Development and nutrient metabolism of embryos from two modern broiler strains

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ABSTRACT A progressive selection for broiler live and processing performance traits has changed broiler growth patterns during the post hatch period. However, limited information is available to understand whether changes have also occurred during the embryonic stages. This study aims to examine influences of broiler strain on nutrient availability, embryonic development, and nutrient metabolism during incubation. Hatching eggs of Ross 308 and Cobb 500 fast feathering were selected from breeder flocks aged 43 to 46 weeks at an egg weight range of 60 to 63 g. Eggs were obtained in 2 batches, 120 eggs per strain per batch. For each batch, 20 eggs per strain were used to determine egg composition and nutrient availability. The remaining eggs were incubated separately in one of 2 climate respiration chambers at an eggshell temperature of 37.8°C. The results showed that Ross 308 eggs had a higher yolk:albumen ratio with 0.9 g more yolk and 0.7 g less albumen than Cobb 500. Albumen + yolk of Ross 308 eggs had a higher dry matter ($\Delta = 0.24$ g) and crude fat ($\Delta = 0.23$ g) than that of Cobb 500 eggs, but a similar amount of crude protein. Albumen and yolk of Ross 308 eggs had a higher energy content ($\Delta = 8.9$ kJ) compared to Cobb 500 eggs. At 3 h after hatch, Ross 308 chicks were 0.2 cm longer and had a 0.6 g heavier yolk free body mass (YFBM) than Cobb 500 chicks. During incubation, Ross 308 embryos used 13.9 kJ more energy than Cobb 500, and the efficiency of converting energy used to YFBM (E_{YFB}) was approximately 7.6% lower compared to Cobb 500. Ross 308 chicks hatched approximately 4 h later and had less hepatic glycogen ($\Delta = 5$ mg) than Cobb 500 chicks. It can be concluded that, Cobb 500 and Ross 308 differ in egg nutrient availability and have different trajectories for embryonic development and nutrient metabolism during incubation.

Key words: broiler strains, nutrient availability, embryonic development, energy utilization, heat production

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INTRODUCTION

Modern broiler strains are genetically selected for improved broiler live and processing performance traits (Zuidhof et al., 2014). Among the commercial available broiler strains, Cobb 500 and Ross 308 are most commonly used. Although the selection criteria for Cobb 500 and Ross 308 will be very similar, the offspring of those strains have shown some differences in the growth trajectory to achieve strain specific target performances (Marcato et al., 2008; Tona et al., 2010). To achieve certain growth trajectories, Tullet and Burton (1983) demonstrated that the growth rate of chickens during the post hatch period can be altered by changing growth patterns throughout their whole developmental period, including the embryonic stage. This suggests that genetic selection of current modern broiler strains Cobb 500 and Ross 308 might not only have changed broiler growth pattern during the post hatch period, but also during the incubation period.

During incubation, embryos rely solely on the nutritional supply from albumen and yolk in the egg. The nutrient reserves of the egg are metabolized with the exchange of oxygen and carbon dioxide through the eggshell and metabolic heat is produced. The combination of heat from the incubation environment and embryonic heat production (**HP**) can influence embryo temperature, reflected as eggshell temperature (**EST**; Meijerhof and Van Beek, 1993). The EST can subsequently affect embryonic development, nutrient utilization, chick quality, and broiler performance (French, 1997; Lourens et al., 2005; Molenaar et al., 2010a; Molenaar et al., 2011). Recently, Nangsuay et al. (2013) demonstrated that embryonic HP increased with an increase of energy utilization. The same authors proposed that the energy utilization of the embryos was influenced by yolk size and a result of a higher use of

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nutrients from the yolk. Although several studies have shown that albumen and yolk are altered by the genetic background of the hen (O'Dea et al., 2004; Joseph and Moran, 2005; Wolanski et al., 2007), there is no study investigating the differences of egg nutrient availability in the eggs of the major broiler strains of Cobb 500 and Ross 308.

