Influence of air composition during egg storage on egg characteristics, embryonic development, hatchability, and chick quality

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ABSTRACT Egg storage beyond 7 d is associated with an increase in incubation duration and a decrease in hatchability and chick quality. Negative effects of prolonged egg storage may be caused by changes in the embryo, by changes in egg characteristics, or by both. An adjustment in storage air composition may reduce negative effects of prolonged egg storage because it may prevent changes in the embryo and in egg characteristics. An experiment was conducted to investigate the effects of high CO2 concentrations or a low O2 concentration in the storage air on egg characteristics, embryonic development, hatchability, and chick quality. Eggs were stored for 14 d in 4 different storage air compositions: normal air (control; 20.9% O2, 0.05% CO2, 78.1% N2), 0.74% CO2 treatment (20.8% O2, 0.74% CO2, 77.5% N2), 1.5% CO2 treatment (20.6% O2, 1.5% CO2, 77.0% N2), or 3.0% O2 treatment (3.0% O2, 0.04% CO2, 96.0% N2). The storage temperature was 16°C and the RH was 75%. Results showed that the change in albumen pH and albumen height between oviposition and the end of storage was less in the 0.74 and 1.5% CO2 treatments than in the control and 3.0% O2 treatments (P < 0.001 and P < 0.001, respectively). None of the treatments affected the stage of embryonic development on d 4 of incubation, hatchability, or chick quality on the day of hatch in terms of BW, chick length, and yolk-free body mass. Although high CO2 concentrations in the storage air had a positive effect on albumen height and albumen pH, it is concluded that the storage air compositions, studied in the current study, do not affect embryonic development, hatchability, or chick quality when eggs are stored for 14 d at a storage temperature of 16°C.

Key words: egg storage, carbon dioxide, oxygen, hatchability, chick quality

INTRODUCTION

Storage of hatching eggs beyond 7 d is associated with an increase in incubation duration and a decrease in hatchability (Mather and Laughlin, 1976; Tona et al., 2003), a decrease in hatchability (Becker, 1964; Merritt, 1964; Fasenko et al., 2001; Tona et al., 2004; Yassin et al., 2008), and a decrease in chick quality (Byng and Nash, 1962; Merritt, 1964; Tona et al., 2003, 2004). The cause of these negative effects is unclear. During egg storage, embryo viability declines, likely due to an increase in cell death (Arora and Kosin, 1968; Bloom et al., 1998). In addition, egg characteristics change due to a loss of CO2 and water from the egg. Albumen pH increases from about 7.6 to about 9.0 within 4 d of storage (Lapão et al., 1999).

Yolk pH increases from about 6.0 to about 6.5 (Shenstone, 1968), albumen viscosity decreases (Shenstone, 1968; Burley and Vadehra, 1989), and the strength of the yolk membrane decreases as well during storage (Fromm, 1966). These changes in embryo viability and egg characteristics may be related to the negative effects of prolonged egg storage on hatchability and chick quality (Becker et al., 1968; Meijerhof, 1992; Reijrink et al., 2008). The negative effects of prolonged egg storage may be decreased by adjustment of the storage air composition. An increased CO2 concentration in the storage air prevents an increase in albumen pH and a decrease in albumen height. A lower albumen pH may have a positive effect on embryo viability because the optimal pH for embryonic development is in the range of 7.9 to 8.4 (Gillespie and McHanwell, 1987). In addition, maintenance of albumen height may have a positive effect on the available proteins during embryonic development (Hurnik et al., 1978). A low O2 concentration in the storage air, which is obtained by an increase

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in the N₂ concentration, may reduce the metabolic rate of the embryo and, therefore, energy use. When energy use is decreased, the embryo may be able to survive a longer storage duration. In several studies, the effects of storage air composition on hatchability (Becker et al., 1963, 1964, 1967, 1968; Proudfoot, 1964a,b, 1965; Krueger et al., 1965; Warren et al., 1965; Gordon and Siegel, 1966; Kosin and Konishi, 1973) and chick quality (Becker, 1964; Proudfoot, 1964a, 1965; Becker et al., 1968) were investigated. In most of these studies, eggs were stored in plastic bags with or without additional N₂ to adjust the storage air composition. These treatments had a positive effect on hatchability when storage duration was beyond 7 d (Becker, 1964; Proudfoot, 1964a,b, 1965; Gordon and Siegel, 1966; Becker et al., 1967, 1968; Kosin and Konishi, 1973). Because the RH and CO₂ concentration both increase when eggs are stored in plastic bags, it is difficult to determine which change or changes in the storage air composition had a positive effect on hatchability.

