INTRODUCTION

The genetic advances of broiler posthatch growth rate over the span of 38 to 46 yr have been explored by Havenstein et al. (1994a,b, 2003a,b). These comparisons were made with the Athens Canadian Random Bred (ACRB) control strain. These meat-type birds are a replication of the Ottawa Meat Control strain, which was developed from commercial broiler strains of the 1950s, and have been regenerated at the University of Georgia since 1958 (Hess, 1962; Merritt, 1968).

Although these studies have increased our understanding of how the broiler has changed from 3 to 12 wk of age posthatch (Havenstein et al., 1994a,b, 2003a,b), only one known paper has evaluated how these genetic changes have affected the broiler embryo during incubation. The comparison of Christensen et al. (1995) of ACRB control chickens to the 1993 Arbor Acres (AA) broiler found that the AA had significantly larger eggs with less percentage yolk and greater percentage albumin. Moisture loss and eggshell conductance was found to be greater in the AA, but conductance constant was not different between the AA and the ACRB. The percentage liver weight was greater for the ACRB from internal pipping (19 d of incubation) to hatch. The percentage heart was greater for the ACRB on d 17 to 19 of incubation, but percentage heart was greater for AA at external pipping (20 d of incubation) and at hatch. These embryos also exhibited differences in metabolism with higher heart glycogen in the ACRB, increased liver glycogen on d 19 of incubation in the ACRB, increased blood glucose on d 19 of incubation for the AA, and higher T₃ concentration for the AA on d 20 at external pipping. These chicks had similar hatch time and hatchability values were 89% for the AA and 93% for the ACRB.

This paper continues the exploration of changes in the modern broiler during incubation. The present study compares egg components, eggshell qualities, hatch performance, and yolk utilization between the Athens...
Canadian Random Bred control strain and a commonly raised modern high-yielding broiler, the 2013 Cobb 500.

**MATERIALS AND METHODS**

Fertile eggs were collected from the ACRB breeder flock at the University of Georgia in May 2013. The ACRB breeders were an average of 43 wk old, so a 43-wk-old commercial Cobb 500 breeder flock was selected for comparison. The ACRB breeders were artificially inseminated with pooled semen 16, 12, and 8 d before the starting incubation date. Fertile ACRB eggs were collected daily and those incubated ranged in storage age from 1 to 8 d. The Cobb 500 eggs were collected on 2 separate days and were stored for 5 and 2 d before incubation. Eggs were stored at the University of Georgia where they were held at 18.4°C and 70% RH.

**Egg Components**

A total of 70 Cobb eggs and 105 ACRB eggs were individually weighed (nearest 0.0001 g) and broken to determine initial relative albumin and yolk weights before incubation. Only eggs with apparently normal shells were used in this study. Yolk and albumin egg components were dried at 65°C for 72 h and then reweighed. Yolks were slit to permit thorough drying. All eggs were stored in an 18.4°C and 70% RH cooler before weighing. At 4 d after lay, 35 intact Cobb 500 eggs (n = 70) were weighed. The shell, yolk, and albumen were separated and the individual components were weighed. Due to the limited egg numbers produced by the ACRB hens, ACRB eggs were collected when the breeders were 46 wk old. At 1, 2, and 8 d of storage, 35 ACRB eggs were weighed (n = 105), and the components separated and weighed.

**Incubation**

From each strain 700 eggs were incubated in 10 trays (90 egg capacity) of 70 eggs each. The ACRB eggs were systematically placed in each tray in order of storage age so that all storage ages were equally represented in each ACRB tray. Additionally, the storage ages did not have the same position in each tray. The Cobb 500 eggs were alternatively placed so the 2 storage ages were not next to each other in a tray. The Cobb 500 storage ages also did not have the same position in all trays. The trays were alternatively placed in the buggy so that trays of each strain were interspersed throughout all levels of the incubator.

All eggs were placed in a 27.2°C room 4 h before placement in a calibrated Natureform NMC2000 incubator at 37.5°C and 53% RH. Air temperatures in the incubator were recorded every 15 min using HOBO temperature probes (HOBO U30 Station, Onset Computer Corporation, Bourne, MA) located at the top, middle, and bottom locations of the incubator. The top probe rested with the incubator’s temperature probe on the ceiling of the incubator, the middle hung in the top of the egg mass, and the bottom probe hung in the bottom of the egg mass. The eggs were placed in the incubator at 2100 h and incubation time began at 2230 h when the average air temperatures reached the set temperature. All eggs were individually weighed immediately before set, at 12 d of incubation, and at 18 d of incubation (nearest 0.01 g). All eggs were candled at 12 d and clear eggs were broken to distinguish infertility, early embryonic mortality (0–7 d of incubation), and middle embryonic mortality (8–12 d of incubation).

