ABSTRACT

Broiler performance is known to be related to embryonic developmental parameters. However, strain or genotype differences with regard to embryo physiological parameters and juvenile growth have received little attention. A total of 1,200 hatching eggs produced by Cobb and Ross broiler breeders of the same age were studied. At setting for incubation and between 66 and 130 h of incubation, egg resonant frequency (RF) was measured as an indicator of embryonic development. Also, eggs were weighed before setting and at d 18. From d 10 to 18 of incubation, remaining albumen was weighed. During the last days of incubation, hatching events such as internal pipping (IP), external pipping, and hatch were monitored every 2 h. Hatched chicks were recorded and weighed. At IP stage, gas partial pressures in the egg air chamber were measured. Hatched chicks were reared for 7 d and weighed. Results indicate that RF of Ross eggs were lower than those of Cobb eggs ($P < 0.01$) and starting time point of RF decrease occurred earlier in Cobb eggs than in Ross eggs. Relative egg weight loss up to 18 d of incubation was lower in Cobb than in Ross ($P < 0.05$). At IP, partial pressure of CO$_2$ was higher in Cobb than in Ross ($P < 0.05$) with shorter incubation duration in Cobb. Between 6 and 60 h posthatch, heat production was higher in Cobb than in Ross ($P < 0.05$). At 7 d posthatch, Cobb chicks were heavier than Ross chicks ($P < 0.05$). It is concluded that Cobb and Ross embryos-chicks have different growth trajectories leading in different patterns of growth resulting from differences in physiological parameters.

INTRODUCTION

During the past 5 decades, intensive selection in broilers has focused on posthatch growth rate and feed conversion to achieve increased meat yield. But, all broiler strains do not have similar physiology or development trajectories, or both. Posthatch performance is known to be related to embryonic developmental parameters (Tona et al., 2003a). Similarly, the effects of incubation conditions, egg storage conditions, and age of breeders on embryonic parameters are well known (O’Dea et al., 2004; Tona et al., 2004). However, strain or genetic line differences with regard to embryo physiological parameters and juvenile growth have received little attention. Crittenden and Bohren (1961), Siegel et al. (1968), and Suarez et al. (1997) showed variations in incubation time with genotype. These variations in incubation time suggest that perhaps optimum incubation conditions need to be established for different genotypes or strains to obtain optimal hatching performance and high-quality chicks. Among the broiler strains used for meat production, Cobb and Ross are actually the most widely produced worldwide. Recently, Sterling et al. (2006) demonstrated that Cobb broilers grew better with a better feed conversion ratio than the Ross strain. It is not clear if the different posthatch performances between the strains are also a reflection of their embryo physiological and hatching parameters.

The aim of this study was to compare 1) embryonic developmental parameters using acoustic resonance methods, 2) embryonic physiological parameters, and 3) the hatching parameters of eggs from Cobb and Ross breeders of the same age.

MATERIALS AND METHODS

Experimental Design

A total of 1,200 hatching eggs, provided by Vervaeke Hatchery Company (Tielt, Belgium) were used for this
study. Half (600) of the eggs were collected from a commercial flock of Cobb broiler breeders and the other half (600) were from a commercial flock of Ross broiler breeders. The age of both broiler breeders’ strains was 52 wk. They were stored for 3 d before setting for incubation. Before setting, eggs were numbered, weighed, assigned into 4 replications of 150 eggs each/strain before setting for incubation, and strains were equally divided over 2 incubators. The eggs were incubated in forced-draft incubators (PasReform, Zeddam, the Netherlands) at 37.6°C, RH of 50%, and turning each hour through an angle of 90°. Before setting for incubation and also between 66 and 130 h of incubation, samples of eggs were used to measure changes in resonant frequency (RF) as an indicator of embryonic development. During incubation, eggs were randomly set to avoid incubator gradients. From d 10 to 18 of incubation, albumen utilization by embryos was measured. At d 18 of incubation, eggs were weighed, candled, and those with evidence of living embryos were transferred from turning trays to hatching baskets. During the last 2 d of incubation, hatching events such as internal pipping (IP), external pipping (EP), and hatch of individual eggs were monitored every 2 h. Hatched chicks were recorded and weighed as well. A sample of eggs was used at the IP stage for measurement of CO₂ and O₂ partial pressures (pCO₂ and pO₂, respectively) in the egg air chamber. Samples of hatched chicks were individually marked and reared for 7 d and were weighed at the end of this period. All unhatched eggs were opened for macroscopic analysis to classify them as infertile eggs or eggs with dead embryos.