The current experiment was conducted to investigate the effects of high CO₂ concentrations or a low O₂ concentration in the storage air on egg characteristics, embryonic development, hatchability, and chick quality when eggs were stored for 14 d at a temperature of 16°C and a RH of 75%.

**MATERIALS AND METHODS**

This experiment was approved by the Institutional Animal Care and Use Committee of Wageningen University.

**Breeder Flock**

Eggs from a Ross 308 broiler breeder flock at 38 wk were used. The male:female ratio was 1:10. Birds were fed restricted diets according to the standard Ross guidelines. The light schedule included 15 h of light and 9 h of darkness. The lights were off between 1800 and 0300 h.

**Egg Collection and Storage**

The day before egg collection, all eggs were cleared from the nests just before the lights were turned off. Eggs were collected the next day (n = 5,890), 5 h after the lights were turned on. Eggs were randomly assigned to 4 separate climate chambers (Verstegen et al., 1987) and stored for 14 d. The storage air composition differed among the 4 climate chambers: control treatment (20.9% O₂, 0.05% CO₂, 78.1% N₂; n = 1,312), 0.74% CO₂ treatment (20.8% O₂, 0.74% CO₂, 77.5% N₂; n = 1,431), 1.5% CO₂ treatment (20.6% O₂, 1.5% CO₂, 77.0% N₂; n = 1,555), and 3.0% O₂ treatment (3.0% O₂, 0.04% CO₂, 96.0% N₂; n = 1,578).

The control treatment was ventilated with 100 L of fresh air per minute, the 0.74% CO₂ treatment with 50 L per minute, the 1.5% CO₂ treatment with 5 L per minute, and the 3.0% O₂ treatment was not ventilated. The O₂ and CO₂ concentrations in the climate chambers were measured every 9 min as described by Lourens et al. (2007). The low O₂ concentration was obtained by injecting N₂ into the climate chamber. The 0.74 and 1.5% CO₂ concentrations were obtained by injecting CO₂ into the climate chambers. Carbon dioxide and N₂ gas were injected in the climate chambers as described by Taylor et al. (1956) with a continuous flow. The flow was adjusted manually when the CO₂ or O₂ concentrations deviated from the desired value. The storage air temperature and RH were measured every 9 min and were maintained at 16°C and 75%, respectively.

**Egg Weight and Egg Weight Loss During Storage.** On the day of oviposition (d −14) and on the day of setting (d 0), 88 eggs per treatment were weighed individually. Egg weight loss during storage was calculated by the following formula:

\[
\frac{\text{[(Egg weight d −14 − Egg weight d 0)}/\text{Egg weight d −14}] × 100}{1}
\]

**Egg Characteristics and Embryonic Development on the Day of Oviposition and During Storage.** On the day of oviposition, before the start of the storage treatment (d −14), albumen height, albumen pH, yolk pH, and the stage of embryonic development were determined in 14 randomly selected eggs. The day before the eggs were set (d −1), the same measurements were performed in 20 eggs per treatment. The height of the thick albumen was measured in the middle (Benton and Brake, 1996) with a tripod meter (QCD device, Technical Services and Supplies, York, UK). Albumen was separated from the yolk and both were homogenized with a vortex (YellowLine TTS2, Omnilabo International B.V., Breda, the Netherlands). Thereafter, albumen and yolk pH were measured with a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerland). The filter ring technique, as described by Gupta and Bakst (1993), was used to isolate the embryo from the yolk membrane. After isolation, the embryo was flushed with buffered saline to remove yolk residue. The dorsal and ventral side of the embryo were examined with a stereo microscope (Olympus SZ61, Olympus Nederland B.V., Zoeterwoude, the Netherlands) to determine the stage of embryonic development according to the classification table of Eyal-Giladi and Kochav (1976) (EG). This classification table measures embryonic development on the basis of morphogenetic movements on the surface of the embryo (Fasenko et al., 1992).

