EDUCATION AND PRODUCTION

Prestorage Incubation of Long-Term Stored Broiler Breeder Eggs:
1. Effects on Hatchability

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ABSTRACT Two thousand eight hundred broiler breeder eggs were used to determine if prestorage incubation (PRESI) treatments of 0, 6, 12, or 18 h (at 37.5 C) could improve the hatchability of eggs stored (at 11.5 C) for 4 vs. 14 d. Embryonic development of 30 eggs was established after exposing the eggs to each PRESI treatment. The remaining eggs were cold-stored for 4 or 14 d and then incubated for 21 d. Unhatched eggs were broken open to determine fertility, and if fertile, stage of embryonic death was determined. Statistical significance was assessed at \( P < 0.05 \). Embryonic development significantly advanced \(( P = 0.00001 \) as the number of PRESI h increased. Therefore, embryos from each of the four PRESI treatments were placed into cold storage at different stages of development. Egg storage for 14 vs. 4 d significantly reduced the hatchability of all eggs set (58.4 and 88.2%, respectively). The PRESI treatments did not have a significant beneficial or detrimental effect on the hatchability of all eggs set for the eggs stored 4 d. However, in eggs stored for 14 d, PRESI for 6 h significantly improved hatchability of all eggs set (79.0%) when compared to eggs that were not PRESI (70.5%). The hatchability of all eggs set for eggs PRESI for 18 h and stored for 14 d was significantly reduced (9.1%) when compared to the other 14-d stored PRESI treatments. The results of this study provide evidence that embryos of eggs that have completed hypoblast formation (PRESI for 6 h) and are stored for 14 d have a survival advantage over embryos of 14-d stored eggs that have not been subjected to any PRESI treatments.

(Key words: broiler breeder, egg storage, embryonic development, hatchability, prestorage incubation)

INTRODUCTION

Hatching eggs are often stored at cool temperatures for extended periods. Egg storage occurs at the broiler breeder farm as well as at the hatchery. The main reason for on-farm storage is to minimize transportation costs incurred by the hatchery, which would be high with daily egg pick-up. Storage at hatcheries occurs for two main reasons. First, hatching eggs are stored until enough eggs are available to fill large incubator racks. Second, stockpiling of eggs occurs in anticipation of fluctuations in egg production or demand during the production year.

Storing fertile eggs at cool temperatures stops embryonic development as assessed by microscopic staging methods (Fasenko et al., 1992; Bakst and Gupta, 1997). The minimum temperature above which embryonic development occurs (physiological zero) has been reported at two different levels. Edwards (1902) reported the minimum temperature for embryonic development to be 21 C, whereas Funk and Biellier (1944) found this minimum temperature to be 28 C. Previous researchers have hypothesized that the minimum temperature for embryonic development is not the same for all developing tissues of the early growing embryo (Kaufman, 1948). Therefore, the objective of storing eggs at temperatures well below physiological zero is to prevent abnormal growth of the embryo that could occur if eggs were held at temperatures between physiological zero and normal incubation temperatures of 37.5 C.

The hatching egg industry has long recognized that egg storage longer than 7 d is detrimental to hatchability. Mayes and Takeballi (1984) and Meijerhof (1992) have reviewed the relevant literature on this topic. In an effort to improve the hatchability of long-term stored eggs, many investigations have been conducted in which hatching eggs are warmed prior to storage. Only three previously published studies have associated improvements in hatchability of chickens (Coleman and Siegel, 1966; Kosin, 1956) and of turkeys (Kosin, 1956; Fasenko et al., 2001) with the stage of embryonic development in the egg at the time the egg is stored. Most recently, Fasenko et al. (2001) determined that when turkey eggs are stored for 14 d, embryos that have completed hypoblast forma-
tion (12 h of PRESI) have a survival advantage over embryos that are undergoing, or have just completed, area pellucida formation (0 h of PRESI).

