Effect of Ascorbic Acid and Cooling During Egg Incubation on Hatchability, Culling, Mortality, and the Body Weights of Broiler Chickens

A. H. ZAKARIA and M. A. AL-ANEZI

Department of Animal Production, College of Agriculture, King Saud University, Riyadh 11451, P. O. Box 2460, Kingdom of Saudi Arabia

ABSTRACT

Five experiments involving 3,100 settable eggs with living embryos of commercial broiler parent stock were conducted to determine the effect of ascorbic acid (AA) and cooling during egg incubation on embryonic weight, hatchability, percentage of cull chicks, embryonic mortality, and body weights of the hatched chicks. The treatments were carried out at 15, 15, 17, 11 and 19 d of incubation for Experiments 1, 2, 3, 4, and 5, respectively. The treatments for the experiments were as follows: 1) eggs injected with 0.1 mL of sterile saline solution; 2) eggs injected with saline containing 0.5, 1, 3, and 12 mg of AA per egg; and 3) control for Experiment 1; 1) eggs injected with 3 mg of AA per egg; 2) eggs cooled at 22°C for 24 h; and 3) control for Experiments 2 and 3; 1) eggs injected with 3 mg AA per egg; and 2) control for Experiments 4 and 5.

Chick embryos may be subjected to stress caused by excessive production of heat during the latter part of egg incubation (Tullett, 1990). If AA is an anti-stress agent, then the addition of AA may be beneficial for conditions of embryonic stress. Data on the effect of AA on the development of chick embryos are lacking. On the other hand, Sarpong and Reinhart (1985) and Lancaster and Jones (1988) suggested that egg cooling during the latter part of incubation to relieve the stress caused by excessive production of metabolic heat. The results of egg cooling showed that the effects were related to temperature, duration, and time of application. Additionally, Webb (1987) stated that embryonic thermal tolerance can be a heritable trait. The work presented here was undertaken to determine the effect of AA and cooling during incubation on the embryonic development of broiler chickens.

MATERIALS AND METHODS

Five experiments were conducted involving more than 3,000 settable eggs with living embryos of commercial broiler parent stock to determine the effect of AA or cooling during egg incubation on embryonic weight,
hatchability, number of cull chicks, embryonic mortality, and body weight of normal hatched chicks. Experiment 1 was carried out at 15 d of incubation with the following treatments: 1) eggs injected with 0.1 mL sterile saline solution; 2) eggs injected with 0.1 mL of saline solution containing 0.5, 1, 3, or 12 mg of AA per egg; and 3) uninjected controls. Experiment 2 was conducted at 15 d of incubation and three treatments were used: 1) eggs injected with 0.1 mL of saline solution containing 3 mg of AA per egg; 2) eggs cooled at 22 C for 24 h; and 3) uninjected controls. Experiment 3 was conducted using the same treatments used in Experiment 2 except that Experiment 3 was carried out at 17 d of incubation. Experiment 4 took place at 11 d of incubation and the treatments were as follows: 1) eggs injected with 0.1 mL of saline containing 3 mg of AA per egg, and 2) uninjected controls. Experiment 5 was conducted using the same treatments used in Experiment 4 except that Experiment 5 was carried out at 19 d of incubation. Five, 4, 4, 5, and 3 independent monthly trials were conducted within Experiment 1 through 5, respectively, and served as replicates over time.

About 385 settable broiler eggs (Hypro) laid the same day were obtained from the same commercial farm for each trial of Experiment 1, 165 eggs for each of Experiments 2 and 3, and 110 eggs for each of Experiments 4 and 5. Groups of eggs were randomly selected and weighed to the nearest 0.1 g. The eggs (385, 165, or 110) were placed at random in the same incubator for Experiment 1 and 30 eggs each for Experiments 2, 3, 4, and 5. Groups of eggs were randomly selected and weighed to the nearest 0.1 g. The eggs (385, 165, or 110) were placed at random in the same incubator for Experiment 1 and 30 eggs each for Experiments 2, 3, 4, and 5)

For the egg injections, 0.1 mL of the freshly prepared solution was injected per egg at 15 d of incubation for Experiments 1 and 2 and at 17, 11, and 19 d of incubation for Experiments 3, 4, and 5, respectively. Two percent tincture of iodine was applied to the injection site on the large end of the egg. A very slight indentation was made in the swabbed shell area using a sharp sterile pin inserted into a rubber stopper. The needle was carefully inserted through the indentation site to a depth of about 8 mm, and 0.1 mL of solution was injected over the inner shell membrane. The puncture was sealed with a small drop of warm paraffin and the eggs returned to the same incubator.