Earlier studies have been conducted to investigate the influence of broiler strain on embryonic development and HP. Hamidu et al. (2007) reported that embryos of Cobb 500 had a higher HP than Ross 308 embryos at incubation day (**E**) 19. The same authors reported a higher HP for Ross 308 embryos than for Cobb 500 embryos at E7, 16, 17, and 18. By using acoustic resonance as a measure for embryonic development, Tona et al. (2010) reported a faster development of Cobb embryos during E4 to E5, whereas Ross embryos developed faster in the second week of incubation. The same authors reported a similar HP of Cobb and Ross strains between 430 to 478 h of incubation. Because these studies were conducted by incubating eggs at the same machine temperature, it can be speculated that HP differences between strains have resulted in differences in EST which in turn affected relative growth rates. While differences in embryonic development between Cobb and Ross strains have been indicated by Tona et al. (2010), there is no study about the influence of broiler strain on nutrient metabolism in relation to embryonic development, which merits further investigation.

The objective of this study was to examine the influence of broiler strain (Cobb 500 vs. Ross 308) on the availability of nutrients, embryonic development, and nutrient metabolism during incubation. Because differences in embryonic development and nutrient metabolism can affect the metabolic status of the embryos, analyses of hepatic glycogen and some metabolic blood variables (glucose, lactate, and uric acid) are included in this study.

MATERIAL AND METHODS

Experimental Design

The experiment was performed in 2 successive batches using hatching eggs of the same egg weight range and breeder flock age of Cobb 500 and Ross 308. Eggs of each strains were incubated separately at an EST of 37.8°C, which has been shown to give the optimal embryonic development for broiler embryos (Lourens et al., 2005). The experimental protocol was approved by the Animal Care and Use Committee of Wageningen University, the Netherlands.

Hatching Egg, Incubation and Determination of Hatching Events

Two batches of in total 480 Ross 308 and Cobb 500 fast feathering hatching eggs from parent stock aging 43 to 46 weeks were obtained from a commercial hatchery. Both broiler breeder strains received commercially available diets. For Cobb 500 the diets contained on average 14.2% CP, 4.5% crude fat, 2,830 kcal/kg ME, and 1.7% linoleic acid and for Ross 308, the diets contained on average 14.3% CP, 4.9% crude fat, 2,900 kcal/kg ME, and 1.8% linoleic acid. Storage duration of the selected eggs was 4 to 5 days. For each batch, a total of 240 eggs (120 eggs of each breeder strain) were selected within an egg weight range of 60 to 63 g. Per batch, 100 eggs of each breeder strain were incubated in 1 of 2 identical small open-circuit climate respiration chambers (**CRC**; Lourens et al., 2006) at a constant EST of 37.8°C and relative humidity of 55%. The remaining 20 eggs per breeder strain were used to determine egg composition.

During incubation, 5 eggs per CRC were equipped with a temperature sensor attached to the equator of the egg, using heat conducting paste and tape, as described by Lourens et al. (2006). The EST was measured every minute, and according to the median EST of the 5 eggs, the CRC temperature was adjusted to maintain EST at 37.8°C. Eggs were candled E11 and E18, and infertile eggs and eggs containing dead embryos were removed. At E18, the CRC temperature, which corresponded with the EST of 37.8°C, was recorded before removal of the EST sensors from the eggs. Fertile eggs were reweighed to determine egg weight loss (**EWL**), transferred to hatching baskets, and placed back in the same CRC. Temperature of the CRC was fixed at the temperature measured before egg transfer at 37.1°C for both strains. The EST after egg transfer until hatching time was allowed to change. Eggs were candled and checked every 3 h from 457 h of incubation onwards to determine the moment of internal pipping (**IP**), external pipping (**EP**), and hatching. The eggshell conductance was calculated as $EWL/\Delta P_{H2O}$ according to Meijerhof and Van Beek (1993); where EWL (mg/h) = EWL at E18/(18 × 24) and ΔP_{H2O} (kPa) = average vapour pressure deficit during E0 to E18.

Heat Production (HP)

To determine HP, oxygen and carbon dioxide concentrations were measured every 9 min in both CRC and in fresh air. Oxygen concentration was measured with a paramagnetic oxygen analyser (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). Carbon dioxide concentration was measured with a non-dispersive infrared CO_2 analyser (type Uras 3G, Hartmann and Braun, Frankfurt, Germany). The refreshed air volume was 5 L/min throughout the incubation period. The exact air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, The Netherlands). Clear eggs from candling at E11 and E18 and dead in shell at hatch were opened to determine true fertility and timing of embryonic mortality as described by Lourens et al. (2006). The HP was calculated from oxygen consumption and carbon dioxide

production (Romijn and Lokhorst, 1961) and adjusted for fertility and day of embryo mortality.