No previously conducted research has tested PRESI as a method for improving the hatchability of long-term stored eggs laid by a modern, high meat yielding breeder strain. The first objective of this study was to determine if PRESI would improve the hatchability of 14-d stored eggs from a modern broiler breeder strain. If PRESI were found to improve hatchability, the second objective would be to use a precise staging method for staging the development of preprimitive streak embryos and determine the optimum stage of embryonic development that produces the best embryo livability during 14 d of storage.

**MATERIALS AND METHODS**

The experimental procedures conducted in this study were in accordance with the principles and guidelines set out by the Canadian Council on Animal Care (1993).

**Flock Information and Egg Collection**

Two thousand eight hundred breeder eggs were collected between 0800 and 1000 h from a commercial breeder flock. All eggs were cleared from the nests prior to egg collection to ensure that eggs laid late on the previous day were not included. One group of 1,400 eggs was collected and stored for 14 d; the second group of 1,400 eggs was collected 10 d after the first set of eggs and cold-stored for 4 d. This method of collection was employed so that the eggs from both storage treatment groups could be set in the incubator at the same time. A 4-d storage was chosen, as this emulates current industry conditions; hatching eggs are commonly stored prior to incubation for 3 to 7 d, whereas storage longer than 7 d is avoided due to reductions in hatchability. The 14-d storage treatment was chosen to simulate a worst-case scenario. At the time of the first collection, the flock was 11 and 12 wk of age and comprised Arbor Acre females (n = 3,823) and Peterson males (n = 330). The eggs were numbered and weighed, and eggs weighing between 50 to 66 g were kept for the experiment. Prior to storage, egg characteristics, including specific gravity and fresh egg weight, shell, albumen, and yolk weights, were assessed on a subsample of 40 fresh eggs from each of the 14- and 4-d stored groups.

**Experimental Design**

Eggs (n = 1,360) designated to each of the two storage treatment groups (4 d and 14 d) were subdivided into four PRESI groups (n = 340) of 0, 6, 12, and 18 h. This procedure resulted in a 4 × 2 factorial experimental design (four PRESI durations × two egg storage lengths). Forty eggs from each of the 4- and 14-d PRESI treatments of 0 h were set aside to stage the embryonic development of 30 embryos. Ten additional eggs were necessary to account for infertile eggs and embryos not able to be staged due to damage incurred during the staging process. The embryo was prepared for developmental staging using the method described by Fasenko et al. (1991), except that the embryo was observed at approximately 16 times magnification. Stage of embryonic development was assessed according to the methods described by Eyal-Giladi and Kochav (1976) and Hamburger and Hamilton (1951). The remaining eggs (n = 300) from each of the 4- and 14-d storage treatment groups, which were not subjected to PRESI, were immediately placed small-end down in an egg cooler maintained at an average of 11.5 C and 60.5% humidity. This egg cooler temperature was chosen to optimize the hatchability of the eggs stored for 14 d. As reviewed by Mayes and Takeballi (1984) several investigations have determined that when eggs are stored longer than 7 d, the optimum storage temperature is between 11 and 12 C.

Eggs stored 4 or 14 d and undergoing PRESI of 6, 12, or 18 h were placed in a room at 24 C for 5 h, to allow the eggs to warm, prior to randomizing the PRESI groups throughout a Robbins incubator2 (dry bulb temperature of 37.5 C and a wet bulb of 30 C). After completion of the PRESI treatments, a subsample of 40 eggs were set aside to stage the embryonic development of 30 embryos from each of the three PRESI treatments. The remaining eggs (n = 300) were placed into storage in the same egg cooler as the 0 h PRESI treatment groups.

**Egg Incubation and Hatchability**

Upon completion of the 4- and 14-d egg storage treatments, all eggs were weighed, the treatments were replicated in 20 groups of 15 eggs, and the treatment groups completely randomized within the same Robbins incubator (dry bulb = 37.5 C, wet bulb = 30 C) for 18 d. At 18 d of incubation, the treatment groups were transferred to a Chick Master3 hatcher (dry bulb = 37.2 C, wet bulb = 33.0 C). After 21 d of incubation, live hatched chicks were counted, and any unhatched eggs were broken open to determine fertility, and if fertile, stage of embryonic death.