**Incubation**

Eggs (n = 5,796: 5,716 + 80 eggs for analysis on d 4 of incubation) were set into 40 setter trays, which contained at least 144 eggs and a maximum of 150 eggs.
The setter were controlled to maintain the CO2 concentration between 45 and 60% and the inlet and outlet valve of the hatchers. Relative humidity varied over the course of incubation. Eggs that were provided with a temperature sensor were used to attach the sensor to the egg’s equator. Average egg-shell temperature was maintained at 37.8°C until d 20 of incubation (Lourens et al., 2005). Fertility of the eggs per setter (4 eggs per setter) was provided with a temperature sensor (NTC Thermistors: type DC 95, Thermometrics, Somerset, UK). Heat-conducting paste (Dow Corning 340 Heat Sink Compound, Dow Corning GmbH, Wiesbaden, Germany) and a piece of tape were used to transfer the eggs per treatment to the hatcher baskets. One setter tray was split in 2 equal halves and eggs in each half were transferred to 1 hatcher basket. Per treatment, 30 eggs were individually placed in hatching boxes (10 × 10 cm) to know which chick hatched from which egg. The chicks that hatched in the individual boxes were used to determine chick quality. The 30 eggs were selected from 2 setter trays out of 1 setter and hatched in 1 hatcher. The remaining eggs were hatched in 2 hatchers (HT-4,800, HatchTech Incubation Technology B.V.). From d 20 of incubation onward, the air temperature was maintained at 36.7°C and RH was maintained above 45% in both hatchers. The inlet and outlet valve of the hatchers were controlled to maintain the CO2 concentration below 0.35%.

**Egg Weight Loss During Incubation.** The 88 eggs that were weighed per treatment at d −14 and 0 were weighed individually again on d 18 of incubation (d 18). Egg weight loss during incubation was determined by the following formula:

\[
\frac{[(\text{Egg weight d } 0 - \text{Egg weight d } 18)]}{\text{Egg weight d } 0} \times 100. \tag{2}
\]

Total egg weight loss was calculated by the following formula:

\[
\frac{[(\text{Egg weight d } -14 - \text{Egg weight d } 18)]}{\text{Egg weight d } -14} \times 100. \tag{3}
\]

**Albumen pH and Embryonic Development During Incubation.** On d 4 of incubation, albumen pH and the stage of embryonic development were measured in 20 eggs per treatment. Albumen pH was measured as described earlier. The embryo was released from extrabrain membranes and the dorsal and ventral side of the embryo were examined with a stereo microscope (Olympus SZ61, Olympus Nederland B.V., Zoeterwoude, the Netherlands) to determine the stage of embryonic development according to the classification table of Hamburger and Hamilton (1951). This classification table measures embryonic development on the basis of external factors, such as the number of somite pairs; changes in the wings, legs, visceral arches, feather germs, and eyelids; and the length of the beak and toes.

**Embryonic Mortality and Hatchability.** On d 8 and 18 of incubation, eggs were candled and after 527 h of incubation, all unhatched eggs were collected. Clear eggs, which were removed during candling, and unhatched eggs were opened to macroscopically determine infertility or the stage of embryonic mortality. The stages of embryonic mortality were determined by the method described by Reijrink et al. (2009). Fertility was calculated as a percentage of set eggs. Hatchability (first- and second-grade chicks) was calculated as a percentage of set eggs and as a percentage of fertile eggs. Embryonic mortality was calculated as a percentage of fertile eggs.

**Incubation Duration and Chick Quality.** From d 19.5 of incubation onward, all chicks that emerged from the eggs were recorded every 4 h to calculate incubation duration. Incubation duration was defined as the interval between the beginning of incubation and the emergence of the chick from the egg. Chicks that hatched in the individual hatching boxes were used to measure chick quality, 12 h after the chick emerged from the egg. Chick quality was measured in terms of BW, chick length, yolk-free body mass, residual yolk weight, and navel quality. Chick length was defined as the length from the tip of the beak to the implantation of the nail on the middle toe (Hill, 2001; Willemsen et al., 2008). Navel quality was analyzed and scored with point 1 if the navel was completely closed and clean; point 2 if the navel was discolored (color different from skin color) or opened to a maximum of 2 mm, or both; and point 3 if the navel was discolored or opened more than 2 mm, or both. After measurement of BW, chick length, and navel quality, chicks were killed with CO2 to measure residual yolk weight. Residual yolk weight was subtracted from BW to calculate yolk-free body mass. Chicks that were not used for chick quality measurements were classified as first- or second-grade chicks based on physical parameters. A chick was classified as a first-grade chick if the chick was clean, dry, free of deformities or lesions, had bright eyes (Tona et al., 2004), and if the chick was given a navel score of 1 or 2. Other chicks were classified as second-grade chicks. The percentage of second-grade chicks was calculated as a percentage of total hatched chicks.