Hatching Egg, Embryo and Chick Measurements

A total of 20 eggs per batch per breeder strain were boiled for 10 min and albumen and yolk for each egg were separated and weighed. Eggshell thickness (without membrane) of 3 regions of the egg, top (blunt end), middle, and bottom (pointed end) was measured, using a digital micrometer (Mitutoyo Corporation, Tokyo). The eggshell without the membrane was dried for 24 h at room temperature and weighed. Albumen weight was calculated as egg weight - yolk weight - shell weight.

Ten fertile eggs per breeder strain per batch were sampled during incubation at E11, E14, and E18. Eggs were opened and yolk free body mass (**YFBM**) was obtained at E11 and E14 and the YFBM and residual yolk (**RSY**) were obtained at E18. At 3 h after hatch (emergence from the egg shell), approximately 50% of the hatchlings were sampled and weighed. From the sampled chicks, chick length was determined by stretching chicks along a ruler and taking the length between the top of the beak and the tip of the middle toe of the right foot (Hill, 2001). Thereafter, chicks were decapitated, blood was collected and the liver was removed, weighed, and immediately stored in liquid nitrogen. The RSY and heart were removed and weighed and the YFBM was calculated by subtracting RSY weight from chick weigh. The RSY and YFBM were stored at -20°C for further analysis.

Blood Metabolites and Hepatic Glycogen Determination

After decapitation, blood was collected in a 4 mL blood tube containing 10 mg of sodium fluoride and 8 mg of potassium oxalate (BD Vacutainer, Franklin Lakes, NJ). An extra droplet (0.02 mL) of 10% heparin was added and mixed into the tube before sampling. Blood was centrifuged at 2,000 × g for 10 min at room temperature, and plasma was decanted and stored at -20°C until further analysis. Plasma glucose, lactate, and uric acid concentrations were determined with a commercially available kit (DiaSys Diagnostic Systems International, Holzheim, Germany).

Determination of hepatic glycogen was carried out on ice as described by Molenaar et al. (2010b). A liver sample of 250 to 300 mg was taken and 1 μL of 7% HCLO4 was added per mg of wet tissue. Thereafter, the liver was homogenized and centrifuged (3,500 × g) at 4°C for 15 min. The supernatant was decanted, cleaned with 1 mL of petroleum ether, and frozen at −80°C until further analysis. Hepatic glycogen was determined by an iodine binding assay (Dreiling et al., 1987), and hepatic bovine glycogen (Type IX, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as a standard.

Chemical Analysis and Nutrient Utilization

To have a sufficient sample for chemical analyses, albumen and yolk samples were pooled from 2 fresh eggs and RSY were pooled from 2 chicks, resulting in 10 samples per strain per batch. Ten YFBM samples per strain per batch were used for analyses. Proximate analyses were performed for dry matter (**DM**; ISO 6496, 1999), crude protein (**CP**; ISO 5983–2, 2005), and crude fat (ISO 6492, ISO, 1999) in albumen and yolk from fresh eggs and RSY and YFBM of chicks at 3 h after hatch. Albumen, yolk, and RSY were freeze dried before analyses of DM, CP, and crude fat. For YFBM analysis, one YFB (without liver) was placed in 150 mL of water and autoclaved for 3 h at 120°C. Thereafter, the YFBM and water suspension was homogenized with an Ultra-Turrax disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) for 10 min and the suspension was used for DM and CP analyses. The remaining suspension was frozen at -20°C and later freeze dried to determine crude fat.

Energy content of protein and fat in albumen and yolk of fresh eggs and RSY and YFBM of chicks at 3 h after hatch was calculated using an energy density for protein and fat of 16.8 and 37.8 MJ/kg of DM (International System of Units, 1998), respectively. Due to a very low amount of carbohydrates in the albumen and yolk of fresh eggs and RSY and YFBM of chicks (Lourens et al., 2006; Molenaar et al., 2010a; Nangsuay et al., 2013) the energy content of carbohydrates was excluded from the calculations.