**Statistical Analysis**

The embryonic development, egg weight loss, fertility, and hatchability data were arranged in a 4 × 2 factorial design with four levels of PRESI (0, 6, 12, and 18 h) and two levels of egg storage (4 and 14 d). The embryonic development data were analyzed using Fisher's exact test (SAS Institute, 1992). As there were no significant effects of storage on embryonic development, the data from the two storage treatments were pooled to test the effects of the four PRESI treatments on embryonic development.
Egg weight loss during storage and incubation, as well as fertility, hatchability of all eggs, hatchability of fertile eggs, and embryonic mortality, were analyzed using the general linear models procedure (SAS Institute, 1992). The hatcher compartments containing 15 eggs per each PRESI x egg storage treatment served as the experimental unit, with 20 groups of 15 eggs per each interaction providing replication for statistical analysis. The main treatments as well as the interactions were analyzed for significance at $P < 0.05$. All data in percentage form were transformed using arc-sine transformations prior to analysis. Least-squares means and SEM were calculated for the main treatment effects of PRESI and egg storage and for the interaction. If significant differences were found by the general linear models procedure, the least-squares means were separated using the least-significant difference procedure (SAS Institute, 1992).

**RESULTS AND DISCUSSION**

**Egg Characteristics of Eggs Collected on Different Days**

Egg characteristics, on a subsample of eggs ($n = 40$) designated to be stored for 4 or 14 d, were conducted to determine if egg component variations existed between the eggs collected 10 d apart. Fresh egg weight, albumen weight, dried shell weight, and specific gravity were not significantly influenced by the egg collection day. Yolk weight was significantly higher ($P = 0.015$) in eggs designated for 4 d of storage (17.4 ± 0.2 g). These eggs were collected 10 d after egg collection of the eggs designated to be stored for 14 d (16.7g ± 0.2 g). Because egg size increases with bird age, it may be possible that the yolk size was affected by the breeder flock aging for an extra 10 d. However, yolk weight as a percentage of total fresh egg weight was not significantly different between the two egg storage groups collected on different days ($P = 0.47$).

**Egg Weight Losses**

Because there were significant interaction effects for the data on fresh egg weight and egg weight loss during storage, only the interaction effects and not the main treatment effects are presented and discussed.

The interaction of egg storage duration and length of PRESI resulted in a significant difference in fresh egg weight with all of the 14-d stored eggs having lighter egg weights than 4-d stored eggs, irrespective of the amount of PRESI (Table 1). This difference may be attributed to the larger yolk weights in eggs designated for 4 d of storage. Because of the egg weight difference, all measurements of weight loss were calculated as a percentage of the fresh egg weight. Egg weight loss during incubation and total egg weight loss were not significantly affected by the storage x PRESI interaction. This result was expected, as all of the eggs, regardless of treatment, were incubated after storage in the same incubator for the same time. The interaction did produce a significant linear relationship in egg weight loss during storage. As egg storage time and length of prestorage incubation increased, egg weight loss during storage significantly increased in a linear fashion. This result was expected, as longer exposure to PRESI and storage would increase the opportunity for water vapor to escape from the egg.

**Embryonic Development Due to PRESI**

Immediately after the PRESI treatments were imposed, the stages of embryonic development were determined for eggs assigned to each storage treatment group. As there was no significant effect of storage length on embryonic development, the results for embryos from eggs stored for 4 or 14 d were pooled to test the effects of the PRESI treatments on embryonic development (Table 2).