**Statistical Analysis**

Data were analyzed as a complete randomized design with 4 treatments. The distribution of the means and
residuals were examined to check model assumptions. Egg weight, egg weight loss, BW, chick length, yolk-free body mass, and residual yolk weight were analyzed using general linear regression (PROC GLM, SAS Institute, 2004) with treatment as class variable and egg or chick as the smallest experimental unit. Unfertilized eggs and eggs containing dead embryos were excluded from the data when egg weights and egg weight loss were analyzed. A log transformation was used for egg weight loss during storage, egg weight loss during incubation, and total egg weight loss to obtain normal distributed data. Egg weight at setting was introduced as a covariate when BW, chick length, yolk-free body mass, and residual yolk weight were analyzed.

To investigate the change (Δ) in albumen height and yolk pH between the day of oviposition (d −14) and last day of storage (d −1), the average measured value on d −14 was subtracted from the values measured on d −1. The Δ values for albumen height and yolk pH were analyzed using general linear regression (PROC GLM, SAS Institute, 2004) with treatment as class variable and egg as the smallest experimental unit. To investigate the change in albumen pH between the day of oviposition and last day of storage and between the day of oviposition and d 4 of incubation, the average measured value on d −14 was subtracted from the values measured on d −1 and d 4. The Δ values for albumen pH were analyzed using general linear regression (PROC GLM, SAS Institute, 2004) with day of analysis (d −1 or 4), treatment, and their interaction as class variables and egg as the smallest experimental unit. The stage of embryonic development on the last day of storage was included as a covariate when yolk-free body mass and residual yolk weight were analyzed.

### Table 1. Fresh egg weights and the effect of storage air composition on egg weight loss during storage and incubation

<table>
<thead>
<tr>
<th>Treatment</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>Control</td>
<td>63.92</td>
<td>1.24b</td>
<td>10.44</td>
<td>11.55</td>
</tr>
<tr>
<td>0.74% CO₂</td>
<td>63.74</td>
<td>1.39a</td>
<td>10.52</td>
<td>11.76</td>
</tr>
<tr>
<td>1.5% CO₂</td>
<td>63.93</td>
<td>1.11c</td>
<td>10.47</td>
<td>11.46</td>
</tr>
<tr>
<td>3.0% O₂</td>
<td>62.97</td>
<td>1.22b</td>
<td>10.81</td>
<td>11.90</td>
</tr>
<tr>
<td>SEM</td>
<td>0.43</td>
<td>0.02</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>n</td>
<td>306</td>
<td>306</td>
<td>306</td>
<td>306</td>
</tr>
<tr>
<td>( P )-value</td>
<td>0.36</td>
<td>&lt;0.001</td>
<td>0.39</td>
<td>0.30</td>
</tr>
</tbody>
</table>

\(^{a}\)Least squares means within a column lacking a common superscript differ \((P \leq 0.05)\).

\(^{1}\)Transformed from log values back to original least squares means.