**Conductance Calculations**

Conductance (\(G_{H2O}\)) was calculated as \(G_{H2O} = \frac{\text{egg weight loss by 12 or 18 d of incubation (mg)}}{\Delta P_{H2O}}\) (Ar et al., 1974; Ar and Rahn, 1978), where \(\Delta P_{H2O}\) is the difference in water vapor pressure between the inside and outside of the egg. The vapor pressure gradient between the incubator and the inside of the egg was determined using the Vaisala online humidity calculator (http://www.vaisala.com) to determine the saturation vapor pressure (egg) and the vapor pressure (incubator environment) using the average air temperatures and RH of the HOBO data loggers within the egg mass and the average barometric pressure recorded for Athens, Georgia, during the experiment. Conductance was also standardized to a 100 g egg weight (\(g_{H2O}\)) as \(g_{H2O} = \frac{G_{H2O}}{\text{set egg weight (g)}} \times 100\) (Peebles and Brake, 1987; Pulikanti et al., 2011). Conductance constants \((K_{H2O})\) were calculated as \(K_{H2O} = \frac{G_{H2O}}{\text{incubation period of each strain (d)}}\times\text{set egg weight (g)}\) (Ar and Rahn, 1978). Moisture loss, conductance \((G_{H2O})\), conductance standardized as a 100 g egg weight \((g_{H2O})\), and conductance constants \((K_{H2O})\) were calculated for all live embryonated eggs that had not yet externally pipped at the time the egg was weighed (12 and 18 d).

**Hatching**

The eggs were transferred to plastic hatching baskets (76.2 cm width, 71.1 cm length, and 11.4 cm deep) in a single calibrated Natureform NMC2000 hatcher on d 18 of incubation at 36.7°C and 53% RH. In each hatching basket, one tray of ACRB eggs and one tray of Cobb 500 eggs were placed in the basket with a divider between the trays. Three Chick Master data loggers (Chick Master Incubator Co., Medina, OH) recorded temperatures in 3 ACRB trays and 2 data loggers recorded temperatures in 2 Cobb trays in the hatcher. One data logger recorded humidity in the hatcher in an ACRB tray.

The numbers of chicks that hatched in each tray was recorded every 6 h starting at 468 h of incubation. A divider was placed behind the eggs, and all hatched chicks were placed behind the divider to eliminate recounting the hatched chicks at each time period. The start time of the hatch was monitored and began once
chicks began externally pipping, and the progress and termination of the hatch was monitored by strain. The hatch was considered complete when pipping chicks were no longer progressing and hatched chicks were dry. At the termination of the hatch, chicks were counted and graded as an A or B quality chick. An A quality chick possessed no exterior defect, whereas a B quality chick had one or more of the following characteristics: enlarged unsealed navel, bloody navel, navel with adhering shell material, navel with protruding dried membrane, inability to stand (including splay legged), appearance of dehydration or mushy chicks, and red hocks. Two trays of hatched chicks per strain were euthanized using carbon dioxide at the time of removal from the hatcher. The sampled chicks were individually weighed, and the yolk sac was removed and weighed (nearest 0.01 g). Yolk sacs were dried at 65°C for 72 h and reweighed.

A residue breakout was completed on all unhatched eggs as described by Wilson (2010). The eggs were assigned categories of early, middle, or late dead embryo, live or dead pip, dead or cull chick, set or transfer crack, contaminated, or live in the shell. Eggs were considered infertile when a fertilized blastoderm was not visible. Early (0–7 d), middle (8–14 d), and late (15–21 d) dead embryos were assigned to eggs that had a fully intact eggshell. Embryos live in the shell had not yet pipped and were still alive at the time of the breakout. Pips were considered live if the chick was still alive at the time the egg was opened or dead if the chick was dead at this time. Set cracks were designated when a crack was present in the eggshell and the internal contents of the egg had dried down to less than half the egg contents. Transfer cracks were designated for eggs with a crack in the eggshell that allowed the shell membrane to dry to a hardened state, making it difficult for the chick to penetrate. Cracked eggs were not assigned a stage (early, middle, late) of embryonic death or designated as fertile or infertile. Contaminated eggs were designated as eggs that had a distinctive black, blue, or red color content with a noticeable odor or eggs that popped when the eggshell was broken. Cull chicks were hatched but were unable to survive due to protruding internal organs (yolk sac, intestines).