Energy utilization (kJ; for protein, fat, and protein plus fat) was calculated as;

$$= \text{Albumen(kJ)} + \text{Yolk(kJ)} - \text{RSY(kJ)}$$

Energy lost (kJ) was calculated as;

$$= [\text{Albumen(kJ)} + \text{Yolk(kJ)}] - [\text{YFBM(kJ)} + \text{RSY(kJ)}]$$

Efficiency of converting energy used to form YFBM (EYFB,%; protein, fat, and protein plus fat) was calculated as;

$$E_{YFB} = \frac{YFBM(kJ)}{Albumen(kJ) + Yolk(kJ) - RSY(kJ)} \times 100\%$$

Statistical Analyses

For statistical analyses of DM, CP, crude fat, and energy of albumen, yolk, albumen plus yolk, and RSY, the experimental unit was a combination of samples from 2 eggs or 2 RSY of 2 chicks. Egg or chicken was used as the experimental unit for other variables, except for

Table 1. Egg weight, egg composition, and shell thickness of Cobb 500 and Ross 308 eggs.[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
Egg weight (g)	62.58	62.17	0.20	0.152
Yolk weight (g)	19.60	20.51	0.16	<0.001
Albumen weight (g)	37.30	36.56	0.22	0.020
Yolk: Albumen	0.53	0.56	0.01	<0.001
Shell weight (g)[2]	5.68	5.10	0.07	<0.001
Shell thickness (mm)[2]	0.38	0.33	0.01	<0.001

[1]For all variables measured, n = 40 per strain.

[2]Without membranes.

the HP, where the CRC was used as the experimental unit. Distributions of the means and residuals were examined to verify model assumptions. HP per day from E1 to E18 was analyzed using the MIXED procedure of SAS 9.2 software package (SAS Institute, 2009) for repeated measurements. The model used was $Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times C_k) + e_{ijkl}$, where Y_{ijkl} is the HP, μ is the overall mean, A_i is the strain (i = Cobb 500 or Ross 308), B_j is the batch (j = 1 or 2) and C_k is the incubation day (k = E1 to E18), $A_i \times C_k$ is the interaction between the strain and incubation day and e_{ijkl} is the error term. All other variables were analyzed with the GLM procedure of SAS 9.2 software package (SAS Institute, 2009). Strain, batch, and their interaction were included as class variables. The interaction between strain and batch was not statistically significant ($P > 0.05$) in any of the analyses and therefore this interaction was excluded from the model. Least square means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as LS means. In all cases, a difference was considered significant at $P \leq 0.05$.

RESULTS

Egg Compositions

At a similar average egg weight, Ross 308 eggs had a higher ratio of yolk:albumen ($P < 0.001$) with a 0.9 g more yolk ($P < 0.001$) and 0.7 g less albumen ($P = 0.020$) than Cobb 500 eggs (Table 1). Ross 308 eggs had 0.5 g lower shell weight and 0.05 mm thinner shells (both $P < 0.001$) than Cobb 500 eggs.

Nutrient and Energy Content in the Eggs and Chicks

Albumen of both strains had a similar amount of DM and CP, whereas the amount of fat in the albumen was higher in Ross 308 eggs ($\Delta = 0.005$ g; $P < 0.001$) than Cobb 500 eggs (Table 2). The yolk of Ross 308 eggs had a higher amount of DM ($\Delta = 0.30$ g; $P = 0.018$) and crude fat ($\Delta = 0.24$ g; $P = 0.009$) than that of Cobb 500 eggs, but the amount of CP did not differ between strains. Albumen plus yolk of Ross 308 had a higher DM ($\Delta = 0.24$ g; $P = 0.040$) and crude fat ($\Delta = 0.23$ g; $P = 0.008$) than that of Cobb 500, whereas the amount of CP was similar. The RSY of Ross 308 and Cobb 500 chicks at 3 h after hatch contained a similar amount of DM, CP, and crude fat. The YFBM of Ross 308 and Cobb 500 chicks at 3 h after hatch did not differ in the amount of DM and CP, but the amount of fat was higher ($\Delta = 0.34$ g; $P < 0.001$) in the YFBM of Cobb 500 chicks than of Ross 308 chicks. The RSY plud YFBM had a similar amount of DM and CP, but the amount of crude fat was higher in Cobb 500 than Ross 308 ($\Delta = 0.44$ g; $P < 0.001$).