### Table 2. Effect of storage air composition on the change (Δ) in albumen height and yolk pH between the day of oviposition and last day of storage and the effect of day of analysis (d −1 and 4), storage air composition, and their interaction on the change (Δ) in albumen pH between the day of oviposition and the last day of storage and between the day of oviposition and d 4 of incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>( \Delta ) Albumen height, mm</th>
<th>( \Delta ) Albumen pH</th>
<th>( \Delta ) Yolk pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of analysis, d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1</td>
<td>0.92</td>
<td>0.28</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>0.98</td>
<td>0.26</td>
<td>0.23</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage air treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>−3.53(^{a})</td>
<td>1.13</td>
<td>0.28</td>
</tr>
<tr>
<td>0.74% CO₂</td>
<td>−1.57(^{b})</td>
<td>0.80</td>
<td>0.26</td>
</tr>
<tr>
<td>1.5% CO₂</td>
<td>−1.44(^{b})</td>
<td>0.66</td>
<td>0.23</td>
</tr>
<tr>
<td>3.0% O₂</td>
<td>−3.52(^{a})</td>
<td>1.20</td>
<td>0.29</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of analysis, d × storage air treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1 × control</td>
<td>1.30(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1 × 0.74% CO₂</td>
<td>0.57(^{d})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1 × 1.5% CO₂</td>
<td>0.35(^{c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−1 × 3.0% O₂</td>
<td>1.46(^{a})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 × control</td>
<td>0.97(^{c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 × 0.74% CO₂</td>
<td>1.03(^{c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 × 1.5% CO₂</td>
<td>0.97(^{c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 × 3.0% O₂</td>
<td>0.97(^{c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.25</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>n</td>
<td>71</td>
<td>149</td>
<td>73</td>
</tr>
<tr>
<td>( P )-value</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage air treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Least squares means within a column and factor lacking a common superscript differ \((P \leq 0.05)\).
day of storage and on d 4 of incubation was analyzed using the Kruskal-Wallis nonparametric test (PROC NPAR1WAY, SAS Institute, 2004) with treatment as class variable. Navel quality was analyzed using logistic regression analysis (PROC LOGISTIC, SAS Institute, 2004) with treatment as class variable. Incubation duration, fertility, hatchability, embryonic mortality, and percentage of second-grade chicks were analyzed using general linear regression (PROC GLM, SAS Institute, 2004) with treatment, incubator, and their interaction as class variables and setter tray as the smallest experimental unit. An arcsine transformation was used for the percentage of second-grade chicks to obtain normal distributed data. For all continuous variables, least squares means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as least squares means ± SEM. The main factors and interaction were analyzed for significance at $P \leq 0.05$. The interaction was excluded from the model when $P > 0.05$.

### RESULTS

**Egg Weight and Egg Weight Loss**

Fresh egg weight did not differ among treatments (Table 1). Eggs of the 0.74% CO$_2$ treatment lost more weight during storage than eggs of the other treatments ($P < 0.001$). Eggs of the control and 3.0% O$_2$ treatment lost more weight during storage than eggs of the 1.5% CO$_2$ treatment. Egg weight loss during incubation and total egg weight loss did not differ among treatments.

**Egg Characteristics**

On day of oviposition, albumen height was 7.13 mm, albumen pH was 8.13, and yolk pH was 6.06. An interaction was found between day of analysis and treatment for the change in albumen pH ($P < 0.001$; Table 2). The change in albumen pH between day of oviposition and last day of storage was different for all treat-

### Table 3.
Number and percentage of embryos in each stage of embryonic development for each storage air composition on the day before setting (d $-1$)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>EG7</th>
<th>EG8</th>
<th>EG9</th>
<th>EG10</th>
<th>EG11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>0.74% CO$_2$</td>
<td>14</td>
<td>0.0</td>
<td>0.0</td>
<td>13.3</td>
<td>80.0</td>
<td>6.7</td>
</tr>
<tr>
<td>1.5% CO$_2$</td>
<td>15</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>3.0% O$_2$</td>
<td>14</td>
<td>1.0</td>
<td>6.7</td>
<td>20.0</td>
<td>46.6</td>
<td>6.7</td>
</tr>
</tbody>
</table>

1According to Eyal-Giladi and Kochav (1976) (EG).

2The distribution among the stages of embryonic development was not different among treatments on the day before setting (d $-1$) ($P = 0.07$).

### Table 4.
Number and percentage of embryos in each stage of embryonic development for each storage air composition on d 4 of incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total</th>
<th>HH19</th>
<th>HH20</th>
<th>HH21</th>
<th>HH22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>0.74% CO$_2$</td>
<td>15</td>
<td>0.0</td>
<td>6.2</td>
<td>18.8</td>
<td>62.5</td>
</tr>
<tr>
<td>1.5% CO$_2$</td>
<td>18</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>3.0% O$_2$</td>
<td>19</td>
<td>0.0</td>
<td>16.7</td>
<td>27.8</td>
<td>50.0</td>
</tr>
</tbody>
</table>

1According to Hamburger and Hamilton (1951) (HH).

2The distribution among the stages of embryonic development were not different among treatments ($P = 0.17$).
ments. However, the change in albumen pH between day of oviposition and d 4 of incubation was not different among the treatments. On the last day of storage, albumen pH was 9.59 for the 3.0% O2 treatment, 9.43 for the control treatment, 8.70 for the 0.74% CO2 treatment, and 8.48 for the 1.5% CO2 treatment. On d 4 of incubation, albumen pH was 9.07 for the 3.0% O2 treatment, 9.10 for the control treatment, 9.16 for the 0.74% CO2 treatment, and 9.10 for the 1.5% CO2 treatment.