**Measurement of Embryonic Development**

Embryo development was monitored by acoustic resonance analysis as described previously by Coucke et al. (1997). Briefly, the method involved the mechanical excitation of the egg by a mechanical impactor. The impactor hit the egg at its equator and the noise of the vibrating egg was recorded by a microphone positioned at the equator at an angle of 90° to the impactor. The recorded signal was then sent to a data acquisition card and transformed by fast Fourier transformation to obtain the RF for the first spherical mode of the vibrating egg. In this experiment, 4 instantaneous excitations with a phase shift of 90° were applied at the equator zone of the eggs. Samples of 30 eggs per replication were used to determine RF. The measurements were done before setting for incubation and were repeated every 2 h between 66 and 130 h of incubation.

**Albumen Utilization and Egg Weight Loss**

Samples of 10 eggs per replication were opened at d 10, 12, 14, 16, and 18 for the assessment of residual thick albumen weights. At d 18 of incubation, all eggs were weighed (W₁₈). Egg weights at d 18 of incubation and at the beginning of incubation (W₀), were used to calculate egg relative weight loss (RWL) up to d 18 of incubation as: 

\[ \text{RWL} = \frac{100 \times (W₀ - W₁₈)}{W₀} \]

**Measurement of Air Cell Gas Partial Pressures**

Within each replication, 8 eggs with evidence of living embryos were used to measure the gas partial pressure (pO₂ and pCO₂) in the air chamber at 10 d of incubation and at IP. These measurements were done directly in the air chamber of the eggs by means of a blood gas analyzer (type 1610, Instrumentation Laboratories, Lexington, IL) for the measurement of pCO₂ and pO₂. This method for the measurement of gas partial pressures in air chamber has previously been described by Dewil et al. (1996), Buyse et al. (1998), and Tona et al. (2003a).

**Measurement of Heat Production**

From 430 to 478 h of incubation, samples of 30 incubated eggs per replication were used to determine heat production (HP). Also from hatch to 3 d posthatch, samples of 10 chicks per replication were used to determine HP. During incubation, samples of 120 eggs per strain of breeder were grouped into 2 replications and placed into separate respiratory units (60 eggs/unit). Likewise, samples of hatched chicks (40 per strain of breeder) were grouped in 2 replications and placed into respiratory units (20 chicks per respiratory unit). The O₂ consumption and CO₂ output of ingoing and outgoing air were calculated, based on paramagnetic and infrared measures, respectively, for O₂ and CO₂. In addition, airflow at standardized conditions for pressure, temperature, and RH were measured. The respiration cells were placed in duplicate in 3 separate climatic chambers. Between 430 and 478 h of incubation, the climatic chambers were set at 37°C and 50% RH for the eggs. For the chicks, from hatch to 3 d, the respiratory chamber temperature was gradually reduced from 35 to 33°C and the chicks were provided water and feed for ad libitum consumption. During measurements, O₂ consumption and CO₂ production for each trial of 60 eggs or 40 chicks were monitored continuously to calculate HP (Buyse et al., 1998).

**Pipping and Hatching Events**

Between 466 and 498 h of incubation, the transferred eggs were checked every 2 h for IP, EP, and the occurrence of hatching events. At IP, EP, or hatching stages, incubation duration was defined as the time between setting and the occurrences of these events for an egg. Then, the timing of the occurrence of hatching events was used to calculate their durations as follows: IP du-
ration = duration between occurrences of IP and EP; EP duration = duration between EP and hatching; and hatching duration = duration between IP and hatching. Spreads of IP, EP, or hatch were defined as the dispersion around the average incubation duration.

Statistical Analysis

The data were processed with the statistical software package SAS Version 8.25 (SAS Institute Inc., Cary, NC). The generalized linear regression model was used to analyze the effects of strains on egg, albumen, and embryo weights; RF; durations of IP, EP, and hatching; and 7 d posthatch weights. When the means of the GLM were statistically different, they were compared using Tukey’s test.

In a second analysis, hatchability was considered as binomial in distribution. A 2-tailed test for comparison of variances was used to analyze the influence of strains on hatchability.

RESULTS

Effects of Strains on RF

Figure 1 shows egg RF variations at setting for incubation and between 66 and 130 h of incubation according to strain. From the hour of setting until 130 h of incubation, the RF of Ross eggs were lower than those of Cobb eggs ($P < 0.01$). In both strains, RF increased slightly between the 70th and 94th hour of incubation and then decreased sharply. Although RF decrease followed a similar trend in both strains, the starting time for the RF decrease was earlier in the Cobb eggs (90th hour) compared with that of Ross eggs (94th hour) ($P < 0.05$).

Strain Effect on Egg, Embryo, and Chick Weights

Table 1 shows the values of egg weights at setting, egg weight loss up to 18 d of incubation, and 1- and 7-d-old chick weights according to the strains. Although egg weights at setting were similar between strains, relative egg weight loss up to 18 d of incubation was lower in Cobb than in Ross ($P < 0.05$). Between d 10 and 18 of incubation, embryo weight was similar between strains (data not shown). Chick weights at hatch were similar between strains, whereas 7-d-old chick weights were heavier in Cobb than in the Ross strain ($P < 0.05$).

