasons. Pre-incubation storage period of eggs is likely to be longer than a week in laying hen producers as well as in poultry breeding companies. It has been recommended that hatching eggs should not be stored more than 7 days since the eggs may lose their hatchability due to spoilage (Soley, 1994). Hatchability decreases under even optimum conditions of eggs stored more than one week (Demircioğlu, 1994, Elibol, 1997, Ipek et al., 1999).

Commercial hatching establishments, especially the ones with high capacity, may have to store hatching eggs for a long period of time before incubation to decrease the cost such as labour and energy, to synchronize the time of hatching, and sometimes to limit the production depending on the market demand. Likewise, breeding establishments producing pedigree parents may sometimes need to store hatching eggs longer before the incubation to produce a number of full-siblings.

Evaporation of water in eggs depends on storage temperature and relative humidity. Increased loss of water content of the hatching eggs during storage may result in underdevelopment or death of embryos at different stages of the incubation. A quail egg may lose approximately 2% of its initial weight after 7 days of storage at room temperature (Soley, 1994). It has been reported that hatchability of quail eggs may decrease by 2-3% after 4 days of storage even under optimum conditions (Imai et al., 1986, Suku-Path and Tanpipat 1991).

The “Physiological zero” for poultry eggs has been reported between 19°C and 27°C (Proudfoot and Hulan, 1976; Decuyper and Michels, 1992). Basal research indicates that during storage there are no discernible embryonic development and the embryo remaining in a state of embryonic diapause when the eggs are held at temperatures below physiological zero. However, embryonic development still occurs during storage, although at a minimum rate, and therefore this fact may contribute to the decline in viability as the storage period increases (Meijerhof, 1992, Mayes et al., 1984).

Survival of embryo depends on many factors including number of blastoderm cells in laid egg, storage period, species, breed, age, egg-weight, and method of eggs collection, position of egg during storage, environmental temperature, relative humidity and environmental gases (Petitte, 1991, Brake et al., 1997). Embryonic survival may be decreased by changes in embryo or changes in albumen and pH (Meijerhof, 1992).

It has been reported that increased storage period caused weight loss of eggs (Paci et al., 1991), increased embryonic mortality (Arkici, 1996), decreased hatchability ratios (Walsh et al., 1995, Brake et al., 1997), delayed embryonic development and hatching (Meijerhof, 1994), decreased in albumen quality (increase in level, increase in pH) (Walsh et al., 1995, Brake et al., 1997), increased embryonic abnormalities (Fasenko et al., 1992) and mortality (Sittman et al., 1971, Fasenko et al., 1992).

There are many published reports indicating that warming of eggs during long-term storage before incubation improved hatchability (Becker and Bearse, 1958; Kucera and Radautz 1980; Parkhurst and Mountney, 1988; Meijerhof, 1994). This practice has also been reported to decrease early-embryonic mortality (Becker and Bearse, 1958, Ar and Meir, 1996). However, some researchers reported that this positive effect of pre-incubation warming of hatching eggs largely depended on genotype, stage of embryonic development at oviposition, age of hen, laying intensity and hatchability, storage conditions and time and length of warming during storage (Ar and Meir, 1996; Elibol, 1997). Elibol et al. (2000) studied effect of six different pre-incubation warming treatments (4.5 h at 24-37.5°C, 50-55% RH) on hatchability of hen-eggs. Their results showed that the lowest hatchability occurred in eggs that received warming treatment on day 1 of 21 days storage (42.7%) where the highest (77.0%) was observed in control eggs (any warming) as well as, in eggs warmed on day 7, and in eggs warmed both, on day 7 and 14 of storage. The lowest embryonic death rate was observed as 12.5% for early-period in group 6 (received warming treatment on days 7 and 14 of the storage) and as 0.5% and 8.7% for middle and late period deaths, respectively, in group 1 (control). The highest embryonic death rates were found as 45.6% for early period in group 2 (warming on day 1 of storage), as 2.5% for middle period in group 4 (warming on days 1, 7 and 14 of storage), and as 12.5% for late-period in group 3 (warming on days 1 and 7 of storage). These researchers concluded that eggs warming at the beginning and during the storage did not improve the hatchability rate. Similar results were reported by other researchers (Domanska and Pawluczuk, 1977; Bowling and Howarth, 1981).
The objective of the present study was to investigate the effects of warming of Japanese quail eggs at the beginning and during the long-term storage on embryonic mortality and hatchability of fertile eggs.