Table 2. Dry matter (DM), crude protein (CP), and crude fat in albumen, yolk, and albumen plus yolk of fresh eggs and RSY, YFBM, and RSY plus YFBM of Cobb 500 and Ross 308 chicks at 3 h after hatch (g).[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
Albumen				
DM	4.44	4.38	0.04	0.307
CP	4.03	3.95	0.04	0.191
Crude fat	0.01	0.02	0.00	<0.001
Yolk				
DM	9.38	9.68	0.09	0.018
CP	2.99	3.06	0.03	0.102
Crude fat	5.79	6.03	0.06	0.009
Albumen + yolk				
DM	13.82	14.06	0.08	0.040
CP	7.02	7.01	0.04	0.928
Crude fat	5.80	6.05	0.06	0.008
RSY				
DM	3.29	3.10	0.07	0.068
CP	1.53	1.45	0.04	0.118
Crude fat	1.323	1.265	0.042	0.334
YFBM[2]				
DM	7.15	7.18	0.08	0.762
CP	4.84	4.88	0.05	0.600
Crude fat	2.19	1.85	0.05	<0.001
RSY + YFBM				
DM	10.44	10.28	0.10	0.225
CP	6.37	6.31	0.05	0.40
Crude fat	3.51	3.07	0.07	<0.001

[1]For variables of albumen, yolk, and albumen plus yolk, n = 20 per strain. For variables of RSY, YFBM, and RSY plus YFBM, n = 30 per strain.

[2]YFBM without liver.

Albumen from both strains had a similar amount of energy from CP, whereas energy from crude fat was higher in albumen of Ross 308 than that of Cobb 500 ($\Delta = 0.2$ kJ; P<0.001; Table 3). Albumen of both strains had a similar amount of energy from CP plus crude fat. Yolk of both strains had a similar amount of energy from CP. The amount of energy from crude fat ($\Delta = 8.76$ kJ; P = 0.009), and CP plud crude fat ($\Delta = 9.98$ kJ; P = 0.010) was higher in the yolk of Ross 308 than that of Cobb 500. The albumen plus yolk of Ross 308 eggs had a higher amount of energy from crude fat ($\Delta = 8.96$ kJ; P = 0.008), and CP + crude fat ($\Delta = 8.88$ kJ; P = 0.014) than that of Cobb 500 eggs.

At 3 h after hatch, RSY of Cobb 500 chicks had a higher amount of energy from CP ($\Delta = 2.46$ kJ; $P = 0.029$) than that of Ross 308 chicks, whereas the amount of energy from crude fat and CP plus crude fat did not differ. YFBM of both strains had a

Table 3. Energy content (kJ) of Cobb 500 and Ross 308 hatching eggs (albumen, yolk, albumen plus yolk) and chicks at 3 h after hatch (RSY, YFBM, and RSY plus YFBM).[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
Albumen				
CP	67.74	66.44	0.69	0.191
Crude fat	0.50	0.70	0.31	<0.001
CP + crude fat	68.24	67.14	0.69	0.268
Yolk				
CP	50.27	51.49	0.51	0.102
Crude fat	219.03	227.79	2.24	0.009
CP +crude fat	269.30	279.28	2.62	0.010
Albumen + yolk				
CP	118.01	117.93	0.69	0.929
Crude fat	219.53	228.49	2.25	0.008
CP + crude fat	337.54	346.42	2.44	0.014
RSY				
CP	26.37	23.91	0.77	0.029
Crude fat	51.31	48.82	1.97	0.379
CP + crude fat	77.68	72.73	2.49	0.168
YFBM[2]				
CP	82.13	81.89	1.04	0.868
Crude fat	82.52	70.39	2.22	<0.001
CP + crude fat	164.65	152.28	2.80	0.004
RSY + YFBM[2]				
CP	108.50	105.80	0.90	0.040
Crude fat	133.83	119.21	2.96	0.001
CP+ crude fat	242.33	225.01	3.27	0.001

[1]For variables of albumen, yolk, and albumen plus yolk, n = 20 per strain. For variables of RSY, YFBM, and RSY plus YFBM, n = 30 per strain.