Eggs that were to be cooled were transferred to ordinary clean plastic trays, large end up, and were held in an incubator at 22 C for 24 h. The incubator was equipped with upper and lower safety thermostat. Setting times for cooling were 24 h in advance of the other treatment groups to ensure synchronization of hatching. At the end of the cooling period, the eggs were returned to the incubator.

On the 19th d of incubation, eggs of each trial of each experiment were removed from the incubator and transferred to separate hatching compartments and placed in the same incubator with temperature and humidity controls (37.5 C, 65 to 70% relative humidity). During the process of egg transfer to hatching compartments, 10 eggs were randomly selected from each group of Experiment 1 only for body weight testing 4 d after starting the treatment. The embryos were weighed individually after removing the yolk sac and wrapped thoroughly with tissue paper.

Observations of the number of saleable chicks hatched, cull chicks, and chick weights to the nearest 0.1 g were made on each treatment at hatch. All unhatched eggs were broken out and examined for each treatment. Embryos were assembled in one of two categories, eggs with dead embryos and eggs with living embryos. These
TABLE 1. The effect of ascorbic acid injection at Day 15 of incubation on embryo weight, hatchability, percentage cull chicks, unhatched egg with dead or live embryos, and hatching body weights of commercial broiler chickens, Experiment 1

<table>
<thead>
<tr>
<th>Injection dose of ascorbic acid (mg)</th>
<th>Embryo weight (g)</th>
<th>Hatchability</th>
<th>Cull chicks (%)</th>
<th>Dead embryos</th>
<th>Live embryos</th>
<th>Chick hatch weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.8 ± 0.3a</td>
<td>89.2 ± 3.5b</td>
<td>1.4 ± 0.8b</td>
<td>6.1 ± 1.9b</td>
<td>3.4 ± 1.9b</td>
<td>39.9 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>24.1 ± 0.2a</td>
<td>90.7 ± 1.9b</td>
<td>1.3 ± 0.8b</td>
<td>4.7 ± 1.3b</td>
<td>3.3 ± 1.1b</td>
<td>40.0 ± 0.2</td>
</tr>
<tr>
<td>0.5</td>
<td>23.3 ± 0.3a</td>
<td>89.3 ± 1.6a</td>
<td>2.7 ± 1.9b</td>
<td>4.0 ± 1.3b</td>
<td>4.0 ± 1.3b</td>
<td>39.7 ± 0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>23.9 ± 0.3a</td>
<td>94.0 ± 1.9b</td>
<td>0.7 ± 0.7b</td>
<td>4.0 ± 1.3b</td>
<td>1.3 ± 1.3b</td>
<td>40.5 ± 0.2</td>
</tr>
<tr>
<td>3.0</td>
<td>23.3 ± 0.4a</td>
<td>96.7 ± 1.1a</td>
<td>0.0 ± 0.0b</td>
<td>1.3 ± 0.8b</td>
<td>2.0 ± 0.8b</td>
<td>39.8 ± 0.2</td>
</tr>
<tr>
<td>12.0</td>
<td>22.1 ± 0.3b</td>
<td>51.0 ± 6.2c</td>
<td>13.4 ± 3.7a</td>
<td>29.6 ± 4.8b</td>
<td>6.0 ± 3.7b</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means ± SEM within a column with no common superscript differ significantly (P < 0.05).
<sup>1</sup>Embryo weight at 19 d of incubation (n = 50 eggs per dose).
<sup>2</sup>The number of saleable chicks hatched as a percentage of the treated eggs with living embryos and transferred to hatching compartments (n = 150 eggs per dose).
<sup>3</sup>Unhatched egg with dead embryo either emerging from the shell or dead in it.
<sup>4</sup>Unhatched egg with live embryo either emerging from the shell or still alive in it.
<sup>5</sup>Body weight at hatching of mixed sexes.

RESULTS

A sample of 10 eggs of each designated treatment group were randomly selected and pooled for normal embryonic weight testing at 11, 15, 17, and 19 d of incubation. The normal embryonic weight represented pooled means before starting treatments at these ages. The normal embryonic weight (X ± SEM) of commercial broiler chickens for 11, 15, 17, and 19 d of incubation was 2.1 ± 0.0, 10.5 ± 0.1, 16.6 ± 0.1, and 20.4 ± 0.2 g, respectively. The results show a dramatic relative increase in embryonic weight between 11 to 15 d of incubation compared with the latter part of incubation.

The average egg weights (X ± SEM) for Experiments 1, 2, 3, 4, and 5 were 57.1 ± 0.1, 57.4 ± 0.1, 56.6 ± 0.1, 58.2 ± 0.1, and 57.7 ± 0.1, respectively.