Materials and methods

The study was conducted between November 2002 and May 2003. A total of 2700 Japanese quail eggs were used for this study. The eggs were collected from 20-week-old laying quails for each trial for 3 consecutive days. The birds were housed as 1 male/3 female per cage of 40x30x30 cm. The Japanese quail were raised in floor pens and fed conventional starter and grower diets until they reached 6 wk of age. A standard layer diet (20% protein and 3.0% calcium) were given ad libitum. Water was available for ad libitum consumption and natural daylight was supplemented with artificial light to give an 18-h photoperiod.

Treatment groups and number of eggs within each group for each of 3 replicates are presented in Table 1. Warming procedure of eggs was conducted as described in Table 2, in order to avoid potential harms of direct heating (Elibol et al., 2000). Holding at 21-23°C for 15 h at the beginning and at the end of the warming process as shown in Table 2, was considered as adaptation period and warming. Eggs were stored at 9-15°C at 70-75% relative humidity in storage room during the remaining period of storage. During storage, the eggs were rotated 2 times every week at a 45° angle to left and right.

In this study, CIMUKA Hatcher of 3000 quail eggs capacity was used. The eggs were disinfected by formaldehyde fumigation for 20 min in the incubator before the incubation was initiated. For the first 15 days of incubation, the temperature was 37.7°C and humidity 55-60%. For the rest period the temperature was 37.5°C and the humidity was 75-80%. During incubation eggs were turned 45° to the right and to the left. They were turned 8 times every 24 hours automatically. Air circulation and the temperature were controlled automatically as well. The eggs were candled on the 6th day of incubation to detect fertile eggs and dead embryos. The embryos of fertile eggs showing the evidence of retarded development and no movement on candling were considered as dead embryos. Unfertile eggs were removed from the incubator. At the end of incubation, unhatched eggs were separated and cracked for determination of the cause including infertile eggs or early embryonic (<6 day) and middle (7-15 day) or late embryonic mortality (16-17 day). Embryonic mortality rate was calculated as percent of the embryonic deaths within the fertile eggs, not within all death embryos (All dead embryos were not taken as 100%). Hatchability of fertile eggs (%) was calculated using the following formula:

\[
\text{Hatchability of fertile eggs} \% = \frac{\text{Number of hatched chicks}}{\text{Total number of fertile eggs}} \times 100
\]

The percentage data within treatment for early, middle, and late mortality classes and for fertility rate and for hatchability were transformed into angles (angle= (arc sin) prior to analysis (Duzgunes et al., 1987). For visualization of results untransformed values are displayed in the tables of results. The effects of pre-incubation warming and replicate on hatchability results were determined by using GLM (General Linear Model) procedure. The model did not include effect of replicate because it was not significant (P<0.05). If significant differences (P<0.05) were found by GLM, least square means for fertility, hatchability, and embry mortality data were separated using Duncan's Multiple Range Test (Snedecor and Cochran, 1980). SPSS software was employed for statistical analysis (SPSS, 1999).

Results

The effects of 5 different pre-incubation warming treatments on hatchability and embryonic mortality in Japanese quail eggs are shown in Table 3.

Overall apparent fertility rate was calculated as 66.5%. The differences between mean apparent fertility rates of the treatment groups were significant (P<0.01).

The lowest hatchability was observed in group 1 (any warming (control group)) (39.9%) while as the highest percentage occurred in groups 4 (warming on day 7 of storage) (56.2%) and 5 (double warming on days 7 and 14 of storage) (57.4%). The hatchability of fertile eggs of group 4 was significantly higher than group 1, 2 (warming on day 7 of storage) and 3 (double warming on days 1 and 7 of storage) (P<0.05). In group 5, the hatchability of fertile eggs was significantly higher than the other groups (except group 4) (P<0.05).

Table 1. Treatment groups and number

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Treatments*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No warming (control group)</td>
</tr>
<tr>
<td>2</td>
<td>Warming on day 1 of storage</td>
</tr>
<tr>
<td>3</td>
<td>Double warming on days 1 and 7 of storage</td>
</tr>
<tr>
<td>4</td>
<td>Warming on day 7 of storage</td>
</tr>
<tr>
<td>5</td>
<td>Double warming on days 7 and 14 of storage</td>
</tr>
<tr>
<td>6</td>
<td>Warming on day 14 of storage</td>
</tr>
</tbody>
</table>

*150 eggs were used for each treatment group within each of 3 replicates. The eggs were stored for 21 days at 9-15 °C