As expected, embryonic development increased significantly ($P = 0.00001$) as the length of the PRESI period increased (Table 2). Incubating eggs for 6, 12, and 18 h exposed the embryos to temperatures above their physiological zero, which allowed embryonic development to progress. The results of this experiment concur with those of Eyal-Giladi and Kochav (1976) and Hamburger and Hamilton (1951) with respect to the number of hours of incubation required to attain certain embryonic developmental stages. The majority of embryos of eggs not exposed to any PRESI treatments were at Stage X of development (Eyal-Giladi and Kochav, 1976). At this stage, area pellucida formation is complete (Eyal-Giladi and Kochav, 1976). PRESI of eggs for 6 h had the effect of progressing embryonic development to the point at which most embryos were at Stage XIII (Eyal-Giladi and Kochav, 1976). At this stage of development, hypoblast formation is complete (Eyal-Giladi and Kochav, 1976). When eggs were PRESI for 12 h, the majority of embryos were determined to be at Hamburger and Hamilton’s (1951) Stage 3. This developmental stage is characterized as having primitive streak formation that is approximately half complete. The majority of embryos of eggs PRESI for 18 h were at Stage 4 (Hamburger and Hamilton, 1951). At this stage of development, primitive streak formation is complete, and the streak is at a maximal length (Hamburger and Hamilton, 1951). The advance in embryonic development as PRESI increased meant that embryos from the eggs exposed to the four PRESI treatments were at different stages of development when they were placed into cool storage for 4 or 14 d.

**Fertility**

Because there were significant interactions in all of the variables presented in Table 3, except mortality from 8 to 14 d of incubation, presentation and discussion of the data will be limited to the interaction effects only.

Fertility should not have been affected by the interaction, as fertilization would or would not have occurred before the eggs were exposed to the treatments. The lower
**TABLE 1. Fresh egg weight and egg weight loss during storage, transfer, and total egg weight loss as a percentage of the fresh egg weight for the interaction of storage × prestorage incubation**

<table>
<thead>
<tr>
<th>Interaction effects</th>
<th>n²</th>
<th>Fresh egg weight (g)</th>
<th>Egg weight loss during storage (%)</th>
<th>Egg weight loss during incubation (%)</th>
<th>Total egg weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage (d) × prestorage incubation (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-0</td>
<td>294</td>
<td>57.82⁶b</td>
<td>0.15⁵h</td>
<td>12.61</td>
<td>12.74</td>
</tr>
<tr>
<td>4-6</td>
<td>292</td>
<td>57.64⁶b</td>
<td>0.32⁶g</td>
<td>12.28</td>
<td>12.55</td>
</tr>
<tr>
<td>4-12</td>
<td>294</td>
<td>58.58⁶f</td>
<td>0.51⁶f</td>
<td>12.35</td>
<td>12.79</td>
</tr>
<tr>
<td>4-18</td>
<td>298</td>
<td>58.47⁶a</td>
<td>0.68⁶e</td>
<td>12.35</td>
<td>12.94</td>
</tr>
<tr>
<td>14-0</td>
<td>300</td>
<td>56.99⁶c</td>
<td>1.20²d</td>
<td>13.16</td>
<td>14.20</td>
</tr>
<tr>
<td>14-6</td>
<td>300</td>
<td>56.84⁶c</td>
<td>1.30²c</td>
<td>13.31</td>
<td>14.43</td>
</tr>
<tr>
<td>14-12</td>
<td>300</td>
<td>56.64⁶c</td>
<td>1.37²b</td>
<td>13.04</td>
<td>14.23</td>
</tr>
<tr>
<td>14-18</td>
<td>298</td>
<td>56.60⁶c</td>
<td>1.67¹a</td>
<td>13.03</td>
<td>14.48</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.18</td>
<td>0.02</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.0004</td>
<td>0.0001</td>
<td>0.2791</td>
<td>0.2694</td>
</tr>
</tbody>
</table>

²Means within columns with no common superscript differ significantly (P < 0.05).
³Values are least-squares means.
⁴n = Number of eggs.