Table 1 shows the embryo weight, hatchability, cull chicks, unhatched eggs with dead embryos and hatch weight of the 1,200 Hypro eggs with living embryos used in Experiment 1. These variables did not differ significantly between saline injection of 0.1 mL and the control. Ascorbic acid at doses of 0.5 to 1 mg did not significantly affect these variables; however, a dose of 3 mg of AA significantly improved hatchability. There was no significant difference between 1 and 3 mg AA for hatchability. There was a significant decrease in embryo weight and hatchability and a significant increase in cull chicks and dead embryos compared to controls, attributable to AA at 12 mg at 15 d of incubation (Table 1).

Data from Experiment 2 indicated that there were no significant differences in percentage hatchability and cull chicks (Table 2). Ascorbic acid injection at a dose of 3 mg at 15 d of incubation increased body weight at hatch compared with the control. Cooling to 22 C for 24 h on Day 15 of incubation significantly increased embryonic deaths compared to AA injection, but not with the control.

Results of the different treatments of Experiment 3 are also given in Table 2. There was no significant difference between groups for hatchability and dead embryos. Ascorbic acid injection at a dose of 3 mg at 17 d of incubation improved hatch weight when compared with the other two treatments. Cooling did not affect body weight at hatch compared with the control. The percentage of cull chicks decreased significantly compared with the control by injecting AA at a dose of 3 mg at 17 d of incubation. Cooling had no significant effect on cull chick percentage relative to the control.

The results on hatchability in Experiments 4 and 5 are shown in Table 3. When AA was injected into eggs at 11...
TABLE 2. The effect of ascorbic acid injection and cooling at Day 15 or 17 of incubation on hatchability, cull chick, non-hatched egg with dead embryo, and body weight of hatched eggs of commercial broiler chickens, Experiments 2 and 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Hatchability (%)</th>
<th>Cull chicks</th>
<th>Unhatched egg with dead embryos</th>
<th>Body weight at hatch (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Control</td>
<td>93.3 ± 2.3</td>
<td>0.8 ± 0.8</td>
<td>4.2 ± 2.1*</td>
<td>38.0 ± 0.3b</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>96.7 ± 1.9</td>
<td>0.0 ± 0.0</td>
<td>1.7 ± 1.7b</td>
<td>38.8 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>Cooling</td>
<td>94.2 ± 0.9</td>
<td>0.8 ± 0.8</td>
<td>4.2 ± 0.9a</td>
<td>38.3 ± 0.3ab</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>92.4 ± 3.6</td>
<td>3.4 ± 1.4a</td>
<td>3.4 ± 1.4ab</td>
<td>37.8 ± 0.2b</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>93.9 ± 2.6</td>
<td>0.0 ± 0.0b</td>
<td>3.5 ± 1.4</td>
<td>38.4 ± 0.2a</td>
</tr>
<tr>
<td></td>
<td>Cooling</td>
<td>93.4 ± 2.4</td>
<td>0.8 ± 0.8ab</td>
<td>4.2 ± 1.6</td>
<td>37.8 ± 0.2b</td>
</tr>
</tbody>
</table>

*Means ± SEM within a column, within the same experiment, with no common superscript differ significantly (P < 0.05).

<table>
<thead>
<tr>
<th>Day of incubation</th>
<th>Treatment</th>
<th>11</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.4 ± 5.4</td>
<td>80.9 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>91.3 ± 1.3**</td>
<td>78.3 ± 6.2</td>
<td></td>
</tr>
</tbody>
</table>

**P < 0.01.

The number of saleable chicks hatched as a percentage of the treated eggs with living embryos and transferred to hatching compartments.

Unhatched egg with dead embryo either emerging from the shell or dead in it.

Body weight at hatching of mixed sexes.

Eggs injected with 0.1 mL of saline containing 3 mg of ascorbic acid per egg.

Eggs cooled at 22 C for 24 h.

do of incubation, AA increased hatchability in comparison with the control; however, AA treatment at 19 d of incubation did not affect hatchability.

DISCUSSION

Egg injection of vitamins, such as pyridoxine and pantothenic acid, has been applied during incubation to study vitamin effects on hatchability of turkey eggs (Robel and Christensen, 1991; Robel, 1993). The present study utilized injection during the latter part of incubation to study the effect of AA on development of broiler chicken embryo.

In this study, there were advantages attributable to AA treatment at a dose of 3 mg during the latter part of egg incubation for some of the measured variables. These advantages were: improvement of hatchability (Tables 1 and 3), an increase in body weight at hatch, and a decrease in cull chick percentage (Table 2). These findings have not been previously reported.