The change in albumen height between day of oviposition and last day of storage was higher for the control and 3.0% O2 treatments than for the 0.74 and 1.5% CO2 treatments (\(P < 0.001\)). The change in yolk pH between day of oviposition and last day of storage did not differ among the treatments.

**Embryonic Development**

On the day of oviposition, 6.7% of the embryos were at developmental stage EG8, 40.0% were at developmental stage EG9, and 53.3% of the embryos were at developmental stage EG10. On the last day of storage and on d 4 of incubation, the stage of embryonic development did not differ among the treatments (Tables 3 and 4, respectively).

**Incubation Duration, Fertility, and Hatchability**

There was a small but significant effect of treatment on incubation duration. Chicks of the 3.0% O2 treatment hatched 2 h earlier than chicks of both CO2 treatments (\(P = 0.002\); Table 5). Incubation duration of the control treatment did not differ from other treatments. Fertility, hatchability, and embryonic mortality did not differ among treatments.

**Chick Quality**

Percentage of second-grade chicks, BW, chick length, and navel quality (Table 6) did not differ among treatments. Chicks of the 3.0% O2 treatment had a higher residual yolk weight than chicks of the control treatment (\(P = 0.002\)). Residual yolk weight of the 0.74 and 1.5% CO2 treatment did not differ from the other treatments.

**DISCUSSION**

One of the hypotheses of the current study was that a high CO2 concentration would improve hatchability and chick quality after prolonged egg storage because an increased CO2 concentration in the storage air reduces CO2 loss from the egg and reduces the changes in albumen pH and albumen height. Another hypothesis of the current study was that a low O2 concentration in the storage air would reduce the embryo’s metabolic rate and, therefore, energy use during storage.
In the current study, the changes in albumen height and albumen pH were reduced by the increase of the CO₂ concentration in the storage air. The CO₂ concentration of 1.5% maintained albumen pH at 8.48, which is near the pH range from 7.9 to 8.4 measured in the extracellular space of the embryo during early incubation by Gillespie and McHanwell (1987). However, the 1.5% CO₂ treatment did not improve hatchability or chick quality. This suggests that the increase in albumen pH to a pH around 9.0, which normally occurs within the first 4 d of storage, and the decrease in albumen height are not involved in the negative effects of prolonged egg storage on hatchability and chick quality.

The 3.0% O₂ treatment also did not affect hatchability and chick quality. This shows that embryos can survive low O₂ concentrations during storage. It also suggests that a storage temperature of 16°C most likely reduced the metabolism of the embryo to a level that was low enough to survive a storage duration of 14 d. A decrease in the O₂ concentration had no additional effect.

As in the current study, Kosin and Konishi (1973) did not find an effect of a 1.5% CO₂ concentration in the storage air on hatchability, when storage time was 14 d and a comparable experimental setup as in the current study was used. On the other hand, several authors found a positive effect of storing eggs in plastic bags, in which CO₂ and H₂O gradually increase, on hatchability when storage time was beyond 7 d (Becker, 1964; Gordon and Siegel, 1966; Becker et al., 1967, 1968). In a few other studies, storage of eggs in plastic bags with or without additional N₂ gas only had a pronounced effect on hatchability when the storage duration was beyond 19 d (Proudfoot, 1964a,b, 1965; Kosin and Konishi, 1973). It is, therefore, possible that an albumen pH around 9.0 has a negative effect on embryo viability but we were unable to prove this in the current study because storage duration was not long enough. An embryo may be able to maintain an effective barrier between the pH of the inside of the embryo and its exterior (albumen and yolk), but this ability may not be endless because cells may run out of energy when storage duration is too long. Consequently, the embryo may not have enough energy left to survive during incubation (Walsh et al., 1995). How long embryos can be stored without negative consequences may depend on factors that affect embryo viability such as age of the breeder flock (Mather and Laughlin, 1979), strain (Yoo and Wientjes, 1991), and storage conditions (Arora and Kosin, 1968). In the current study, hatchability was relatively high in comparison to hatchability results of other studies in which egg storage was prolonged (Fasenko et al., 2001; Reijrink et al., 2009). This suggests that the age of the breeder flock or strain, or both, that was used in the current study positively affected embryo viability and the sensitivity of the embryo to prolonged egg storage.