Table 2. Warming procedure of eggs during storage

<table>
<thead>
<tr>
<th>Location</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Relative humidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg storage room</td>
<td>15</td>
<td>21-23</td>
<td>70-75</td>
</tr>
<tr>
<td>Incubator</td>
<td>1.5</td>
<td>24-37.5</td>
<td>50-55</td>
</tr>
<tr>
<td>Incubator</td>
<td>3</td>
<td>37.5</td>
<td>50-55</td>
</tr>
<tr>
<td>Incubator (doors open)</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Egg storage room</td>
<td>15</td>
<td>21-23</td>
<td>70-75</td>
</tr>
</tbody>
</table>

In total 36.5 h in temperatures above “physiological zero”
Table 3. Influence of pre-incubation warming of eggs of Japanese quail on hatchability of fertile eggs and embryonic mortality

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Number of set eggs</th>
<th>Number of fertile eggs</th>
<th>Number of hatched chicks</th>
<th>Apparent Fertility (%)</th>
<th>Hatchability of fertile eggs (%)</th>
<th>Embryonic mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X ± S</td>
<td>X ± S</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Early dead (%)</td>
<td>Middle dead (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X ± S</td>
<td>X ± S</td>
</tr>
<tr>
<td>1 No warming (control group)</td>
<td>450</td>
<td>273</td>
<td>109</td>
<td>60.67 ± 13.92a</td>
<td>39.93 ± 10.72a</td>
<td>77</td>
</tr>
<tr>
<td>2 Warming only on day 1 of storage</td>
<td>450</td>
<td>277</td>
<td>126</td>
<td>61.56 ± 6.48b</td>
<td>45.49 ± 23.61a,b</td>
<td>68</td>
</tr>
<tr>
<td>3 Double warming on days 1 and on day 7 of storage</td>
<td>450</td>
<td>307</td>
<td>123</td>
<td>68.22 ± 3.85b</td>
<td>40.07 ± 12.95a,b</td>
<td>72</td>
</tr>
<tr>
<td>4 Warming only on day 7 of storage</td>
<td>450</td>
<td>308</td>
<td>173</td>
<td>68.44 ± 13.68b</td>
<td>56.17 ± 12.48c,d</td>
<td>65</td>
</tr>
<tr>
<td>5 Double warming on days 7 and 14 of storage</td>
<td>450</td>
<td>319</td>
<td>183</td>
<td>70.89 ± 4.07b</td>
<td>57.37 ± 7.48c,d</td>
<td>61</td>
</tr>
<tr>
<td>6 Warming only on day 14 of storage</td>
<td>450</td>
<td>312</td>
<td>147</td>
<td>69.33 ± 4.67b</td>
<td>47.12 ± 6.71a,c</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>2700</td>
<td>1796</td>
<td>861</td>
<td>66.52 ± 8.51</td>
<td>47.94 ± 13.54</td>
<td>403</td>
</tr>
</tbody>
</table>

- - Not significant, *: Significant difference (P<0.05), **: Significant difference (P<0.01)

a-d: Means within columns with no common superscript differ significantly (P<0.05).
The highest embryonic mortality rate was observed in early stage of group 1, last stage of group 1 and in middle stage of group 3 while the lowest rates were determined in early stage of group 5, and in middle stage of group 5 and late stages of group 4. There were no significant differences (P>0.05) in early embryonic mortality between treatment groups.

Discussion

Fertility rates calculated in the present study (60.7%-70.9%) were found to be lower than those reported for quails in the literature. For example, SAYLAM (1999) studied the effect of pre-incubation storage of eggs on fertility rate of quails of German origin. The eggs were obtained from 11 week old quails housed as 1 male:2 females. The researcher reported fertility rates as 94.1, 91.2, 88.0, 88.4, 88.9 and 72.2% in eggs stored for 1, 3, 5, 7, 9 and 11 days before incubation. Similarly, WOODARD et al. (1983) reported fertility rate as 79% in quail eggs stored up to 8 days.

The low fertility rate observed in the present study can be explained by death of the embryos at a very early stage during storage due to various factors (temperature, humidity, rotation) or by the fact that fertility of these eggs could not be determined during the fertility control conducted on day 6 of the incubation and recorded as infertile. In addition, low fertility might be resulted from differences in parent quails in terms of age, genotype, male: female ratio, egg weight, environmental temperature, caring and handling conditions.

In general, results indicated that hatchability rates were significantly higher in groups that received a warming treatment on day 7 only or 14 only or on day 7 and 14 of 21 days storage. These results were consistent with those reported by AKINCI (1996) who observed positive effects of pre-incubation warming (at 27°C for 3 d) during short-term storage, regardless of application at the beginning or at the end of storage. AKINCI (1996) reported that when storage period was prolonged (22 day), pre-incubation warming at the beginning of the storage resulted in negative effects (low hatchability) while positive effects from warming during the incubation were still observed. Similarly, our results were in consistency with those of other studies that indicated pre-incubation warming at the beginning and during the storage of hatching eggs improved the hatchability rates (BECKER and BEARSE, 1958; KUCERA and RADDATZ, 1980; PAREMBOUR and MOUNTNEY 1988; MEIJERHOF, 1994, ELIBOL, 1997).