### Results

Cobb 500 eggs were an average 12.64 g (20%) heavier than ACRB eggs (Table 1). By 18 d of incubation, ACRB eggs had 2.7% greater average moisture loss than Cobb 500 eggs. The greater moisture loss and smaller egg size of the ACRB calculated to a higher vapor conductance of the eggshell ($G_{H2O}$) at both 12 and 18 d of incubation (Table 1). Conductance standardized to a 100 g egg weight ($G_{H2O}$) and conductance constant ($K_{H2O}$) were also significantly greater for the ACRB eggs (Table 1).

Storage times of the ACRB eggs had no effect on egg weight, moisture loss, $G_{H2O}$, $G_{H2O}$, or $K_{H2O}$ at 12 or 18 d. The Cobb 500 eggs stored for 2 d were 0.62 g heavier than the Cobb 500 eggs stored for 5 d ($P = 0.0405$). Moisture loss and conductance measurements at 12 and 18 d of incubation did not differ between the 2 storage ages of the Cobb 500 eggs.

The Cobb 500 chicks hatched 6 h earlier than the ACRB chicks (Figure 1). Cobb 500 chicks were removed from the hatcher at 498 h of incubation. The ACRB chicks were removed at 504 h of incubation. Once the Cobb 500 chicks were removed from the hatcher, ACRB chicks were moved to the empty half of the hatch basket to limit change in heating and airflow.

Both strains had similar percent fertility and hatch (Table 2). From the residue analysis, there was a greater percentage of cull chicks in the Cobb 500 hatch than the ACRB. Percentages of salable chicks, however, was not different between the strains ($P = 0.2891$). Cobb 500 had 91.0% A quality chicks and ACRB had 92.1% A quality chicks.

The HOBO temperature probes logged 1,788 measurements over the first 18 d of incubation. The top probe, aligned with the incubator’s temperature probe, averaged 37.54°C, right on target temperature. The middle and bottom temperature probes positioned in the egg mass recorded average air temperatures of 37.76 and 37.84°C, respectively. Relative humidity averaged 53.44%. In the hatcher, the 3 ACRB eggloggers averaged 36.94°C over 159 logged temperatures, and the 2 eggloggers in the Cobb trays averaged 36.98°C from 141 logged temperatures. The average RH, recorded by an egglogger in an ACRB tray, was 54.4% during hatching. The average pressure recorded during the experiment was 764.54 mmHg.

The eggs selected for preincubation egg components were representative weights of those incubated (Table 3). The ACRB eggs had a significantly greater percentage of solids, whereas Cobb eggs had larger percentage of water in the egg. The ACRB eggs had 2.1% more yolk solids than the Cobb 500, whereas Cobb eggs devoted a 1.6% greater solid percentage to albumin. The calculated shell percentage was not different between the strains. Storage time had no effect on dry egg components for the ACRB. Cobb eggs stored for 6 d had 0.4% greater percentage dry albumin than 4 d stored.

### Statistical Analysis

All data were analyzed using the general linear model procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). Hatch performance and chick quality were analyzed as percentages by tray (n = 10 per strain). Egg components, egg quality measurements, and chick and yolk measurements were analyzed on an individual egg and chick basis. Significance between the 2 strains was based on $P < 0.05$. Egg components and egg quality measurements were also analyzed within each strain to determine any effect of egg storage time.
No other Cobb dry egg components were significantly altered due to storage time. At hatch, 125 Cobb chicks and 129 ACRB chicks were sampled for chick and residual yolk weights. The Cobb 500 had 1.6% greater residual yolk solids than the ACRB. Thus, the ACRB started incubation with Cobb eggs \( (P = 0.0037) \). No other Cobb dry egg components were significantly altered due to storage time.

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a larger percentage of yolk solids and hatched with a smaller percentage of yolk solids compared with the Cobb 500 (Table 4). Based on the initial and final yolk percentages, ACRB chicks used 69.1% of their yolk solids during incubation, whereas Cobb 500 chicks used 54.8% of their yolk solids. Cobb yolk-free chick weights averaged 39.07 ± 0.21 g, and ACRB yolk-free chicks averaged 30.81 ± 0.20 g (P < 0.0001).