Although albumen pH was different among the treatments on the last day of storage, albumen pH on d 4 of incubation was comparable for all treatments. It is known that albumen pH increases to a pH around 9.0 during the first 24 h of incubation (Benton and Brake, 1996). In the current study, albumen pH was only measured on the last day of storage and on d 4 of incubation. Therefore, it is unknown how the pH of the different treatments changed during early incubation. However, it can be assumed that the albumen pH increased to a pH level around 9.0 during the first 24 h of incubation in all treatments. The albumen pH during egg storage may not be important for embryo survival during incubation after prolonged egg storage, but the albumen pH during early incubation may be crucial. Maintenance of the albumen pH within the range of 7.9 to 8.4 during early incubation may prevent abnormal development or embryonic mortality, or both. In the current study, albumen pH of the different treatments was probably comparable soon after the onset of incubation and this may also be the reason why the treatments did not affect hatchability and chick quality.

In the current study, egg weight loss during storage differed among the treatments. Egg weight loss is influenced by RH, temperature, and the conductivity of

### Table 6. Effect of storage air composition on chick quality variables

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Second-grade chicks, arsin</th>
<th>Second-grade chicks, %</th>
<th>BW, g</th>
<th>Chick length, cm</th>
<th>Yolk-free body mass, g</th>
<th>Residual yolk, g</th>
<th>Navel quality, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.02</td>
<td>0.04</td>
<td>45.3</td>
<td>19.6</td>
<td>40.3</td>
<td>5.09</td>
<td>39.7</td>
</tr>
<tr>
<td>0.74% CO₂</td>
<td>0.02</td>
<td>0.04</td>
<td>45.5</td>
<td>19.6</td>
<td>40.3</td>
<td>5.31</td>
<td>54.4</td>
</tr>
<tr>
<td>1.5% CO₂</td>
<td>0.02</td>
<td>0.04</td>
<td>45.4</td>
<td>19.5</td>
<td>40.1</td>
<td>5.39</td>
<td>56.1</td>
</tr>
<tr>
<td>3.0% O₂</td>
<td>0.01</td>
<td>0.01</td>
<td>45.5</td>
<td>19.6</td>
<td>39.9</td>
<td>5.70</td>
<td>56.1</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>0.1</td>
<td>0.04</td>
<td>0.2</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td>229</td>
<td>229</td>
<td>221</td>
<td>221</td>
<td>229</td>
</tr>
<tr>
<td>P-value</td>
<td>0.82</td>
<td></td>
<td>0.61</td>
<td>0.56</td>
<td>0.23</td>
<td>0.002</td>
<td>0.14</td>
</tr>
</tbody>
</table>

1a,bLeast squares means within a column lacking a common superscript differ (P ≤ 0.05).
1Transformed back to original least squares means.
2Number of second-grade chicks as a percentage of total number of hatched chicks.
3Corrected in the statistical model for egg weight at setting.
4Navel quality scored with score 1 to 3.
5Percentage of chicks with navel score 1, 2, or 3 (total percentage per row is 100%).
the eggshell (Meijerhof, 1994). Relative humidity and temperature were comparable in the different climate chambers. Therefore, conductivity of the eggshell must have been different among the treatments, although eggs were randomized and egg weight loss during incubation did not differ among the treatments. However, we assume that the differences in egg weight loss during storage did not affect hatchability or chick quality because total egg weight loss was not different among the treatments and total egg weight loss of all treatments was close to the optimal egg weight loss of 11.5% (Hulet et al., 1987; Meir and Ar, 1987).

In the current study, the storage air composition did not affect chick quality on the day of hatch in terms of BW, chick length, and yolk-free body mass. Becker (1964), Proudfoot (1964a, 1965), and Becker et al. (1968), also did not find an effect of storage air composition on chick quality in terms of BW at hatch and Proudfoot (1965) and Becker et al. (1968) also did not find an effect of storage air composition on subsequent performance.

In conclusion, the storage air compositions, studied in the current study, do not affect embryonic development, hatchability, and chick quality on the day of hatch in terms of BW, chick length, and yolk-free body mass when eggs are stored for 14 d at a storage temperature of 16°C.

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