Strain Effect on Albumen Utilization During Incubation

Figure 2 shows changes in albumen weights of the eggs of different strains at different stages of incubation. At d 10 and 12 of incubation, albumen weights were not different between strains. Between d 12 and 16 of incubation, there was a rapid decrease in albu-
men weight in both strains. However, at d 14 and 16 of incubation, albumen weights were lower in Ross eggs than in Cobb eggs ($P < 0.05$). At d 18 of incubation, albumen weight was at nadir level in both strains.

**Strain Effect on Partial Gas Pressures in Air Chamber and HP by Eggs and Chicks**

Table 2 shows partial gas pressures in the air chamber of eggs at d 18 of incubation and at the IP stage of the different strains. Partial pressures of CO$_2$ or O$_2$ at d 18 of incubation were similar between strains. However, at the IP stage, pCO$_2$ was higher, whereas pO$_2$ was lower in Cobb than in Ross ($P < 0.05$).

Figure 3 shows the changes in embryonic HP between 430 and 478 h of incubation according to strain. Overall, embryonic HP was comparable between Cobb (0.64 ± 0.01 kJ/h per egg) and Ross (0.62 ± 0.01 kJ/h per egg). Heat production increased sharply from 460 h of incubation onward in both strains. However, close to the end of incubation, HP of Ross eggs was slightly lower compared with that of Cobb eggs.

During posthatch development, HP increased with the age of the chicks. Between 6 and 60 h, HP increased from 2.23 to 5.58 kJ/h per chick for the Cobb and from 2.09 to 5.11 kJ/h per chick for the Ross (Figure 4). Overall, during this posthatch period, HP was significantly higher in Cobb than in Ross from 16 until 60

![Figure 2. Remaining albumen weights according to incubation day and strains. At each incubation day, an asterisk (*) indicates difference between albumen weights.](image)

**Table 1.** Relative egg weight loss and weights of egg at setting and of 1- and 7-d-old chicks in relation to strains

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Egg/chick number per strain</th>
<th>Cobb</th>
<th>Ross</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg weights at setting</td>
<td>600</td>
<td>70.15 ± 0.34</td>
<td>70.68 ± 0.33</td>
</tr>
<tr>
<td>Egg weight losses up to 18 d of incubation</td>
<td>450</td>
<td>9.13 ± 0.24$^b$</td>
<td>10.08 ± 0.14$^a$</td>
</tr>
<tr>
<td>1-d-old chick weight</td>
<td>350</td>
<td>52.73 ± 0.32$^a$</td>
<td>52.61 ± 0.37$^a$</td>
</tr>
<tr>
<td>7-d-old chick weight</td>
<td>300</td>
<td>151.84 ± 2.55$^a$</td>
<td>145.08 ± 2.41$^b$</td>
</tr>
</tbody>
</table>

$^{a,b}$Within rows, data sharing no common superscripts are different ($P < 0.05$).
Moreover, the difference between the 2 strains increased with posthatch age.

**Effects of Strains on Hatching Events**

Table 3 shows the durations of incubation, duration of each hatching event (IP, EP, and hatch), and egg hatchability. Total incubation duration to attain 50% of eggs at IP, EP, and hatch was delayed by 2 h in Ross eggs compared with Cobb eggs \((P < 0.05)\), although the durations of IP, EP, and hatch were similar between strains. Hatchability, at the end of incubation, was similar between strains.

**DISCUSSION**

Overall, a differential embryonic developmental pattern between both lines could be related to differences in hatching process and early postnatal growth (Tona et al., 2004). Although Cobb had a somewhat faster development in the first 4 to 5 d, a faster development was observed in the second incubation week for Ross. Although again, a catching up and higher metabolism and developmental speed was observed during the last days and in the perinatal period for the Cobb strain. This higher metabolism and faster growth for Cobb was prolonged in the first week posthatch.

Higher RF of Cobb eggs compared with Ross eggs before and during incubation may be due to differences in eggshell characteristics. This observation is in line with the findings of De Ketelaere et al. (2002), who reported differences in eggshell characteristics between 6 different lines of chickens. Bamelis et al. (2002) had shown that changes in RF can be related with embryo fluid formation during early embryonic development and hence is a marker for a well-defined developmental stage. The earlier general decrease in the RF of the egg of the Cobb strain (at 90 h of incubation) compared

![Figure 3. Changes in heat production (HP) in relation to incubation duration and according to strains.](image)

### Table 2. Partial pressures of CO\(_2\) and O\(_2\) (pCO\(_2\) and pO\(_2\), respectively) according to strains and developmental stages

<table>
<thead>
<tr>
<th></th>
<th>d 18 of incubation</th>
<th>IP stage(^{1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pCO(_2) (mmHg)</td>
<td>pO(_2) (mmHg)</td>
</tr>
<tr>
<td>Cobb</td>
<td>39.97 ± 2.04(^a)</td>
<td>110.87 ± 2.46(^a)</td>
</tr>
<tr>
<td>Ross</td>
<td>37.8 ± 1.77(^a)</td>
<td>112.69 ± 2.13(^a)</td>
</tr>
</tbody>
</table>

\(^{a,b}\)Within columns, data sharing no common superscripts are different \((P < 0.05)\).