The percentage fertility of the eggs stored for 14 d and PRESI for 18 h occurred as a result of an underestimation of fertility; germinal discs that were actually fertile, but had died very early during development were likely misclassified as infertile. This overestimation of infertility occurred because of the difficulty in distinguishing between fertile gers and embryos that died at very early stages of development. Establishing the difference between a fertile germ and an embryo that had died prior to significant extra-embryonic membrane formation was made more difficult because the nonhatching eggs were examined after incubation for 21 d.

**Hatchability of All Eggs Set and of Fertile Eggs**

The interaction between egg storage length and duration of PRESI influenced the hatchability of all eggs set and of fertile eggs (Table 3). The values for hatchability of all eggs set from the 4 d-0 h treatment group (87.5%) and the 14 d-0 h group (70.5%) represent the levels of hatchability that would be expected in industry, as PRESI is not a practice that is currently employed by commercial hatcheries. The results from this experiment show that, in eggs stored for 4 d, the PRESI treatments of 6, 12, or 18 h had neither a beneficial nor a detrimental effect on hatchability when compared to the 0-h PRESI treatment. However, for the eggs stored for 14 d, the hatchability of eggs PRESI for 6 h improved significantly when compared to eggs with 0 h of PRESI. Long-term egg storage increases the incubation time required for eggs to hatch (Kirk et al., 1980). Without further examination of the data from the present experiment, it could be argued that the reason PRESI improves hatchability is because it provides more incubation time for the egg to hatch. However, the significant reduction in hatchability in eggs stored for 14 d and PRESI for 18 h shows that embryos advancing to the developmental level at which primitive streak formation is complete have a survival disadvantage when placed into cool storage. If improvements in hatchability of PRESI long-term stored eggs were due simply to an increase in incubation time, the 18-h PRESI...
## TABLE 3. The effect of the interaction between egg storage and prestorage incubation on fertility, hatchability of all eggs set, hatchability of fertile eggs, and broiler embryonic mortality

<table>
<thead>
<tr>
<th>Interaction effects</th>
<th>n²</th>
<th>Fertility</th>
<th>Hatchability of all eggs set</th>
<th>Hatchability of fertile eggs</th>
<th>Incubation periods when embryonic mortality occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage (d) × prestorage incubation (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1-7 d</td>
</tr>
<tr>
<td>4-0</td>
<td>20</td>
<td>97.6 a</td>
<td>87.5 a</td>
<td>89.7 b</td>
<td>6.2 bcd</td>
</tr>
<tr>
<td>4-6</td>
<td>20</td>
<td>95.6 a</td>
<td>89.6 a</td>
<td>93.7 a</td>
<td>5.6 d</td>
</tr>
<tr>
<td>4-12</td>
<td>20</td>
<td>97.3 a</td>
<td>85.9 b</td>
<td>94.4 a</td>
<td>2.9 d</td>
</tr>
<tr>
<td>4-18</td>
<td>20</td>
<td>97.3 a</td>
<td>70.6 d</td>
<td>72.2 a</td>
<td>11.3 b</td>
</tr>
<tr>
<td>14-0</td>
<td>20</td>
<td>96.6 a</td>
<td>79.6 b</td>
<td>81.9 d</td>
<td>5.8 bcd</td>
</tr>
<tr>
<td>14-6</td>
<td>20</td>
<td>96.0 a</td>
<td>74.9 b</td>
<td>78.1 b</td>
<td>10.8 bcd</td>
</tr>
<tr>
<td>14-12</td>
<td>20</td>
<td>80.1 b</td>
<td>9.1 a</td>
<td>11.5 b</td>
<td>46.3 a</td>
</tr>
<tr>
<td>14-18</td>
<td>20</td>
<td>80.1 b</td>
<td>9.1 a</td>
<td>11.5 b</td>
<td>46.3 a</td>
</tr>
<tr>
<td>SEM</td>
<td>1.2</td>
<td>2.2</td>
<td>2.2</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Probability</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.7381</td>
</tr>
</tbody>
</table>

a–f Means within columns with no common superscript differ significantly (*P < 0.05).