Tullett (1990) summarized results to indicate that during the latter part of incubation the chicken embryos are subjected to stressors due to an increase in metabolic heat. Wilson and Jaworski (1992) stated that plasma AA concentration declined at 15 d of incubation in White Leghorn chicken eggs. Elevation of blood corticosterone is usually associated with stress and with the reduction of AA biosynthesis in poultry (Pardue and Thaxton, 1986; Kutlu and Forbes, 1994). Generally, AA may be regarded as an anti-stress agent (Pardue and Thaxton, 1986), because its treatment led to the reduction of corticosterone (Satterlee et al., 1994; Kutlu and Forbes, 1994). Additionally, AA has a role in collagen synthesis (Weiser et al., 1988), the metabolism of minerals (Roberson and Edwards, 1994), and vitamin D metabolism (Weiser et al., 1988).

Based on the previous considerations and the findings of the present study, with the exception of Day 19 of incubation, which was probably too late to have an effect, it may be suggested that AA injection at a dose of 3 mg per egg during the later stages of incubation may have a role in reducing stress. The increase in hatchability and body weight at hatch and the decrease in the numbers of cull chicks are indicative of the effect.

Vitamin C supplementation at a dose of 12 mg per egg at the 15th d of incubation adversely affected some variables in this study. The results showed that the vitamin decreased embryo weight and hatchability increased cull chick numbers, and dead embryos (Table 1).

Studies have not been made of the effect of high doses of AA during the latter stages of chicken
embryonic development; however, reports indicate that increasing the concentration of AA is accompanied by a selective toxic action on the pancreatic beta cells (Meglasson and Hazelwood, 1982), death of resting cells, and reversal of differentiation (Iyengar and Lai, 1982), death of mesenchymal cells, and hence a decrease in mineralization (Boskey et al., 1991), and reduced the viability of cultured fibroblasts (Murakami et al., 1992).

In the present study, preliminary observations on dead embryos receiving AA at a dose of 12 mg per egg showed severe body hemorrhages, but no attempt was made to elucidate the reason. Hence, this may be an area for active research.

The results of this study on hatchability concerning egg cooling during the latter part of incubation were not in conflict with the previously reported by Lancaster and Jones (1988). Lancaster and Jones (1988) in which broiler hatching eggs of commercial parent stock (Ross 208 and Cobb 500) were subjected to cooling from 13 to 18 d of incubation for 8 to 72 h at 18.3 to 26.7 C. They concluded that cooling broiler hatching eggs at these temperatures and ages did not have significant effects on hatchability. The results of the present study on the comparison between egg cooling and the control on 15 and 17 d of incubation agree with Lancaster and Jones (1988). The two works share the view that there is no effect of egg cooling on hatchability during the latter part of incubation.

Male broiler body weight at hatch (Sarpong and Reinhart, 1985) and chick quality (Lancaster and Jones, 1988) were not affected by cooling eggs on Day 16 of incubation to 22 C for 24 h. The results of the present study on body weight of commercial broiler chicks of mixed sexes are consistent with the previous findings of Sarpong and Reinhart (1985) and Lancaster and Jones (1988).

Lancaster and Jones (1988) found no differences in embryonic mortality by cooling eggs on 14 to 17 d of incubation in comparison with 13 d of incubation. Sarpong and Reinhart (1985) linked embryonic mortality during the latter part of egg incubation with egg size and found that 24 h of cooling resulted in lower mortality in small (57.3 g) and medium (61.4 g) eggs, but not in large (65 g) eggs. In the present study, there was no significant difference in embryonic mortality by cooling eggs to 22 C for 24 h on Days 15 and 17 of incubation on egg weight.

Sarpong and Reinhart (1985) proposed that cooling slowed the metabolic rate of embryos during the latter part of incubation, thus reducing the effect of excess heat in embryos. The results of the present investigation on the development of chicken embryo showed a pronounced increase in chick weight during the latter part of incubation particularly on Day 15 of incubation. It seems that cooling of broiler hatching eggs to 22 C for 24 h between 15 to 17 d of incubation reduced stress without causing harmful effects on hatchability, hatch weight of mixed sexes, numbers of cull chicks and embryonic death. As as result, egg cooling may be used as a management tool to time hatching for the convenience of hatchery staff as well as broiler producers.

Comparison of AA injection at a dose of 3 mg per egg and egg cooling to 22 C for 24 h at 15 to 17 d of incubation led to the conclusion that AA had two advantages over cooling. These advantages were lower numbers of embryonic death and higher chick weights at hatch. Further investigation on AA effects during the latter part of egg incubation are needed at doses between 3 to 6 mg per egg.

REFERENCES


Takahashi, K., Y. Akiba, and M. Horiguchi, 1991. Effects of supplemental ascorbic acid on performance, organ weight