In the present study, positive effect of warming treatment in groups 4, 5 and 6 can be explained by increased metabolic activity of embryo due to periodical warming. Similar approaches were reported by some other researchers (KUCERA and RADDATZ, 1980). Although a relative reduction in embryonic mortality rates was observed in groups received a warming treatment, the differences between groups were not significant for early embryonic mortality. It can be speculated that warming during the long-term storage may have stimulate the embryos stored under physiological zero, therefore, leads embryos to perceive long-term storage as a short one.

Hatching performance values in groups 4 (warming only on day 7 of storage) and 5 (double warming on days 7 and 14 of storage), in which pre-incubation warming showed a positive effect, were found to be lower than those of 1-week-regular-stored eggs, which is accepted as a normal storage regime for quail eggs.

SAYLAM (1999) reported hatching performance values as 68.5, 66.6, 66.3, 71.98, 58.2 and 63.3% in eggs stored for 1, 3, 5, 7, 9 and 11 days before incubation. Similarly, CAMCI (1995) reported hatching performance rates as 73.3, 76.6, 67.7 and 88.3% in egg groups stored up to 1 week. Hatching performance in groups 4 and 5 of the present study were lower than those reported by WOODARD et al. (1983) for eggs stored for 2-8 days (69%). However, they were found higher than those reported by the same researchers for eggs stored for 9-15 days (53%) and for 16-22 days (26%). This result indicates that pre-incubation warming of quail eggs stored for 21 days results in a lower hatching performance compared to those stored for up to 1 week, while a higher hatching performance in eggs stored longer than 9 days may occur.

It is known that sudden changes in environmental conditions affect embryonic development negatively and even cause embryonic deaths. Based on this fact, warming process was conducted precisely to avoid any sudden temperature changes by gradually increasing and decreasing the temperature. Similar results were reported by other researcher (DEMIRCOGLU, 1994). On the other hand, results of this study were different from those reported by some researchers who claimed that warming of eggs did not affect hatchability at all (DOMANSKA and PAWLUCZUK, 1977; BOWLING and HOWARTH, 1981). These differences might be due to variations in genotype, developmental stage of eggs when laid, flock age, egg yield, hatching performance, storage period and conditions, time of warming, as stated by other researchers (AR and MEIR, 1996, ELIBOL, 1997).

It should be considered that pre-incubation warming of long-term storage of hatching eggs may cause additional difficulties due to gradual heating and cooling which may not be practical under commercial conditions (ELIBOL et al., 2000).

As a result, pre-incubation warming at the beginning and during the storage of 21 days resulted in an increased hatching performance. This positive effect was significantly higher in groups 4 and 5 compared to other groups.

Although there was no significant difference in embryonic deaths between groups, effect of pre-incubation warming treatment in decreasing early stage embryonic deaths was observed. Effect of pre-incubation warming in decreasing middle and late stage embryonic deaths were significant. In middle period, the lowest rate of embryonic death was seen in group 5 (double warming on days 7 and 14 of storage) while the highest was in group 3 (double warming on days 1 and 7 of storage). In the late period, the lowest rate of embryonic death was observed in group 4 (warming on day 7) while the highest was in control group.

In the present study, the highest hatching performance and the lowest rate of embryonic deaths (early, middle, and late) were observed in group 5. In control group, however, hatching performance was the lowest and embryonic deaths were the highest. Since there was no significant difference in hatchability between groups warmed on day 7 only and warmed on both days 7 and 14, it can be advantageous to warm eggs on day 7 only to avoid unnecessary expenses in energy and labour. Further studies are warranted to understand the effects of genotype, flock age, laying intensity, storage conditions, storage period, and time and length of warming on pre-incubation heating application of quail eggs that will be stored long-term.

Acknowledgement

This investigation was supported by Firat University, Scientific Research Foundation (FUBAP), and Project num-
Summary

The objective of the present study was to investigate the effects of warming of Japanese quail eggs at the beginning and during the long-term storage on embryonic mortality and hatchability of fertile eggs.