[2]YFBM without liver.

similar amount of energy from CP. YFBM of Cobb 500 chicks had a higher amount of energy from crude fat (Δ = 12.13 kJ; $P < 0.001$), and CP plus crude fat (Δ = 12.37 kJ; $P = 0.004$) than that of Ross 308 chicks. A combination of RSY plus YFBM of Cobb 500 chicks at 3 h after hatch contained a higher amount of energy from CP (Δ = 2.7 kJ; $P = 0.040$), crude fat (Δ = 14.62 kJ; $P = 0.001$), and CP plus crude fat (Δ = 17.32 kJ; $P = 0.001$) than that of Ross 308 chicks.

Developmental and Physiological Status

The weight of YFBM of embryos at E11, E14, and E18 and total chick weight at 3 h after hatch did not differ between strains (Table 4). At 3 h after hatch, Ross 308 chicks were 0.6 g heavier in YFBM ($P = 0.001$) and 0.2 cm longer ($P = 0.003$) than Cobb 500 chicks. RSY at E18 did not differ between strains, but RSY weight of chicks at 3 h after hatch tended to be higher in Cobb 500 chicks than in Ross 308 chicks (Δ = 0.37g; $P = 0.06$).

Weight loss from onset of incubation to E18 ($P = 0.071$) and the eggshell conductance ($P = 0.057$) tended to be higher in Ross 308 than in Cobb 500, whereas the moment of IP did not differ between strains (Table 5). External pipping occurred at approximately 4 h earlier ($P = 0.010$) in Cobb 500 than in Ross 308 chicks. Cobb 500 chicks hatched approximately 4 h earlier ($P < 0.001$) than Ross 308 chicks. At 3 h after hatch,

Table 4. Yolk free body mass (YFBM) at incubation day (E)11, E14, E18, and at 3 h after hatch, chick weight, chick length, heart weight, heart/YFBM, liver weigh, and liver/YFBM at 3 h after hatch and residual yolk (RSY) weight at E18 and at 3 h after hatch of Cobb 500 and Ross 308.[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
YFBM (g)				
E11	4.67	4.58	0.10	0.490
E14	13.76	13.43	0.29	0.434
E18	30.02	30.64	0.26	0.099
RSY (g)				
E18	13.17	13.13	0.29	0.923
3 h after hatch	6.63	6.26	0.14	0.060
3 h after hatch				
YFBM (g)	38.87	39.47	0.160	0.001
Chick wt. (g)	45.51	45.76	0.16	0.277
Chick length (cm)	19.29	19.49	0.04	0.003
Heart wt. (g)	0.34	0.36	0.006	0.073
Heart/ YFBM (%)	0.89	0.91	0.02	0.265
Liver wt. (g)	0.86	0.89	0.12	0.069
Liver/ YFBM (%)	2.22	2.27	0.03	0.281

[1]For all variables measured at E11, E14, and E18, n = 20 per strain. All variables measured at 3 h after hatch, n = 60 per strain.

heart, and liver weights in absolute values and relative to YFBM did not differ between strains.

At 3 h after hatch, hepatic glycogen concentration (Δ = 6.3 mg/g, $P < 0.001$) and total hepatic glycogen (Δ = 4.9 mmol/L, $P = 0.002$) were higher in Cobb 500 than in Ross 308 chicks (Table 6). The levels of glucose, lactate, and uric acid in blood plasma did not differ between strains.

Nutrient Metabolism

The total amount of energy from CP plus crude fat used by Ross 308 embryos was higher than for Cobb 500 embryos (Table 7; Δ = 13.85 kJ; $P = 0.013$). The amount of energy lost was higher in Ross 308 than in Cobb 500 (Δ = 26.21 kJ; $P < 0.001$). The E_{YFB} for energy from CP did not differ between strains. The E_{YFB} for energy from crude fat (Δ = 9.57 kJ) and for CP plus crude fat (Δ = 7.59 kJ) was higher in Cobb 500 than in Ross 308 (both $P < 0.001$). Heat production of Cobb 500 and Ross 308 embryos from E1 to E18 did not differ (Figure 1).