**DISCUSSION**

The genetic selection of a larger broiler (Havenstein et al., 1994a,b, 2003a,b) has also resulted in a larger egg. Like Christensen et al. (1995), this study found a significantly greater percentage of water in the initial eggs of the Cobb 500 and significantly greater percentage of solids in the ACRB eggs. This study also agrees with Christensen et al. (1995) with significantly greater albumin in the Cobb 500 eggs and significantly greater percentage yolk in the ACRB eggs. The egg component percentages between the 2013 Cobb 500 and the 1995 AA appear similar, which suggests that broiler egg composition has not drastically changed over the past 18 yr like it has over the past 58 yr. Within a strain and age group, smaller eggs have been found to have greater amount of yolk than larger eggs, which is in agreement with this study: the smaller ACRB eggs had a greater amount of yolk than the larger Cobb 500 eggs (Jull, 1924; Scott and Warren, 1941; Marion et al., 1964; Curtis et al., 1986).

Unlike Christensen et al. (1995), the ACRB of this study had greater moisture loss, conductance, and conductance adjusted to egg weight, via both gH2O and K_{H2O}, than Cobb 500 eggs. The ACRB eggs lost the average ideal egg weight loss of 12% at 18 d of incubation (Tullett, 1981). Studies have found that decreased moisture loss and conductance coincides with higher late embryonic mortality in turkeys (Christensen and McCorkle, 1982; Christensen et al., 1993; Christensen and Nestor, 1994); however, both chicken strains in the present study had equivalent embryonic mortality despite the lower calculated conductance of the Cobb 500 eggs.

Because eggshell water vapor conductance indicates the ability for gas exchange through the eggshell (Paganeli et al., 1978; Rahn et al., 1979), the higher conductance values of the ACRB eggs of the present study indicate a greater level of gas exchange for the 1955 broiler than the 2013 Cobb 500 broiler. This finding is slightly unexpected because the Cobb 500 hatched 6 h faster than the ACRB. Larger eggs usually have reduced water loss and lower conductance, which was seen in the Cobb 500 eggs, but larger eggs usually have longer incubation time (Ar et al., 1974; Rahn and Ar, 1974). The lower conductance in the larger Cobb 500 egg may be due to a difference in pore length or diameter or a difference in number of pores in the eggshell compared with the ACRB, which was not measured in this current study (Rahn et al., 1979). Future work could further explore the difference in shell characteristics between the ACRB and the Cobb 500.

This study found that ACRB chicks used 14.3% greater percentage yolk than Cobb 500 chicks. To our knowledge, no previous study has discovered decreased yolk utilization in modern Cobb 500 broiler embryos compared with a meat-type control strain. A decrease in oxygen available to Cobb 500 embryos for fatty acid oxidation is a potential reason for the decreased yolk utilization. Pulikanti et al. (2012) found that Ross 308 broiler eggs with higher g_{H2O} produced larger 3 d posthatch chicks that used a greater percentage of yolk than eggs with lower g_{H2O} values. The higher g_{H2O} of the ACRB with greater yolk utilization of this study agrees with the finding of Pulikanti et al. (2012). Unlike the 3 d posthatch weights of Pulikanti et al. (2012), however, the ACRB, with higher g_{H2O}, did not have a greater yolk-free chick weight as a percentage of initial egg weight at hatch compared with the Cobb 500. At 8 wk posthatch, despite a significantly larger BW (Havenstein et al., 1994a,b), modern commercially raised

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**Table 3.** Initial\(^1\) egg components of Athens Canadian Random Bred and Cobb 500 hatching eggs

<table>
<thead>
<tr>
<th>Strain</th>
<th>Weight</th>
<th>Water</th>
<th>Solids</th>
<th>Albumen</th>
<th>Yolk</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>%</td>
<td></td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>ACRB(^2)</td>
<td>50.0183</td>
<td>62.5</td>
<td>37.5</td>
<td>5.9</td>
<td>17.8</td>
<td>13.8</td>
</tr>
<tr>
<td>Cobb(^3)</td>
<td>63.1817</td>
<td>63.4</td>
<td>36.6</td>
<td>7.5</td>
<td>15.7</td>
<td>13.4</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>55.3285 ± 0.53</td>
<td>62.8 ± 0.13</td>
<td>37.2 ± 0.13</td>
<td>6.6 ± 0.07</td>
<td>17.0 ± 0.11</td>
<td>13.6 ± 0.12</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^1\)Prior to incubation.