1Values are least-squares means.

2n = Number of hatcher compartments (experimental unit); 15 eggs per compartment.

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**Embryonic Mortality**

Early (1 to 7 d) and late (15 to 18 d) embryonic mortality and embryonic mortality at external pipping were significantly affected by the interaction between egg storage and prestorage incubation length. At this point it should be reiterated that in the interaction of eggs stored for 14 d and no given PRESI, very few embryos from the 14-4 storage—18-h treatment survived to late stages of embryonic development. This conclusion only serves to reinforce the observation that embryos at the stage of development when the primitive streak is at a maximal length are placed in storage for 14 d. Early embryonic mortality at external pipping in the 14-d-18-h treatment and mortality at external pipping in the 14-d-18-h treatment were significantly reduced compared to the other interaction treatment groups. This result accounts for 4 d with 0 h of PRESI; this result is in contrast to eggs stored for 14 d and PRESI for 18 h that had significantly reduced hatchability compared to eggs stored for 4 d with 0 h of PRESI.

**Determining the Best Stage of Embryonic Development at Which to Store Eggs for 14 d to Obtain Maximum Hatchability**

The results of the present experiment provide evidence that PRESI does improve the hatchability compared to eggs stored for 4 d with no PRESI. It was further determined that PRESI for 12 h prior to storage for 14 d should have had the most beneficial effect on the hatchability of 14-d stored eggs.
brought the hatchability of these eggs up to the same level as the 0-h PRESI—4-d stored eggs. Based on the results of the present experiment, the optimal length of PRESI appears to be 6 h for the broiler breeder hatching egg industry when storage is 4 or 14 d. This 6-h PRESI treatment prior to storage for 14 d does not improve the hatchability of all eggs set to the same level as eggs stored for 4 d with 0 h of PRESI. However, in eggs stored for 14 d, there is still a significant improvement in hatchability of all eggs set of 8.5% when eggs are provided with 6 h of PRESI compared to 0 h PRESI.

Interestingly, the optimum PRESI lengths of 12 h for turkeys (Fasenko et al., 2001) and 6 h for broiler breeders advanced the turkey and broiler breeder embryos to the stage of development at which hypoblast formation is complete. In contrast to this stage, broiler breeder eggs stored for 14 d and PRESI for 18 h had significantly reduced hatchability due to extremely high early embryonic mortality. After 18 h of PRESI, the majority of broiler breeder embryos were at developmental Stage 4 of Hamburger and Hamilton (1951). At this stage of development, primitive streak formation is complete (Hamburger and Hamilton, 1951). This period of development is characterized as an extremely active period of cellular migration and differentiation (Bellairs, 1986). During this developmental stage, surface cells on the outside of the embryo ingress through the primitive streak to the inside of the embryo and differentiate into mesoderm and endoderm (Bellairs, 1986). We hypothesize that because embryos at Stage XIII (Eyal-Giladi and Kochav, 1976) are at a relatively quiescent developmental period, they are better able to withstand developmental arrest during cool storage. In contrast, the active cellular migration and differentiation of embryonic cells at Stage 4 (Hamburger and Hamilton, 1951) may not respond favorably to developmental arrest during 14 d of storage.

In conclusion, PRESI of commercial broiler breeder eggs could be used by the industry as a method to improve hatchability of hatching eggs stored for 14 d. Further research needs to more precisely determine the number of hours of PRESI required to obtain maximum hatchability. Data from the present experiment suggest that a 6-h PRESI is optimum; however, the actual optimum PRESI is somewhere between 0 and 12 h. This time frame should be investigated further. In addition, it should be noted that the present study examined specific storage durations of 4 and 14 d; exposure of eggs to other egg storage durations could alter the effectiveness of the PRESI treatments.

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