A total of 2700 Japanese quail eggs were used for this study. The eggs were collected from 20-week-old laying quails for each trial for 3 consecutive days. The birds were housed as 1 male/3 female per cage of 40x30x30 cm. A standard layer diet (20% protein and 3.0% calcium) was given ad libitum. Natural daylight was supplemented with artificial light to give an 18-h photoperiod. Eggs were stored at 9-15°C at 70-75% relative humidity in storage room during the remaining period of storage. The eggs were candled on the 6th day of incubation to detect fertile eggs and dead embryos. At the end of incubation, unhatched eggs were separated and cracked for determination of the cause including infertile eggs or embryonic mortality. Embryonic mortality rate was calculated as percent of the embryonic deaths within the fertile eggs, not within all death embryos.

In this study, overall apparent fertility rate was calculated as 66.5%. The differences between mean apparent fertility rates of the treatment groups were significant (P<0.01). The lowest hatchability was observed in group 1 (any warming (control group)) (39.9%) while as the highest percentage occurred in groups 4 (warming on day 7 of storage) (56.2%) and 5 (double warming on days 7 and 14 of storage) (57.4%). The hatchability of fertile eggs of group 4 was significantly higher than for groups 1, 2 (warming on day 1 of storage) and 3 (double warming on days 1 and 7 of storage) (P<0.05). In group 5, the hatchability of fertile eggs was significantly higher than in the other groups (except group 4) (P<0.05).

The highest embryonic mortality rate was observed in early stage of group 1 while the lowest rate was determined in early stage of group 5. Although there was no significant difference in embryonic deaths between groups, effect of pre-incubation warming treatment in decreasing early stage embryonic deaths was observed. Effect of pre-incubation warming in decreasing middle and late stage embryonic deaths was significant. In middle period, the lowest embryonic death rate was seen in group 5 (double warming on days 7 and 14 of storage) while the highest was in group 3 (double warming on days 1 and 7 of storage). In the late period, the lowest embryonic death rate was observed in group 4 (warming on day 7) while the highest was in control group.

In the present study, the highest hatching performance and the lowest rate of embryonic deaths (early, middle, and late) were observed in group 5. In control group, however, hatching performance was the lowest and embryonic deaths were the highest. Since there was no significant difference in hatchability between groups warmed on day 7 only and warmed on both days 7 and 14, it can be advantageous to warm eggs on day 7 only to avoid unnecessary expenses in energy and labour.

Key words

Japanese quail, egg warming, storage, hatching eggs, hatchability, embryonic mortality

Einfluss einer langen Lagerdauer und des Anwärmen von Wachtelleiern (Coturnix coturnix japonica) vor der Brut auf den Bruterfolg

Das Ziel der vorliegenden Studie war die Untersuchung der Auswirkungen des Anwärmen von Wachtel-Bruteiern zu Beginn und während der Langzeitlagerung auf die Embryonalsterblichkeit und die Schlupfrate der befruchteten Eier.


In der Untersuchung wurde ein durchschnittlicher Anteil an befruchteten Eiern von 66,5% ermittelt. Die Behandlungsgruppen unterschieden sich dabei signifikant im Anteil befruchteter Eier (P<0.01). Die geringste Schlupfrate wurde in Gruppe 1 (kein Anwärmen = Kontrolle; 39,9%) beobachtet, während die höchsten Schlupfraten in den Gruppen 4 (Anwärmen am 7. Tag; 56,2%) und in Gruppe 5 (Anwärmen am 7. und 14. Tag; 57,4%) vorlagen. Die Schlupfrate der befruchteten Eier war in Gruppe 4 signifikant höher als in den Gruppen 1 und 2 (Anwärmen am 1. Tag) sowie 3 (Anwärmen am 1. und 7. Tag)(P<0,05). In Gruppe 5 wurde die höchste Schlupfrate der befruchteten Eier im Vergleich zu den anderen Gruppen (außer 4) erzielt (P<0,05).


In der vorliegenden Untersuchung wurden der beste Bruterfolg und die geringste Embryonalsterblichkeit (frühe, mittlere und letzte Phase) in Behandlung 5 beobachtet. In der Kontrollgruppe lagen dagegen die Schlupfrate am niedrigsten und die Embryonalsterblichkeit am höchsten. Nachdem keine signifikanten Unterschiede im Bruterfolg zwischen den Gruppen, bei denen die Eier nur am 7. oder am 7. und 14. Tag angewärmt wurden, vorlagen, wird zur Kostenminimierung die Anwärmung nur am 7. Tag empfohlen.
Stichworte
Japanische Wachtel, Anwärmen, Lagerung, Bruteier, Bruterfolg, Embryonalsterblichkeit

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Arch.Geflügelk. 1/2006