\(^2\)ACRB = Athens Canadian Random Bred, 1955 meat-type chicken control strain; n = 105 eggs.

\(^3\)Cobb = 2013 Cobb 500 broiler; n = 70 eggs.

**Table 4.** Yolk utilization of Athens Canadian Random Bred and Cobb 500 broiler embryos

<table>
<thead>
<tr>
<th>Item</th>
<th>Initial % of dry yolk (of egg weight)</th>
<th>Final % of dry yolk (of chick weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACRB(^1)</td>
<td>17.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Cobb(^2)</td>
<td>15.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>17.0 ± 0.11</td>
<td>6.3 ± 0.12</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^1\)ACRB = Athens Canadian Random Bred, 1955 meat-type chicken control strain; initial n = 105; final n = 129.

\(^2\)Cobb = 2013 Cobb 500 broiler; initial n = 35; final n = 125.
broilers have also been found to have double the occurrence of unabsorbed yolk sacs compared with ACRB chickens (Buhr et al., 2006). The embryonic yolk utilization findings of the present study may help explain the greater percentage of unabsorbed yolk sacs present at processing age in modern broilers.

Despite the significantly lower percentage yolk utilization in the Cobb 500, average yolk-free chick weights were approximately 61% of the average initial egg weights for both strains. Thus, both strains obtained the same final chick composition of egg weight, while potentially relying on different metabolic means.

Speake et al. (1998) reviewed the yolk utilization processes of the chicken embryo. The yolk is thought to provide 90 to 94% of the energy required by the developing chick embryo with the majority of yolk utilization starting at 12 d of incubation via fatty acid oxidation (Speake et al., 1998). The results of the present study indicate that either modern Cobb 500 broiler embryos utilize the greater percentage albumen present in their eggs in ways to compensate for the lower yolk utilization, or modern broilers are not only efficient during posthatch growth (Havenstein et al., 1994a, 2003a), but also as an embryo. Perhaps the modern Cobb 500 broiler embryo makes such efficient use of their energy resources that a higher percentage of yolk utilization is not necessary to obtain the same relative chick body mass as the ACRB.

The decreased gas exchange through the eggshell may have also contributed to the earlier hatch of the Cobb 500 because decreased oxygen and increased carbon dioxide are proposed mechanisms for the cue to pip (Freeman, 1962; Visschedijk, 1968). Hormone signaling, including thyroid hormones, is thought to induce hatching (Decuyper et al., 1991). The higher T₃:T₄ ratio found by Christensen et al. (1995) in AA broilers than ACRB at external pipping and at hatch could potentially still be at higher levels in the modern Cobb 500 broiler embryo near hatch, and may have contributed to the Cobb 500s earlier hatch.

Earlier hatches, larger egg size with lower conductance, and larger residual yolk sac have all been found to occur with higher air and eggshell temperatures (Romanoff, 1936; Barott, 1937; French, 1997; Willemsen et al., 2010, 2011). Eggshell temperatures were not recorded in the current study. Preliminary work comparing the ACRB and the Cobb 500 found no difference in eggshell temperatures between the strains; however, this preliminary work had a small sample size due to the drop in eggshell temperature upon opening the incubator door and the strains were not equally distributed throughout the incubator (Collins, 2013). Researchers have estimated that heat production has increased in modern broiler eggs (Hamidu et al., 2007; Hulet, 2007) particularly with findings of differential heat production of embryos of different strains (Tona et al., 2004, 2010). Further investigation is needed to definitively conclude if eggshell temperatures and heat production are higher in the modern Cobb 500 during incubation compared with the 1955 ACRB during incubation.

In summary, in comparison with the 1955 ACRB meat-type control strain, modern Cobb 500 broilers have larger eggs with lower K H₂O and e H₂O, which indicates decreased gas exchange during incubation. This eggshell characteristic may have contributed to the earlier hatch and decreased embryonic yolk utilization found in this study. Despite these effects, the modern Cobb 500 broiler embryo continues to metabolize enough energy to hatch at a similar percentage of the egg as the 1955 broiler with equivalent embryonic mortality and salable chicks.

REFERENCES