DISCUSSION

The objective of this study was to examine influences of broiler strain on nutrient availability, embryonic development, and nutrient metabolism during incubation. To minimize confounding effects, Cobb 500 and Ross 308 eggs were obtained from the same breeder age and at the same egg weight range. According to standard breeder performance, an average egg during 43 to 46 weeks is weighing at 65.1 to 66.3 g for Cobb 500 fast feathering and 64.8 to 65.8 g for Ross 308. This means that selected eggs at weight range of 60 to 63 g were in a similar egg weight distribution range of both strains. Eggs of both strains were incubated at the same EST of

Table 5. Weight loss and eggshell conductance at E18 and hatching events of Cobb 500 and Ross 308 eggs.[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
Weight loss E18 (%)	9.78	10.08	0.12	0.071
Eggshell conductance (mg/h/kPa)	4.34	4.48	0.05	0.057
IP (h)	466.43	466.65	0.61	0.798
EP (h)	481.79	486.02	0.88	0.010
Hatch (h)	488.58	492.82	0.72	<0.001

[1]For weight loss and eggshell conductance at E18, n = 141 for Cobb 500 and 144 for Ross 308. For variable of hatching events, n = 123 for Cobb 500 and 124 for Ross 308.

Table 6. Total hepatic glycogen (mg), hepatic glycogen concentration (mg/g), glucose, lactate, and uric acid in plasma (mmol/L) of Cobb 500 and Ross 308 chicks at 3 h after hatch.[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
Total hepatic glycogen (mg)	15.85	10.89	1.08	0.002
Hepatic glycogen concentration (mg/g)	18.11	11.81	1.12	<0.001
Glucose (mmol/L)	9.90	10.14	0.15	0.259
Lactate (mmol/L)	3.12	3.14	0.13	0.918
Uric acid (mmol/L)	0.18	0.17	0.01	0.358

[1]For all variables measured, n = 60 per strain.

Table 7. Energy utilization (kJ), energy lost (kJ), and E_{YFB} (%) of Cobb 500 and Ross 308 chicks at 3 h after hatch.[1]

Variable	Cobb 500	Ross 308	SEM	*P*-value
Energy utilization				
CP	91.63	94.01	1.09	0.131
Crude fat	168.21	179.67	3.21	0.16
CP + crude fat	259.84	273.69	3.75	0.013
Energy lost	95.19	121.40	4.11	<0.001
E_{YFB}				
CP	89.71	87.26	1.12	0.130
Crude fat	49.18	39.61	1.49	<0.001
CP + crude fat	63.47	55.88	1.16	<0.001

[1]For all variables measured, n = 20 per strain.

37.8°C, which is shown to give optimal embryonic development (Lourens et al., 2005). The results showed that Cobb 500 and Ross 308 differed in nutrient availability, embryonic development, and nutrient metabolism during incubation.

Although eggs were selected at the same egg weight range, the eggs of Ross 308 had a higher energy content compared to Cobb 500. This can be explained by a higher ratio of yolk:albumen in Ross 308 than in Cobb 500 eggs. Egg yolk functions as the main energy source providing approximately 90% of the total energy requirement of an embryo (Noble and Cocchi, 1990), whereas albumen has limited contribution to egg energy availability due to a low DM content and the almost absence of fat (Ar et al., 1987; Nangsuay et al.,

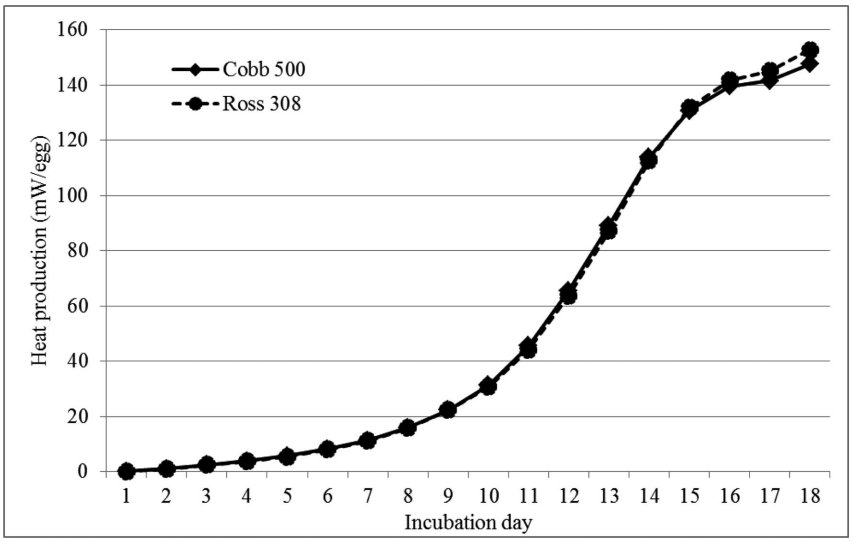

Figure 1. Heat production (HP; mW/egg) of Cobb 500 and Ross 308 embryos from E1 to E18 (strain × incubation day $P = 0.469$).