\(^{1}\)IP = internal pipping.
with the Ross eggs (at 94 h of incubation) indicates a quicker initiation of embryonic development, which might have been correlated with shorter incubation duration for this strain. Kemps et al. (2003) previously reported a positive correlation between the timing of RF decrease during early incubation and hatching time. Consequently, at the same incubation time point, Cobb embryos and Ross embryos may not have been at a similar physiological stage of development.

Between d 12 and 16 of incubation, remaining albumen weight was lower in the Ross strain than in the Cobb strain, indicating that during this stage the Ross embryo uses faster albumen for growth. Indeed, during incubation, albumen proteins move into the amniotic fluid and are swallowed by the embryo. These proteins are then either digested in the gut of the embryo for growth or transferred into the yolk sac where they can be used after hatching (Deeming, 1989).

The trend for higher pCO2 at d 18 of incubation and the significantly higher pCO2 at the IP stage in the Cobb egg air chamber compared with that of Ross eggs may be due to more advanced physiological stage of the Cobb compared with Ross. This advanced physiological stage led to the slightly higher HP and shorter incubation duration. This observation is in line with the previous reports of Dewil et al. (1996) and Tona et al. (2003a), who demonstrated that higher pCO2 in the egg air chamber at IP increased the chances for early hatching. Indeed, at median (50%) occurrence of IP, EP, or hatch, eggs from Cobb hatched 2 h earlier than those of Ross. Furthermore, the occurrence of this early hatch can be linked to the timing of the decrease

<table>
<thead>
<tr>
<th>Hatching event</th>
<th>Cobb</th>
<th>Ross</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation duration for 50% of IP</td>
<td>464b</td>
<td>466a</td>
</tr>
<tr>
<td>Incubation duration for 50% of EP</td>
<td>470b</td>
<td>472a</td>
</tr>
<tr>
<td>Incubation duration for 50% of hatch</td>
<td>484b</td>
<td>486a</td>
</tr>
<tr>
<td>IP duration</td>
<td>8.00 ± 0.66</td>
<td>7.94 ± 0.63</td>
</tr>
<tr>
<td>EP duration</td>
<td>12.89 ± 0.44</td>
<td>11.65 ± 0.38</td>
</tr>
<tr>
<td>Hatching time</td>
<td>19.32 ± 0.67</td>
<td>18.84 ± 0.43</td>
</tr>
<tr>
<td>Hatchability</td>
<td>91.51</td>
<td>90.10</td>
</tr>
</tbody>
</table>

a,bWithin rows, data sharing no common superscripts are different (P < 0.05).

1IP = internal pipping; EP = external pipping.

Figure 4. Changes in heat production (HP) in relation to posthatch stage and according to strains. At each posthatch time, an asterisk (*) indicates difference between chick heat production.
of RF early in embryonic development. The lack of differences in hatching events between the strains clearly demonstrates that the differences in incubation duration occurred during the developmental stage before the beginning of the hatching process.

During the perinatal stage, HP increased rapidly irrespective of strains. Heat production was slightly higher in Cobb before hatch. This agrees with the results of Hamidu et al. (2007), who reported higher HP in Cobb embryos compared with Ross embryos. Also, it has been shown previously that broiler chickens selected for rapid growth rate have higher metabolism toward the end of incubation (Jørgensen et al., 1990; Buyse et al., 1998; Tona et al., 2004).

Even though chicks had similar weights at hatch, 7 d posthatch weights were higher in Cobb chicks than in the Ross chicks. Heat production was also higher in the Cobb during the first few days posthatch, suggesting higher metabolic rate. This finding is consistent with our previous reports, which showed that embryos with higher levels of this physiological indicator had better posthatch chick growth performance (Tona et al., 2003a,b). It can be concluded that Cobb and Ross embryos-chicks have differential growth trajectories. These different patterns of growth are deduced from differences in physiological parameters. These findings suggest that differences in physiological parameters during embryonic development and also in physical parameters of the eggs may lead to the hypothesis that incubation conditions could be improved in a strain-dependent manner. Moreover, the observed differences in gas partial pressures and HP rates during late incubation or posthatch growth suggest consistency with the posthatch growth speed of both strains.

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REFERENCES