2013). Similar to the results of Nangsuay et al. (2013), the present results indicate that the amount of energy in the eggs was determined mainly by the amount of energy in the yolk. The albumen weight was higher in Cobb 500 than in Ross 308, but the amount of energy in the albumen was similar. This was due to a similar amount of DM and CP content in the albumen of both strains. A one gram heavier yolk weight accompanied with a higher amount of DM and crude fat resulted in a higher amount of energy in the yolk of Ross 308 than Cobb 500 eggs. The amount of CP and energy derived from CP in the yolk did not differ between strains. In total, Ross 308 eggs had a higher DM and crude fat and thus a higher energy content than Cobb 500. It can be questioned if the commercial diets for both breeder strains have affected the egg compositions and egg energy content. Peebles et al. (2000) demonstrated that there is no effect of the diets containing ME in the ranges at 2,709 to 2,940 kcal/kg and crude fat at 2.26 to 5.28% on the yolk:albumen ratio of broiler breeder eggs of 31 to 47 weeks old hens. Since differences of commercial diets provided for breeders in the current study were minimal, it can be assumed that the nutrient availability for embryos at the start of incubation is influenced by broiler strains. However, we did not know the actual energy intake of the breeders which might have an influence on yolk:albumen ratio (Peebles et al., 2000).

During incubation, Cobb 500 and Ross 308 embryos showed differences in developmental pattern especially at the last stage of incubation. The YFBM at E11 and E14 did not differ, but at E18 Ross 308 embryos tended to have a heavier YFBM than Cobb 500 embryos. This might be caused by differences in growth rate between E14 and E18, where Ross 308 grew approximately 5% faster than Cobb 500. From E18 to hatch, the increase in YFBM was similar for both strains. However, at hatch Ross 308 chicks had a higher YFBM and a longer chick length than Cobb 500 chicks. Our findings are in agreement with Tona et al. (2010) who reported a faster development of Ross than Cobb strain during the second week of incubation. Although differences in embryonic development in the current study occurred only during the last stage of incubation, the results indicate an influence of genetic background of broiler strains on developmental pattern during embryonic stages.

During the hatching process, the moments of EP and hatching were approximately 4 h earlier in Cobb 500 than in Ross 308, whereas the moment of IP was similar. A tendency of a lower eggshell conductance and egg weight loss at E18 in Cobb 500 than in Ross 308 might have an influence on respiration gas exchanges and consequently the hatching events might be affected. Visschedijk (1968) demonstrated that external pipping will be immediately or within a very short time followed by a sudden sharp rise of the total O_2 consumption and CO_2 production in the air cell. It is possible that after E18 Cobb 500 embryos had to deal with a higher magnitude of insufficient O_2 and an increase of CO_2 than the embryos of Ross 308. A trigger for an early EP and consequently early hatching of Cobb 500 embryos might occur by a limited supply of O_2 in the air cell. In addition, a higher hepatic glycogen in Cobb 500 than in Ross 308 might play a role in hatching process. It could mean that Cobb 500 embryos have more glycogen storage, which can provide a comparatively greater supply of glucose as energy source for a success of hatching in a shorter time. Our findings are in agreement with Tona et al. (2010) who reported approximately 2 h shorter in incubation duration of Cobb than Ross strain.

Cobb 500 and Ross 308 embryos differed in nutrient metabolism during incubation. This was shown in energy utilization, energy lost, and the efficiency of converting energy used to YFBM (E_{YFB}). However, the observed differences in nutrient metabolism did not result in differences in metabolic blood parameters like glucose, lactate, and uric acid at 3 h after hatch. The differences in embryonic development pattern, nutrient availability, and gaseous exchanges might be reasons for the differences in nutrient metabolism. The available egg nutrients are catabolized and used by the embryos for the purposes of synthesis of new body tissues, maintenance of existing tissues and for muscular activity to sustain embryonic development through hatching (Vleck, 1991). Although we did not measure energy used before hatching, an increase in growth rate of Ross 308 embryos during E14 to E18 might have increased the use of energy for these embryos in this period compared to Cobb 500 embryos. As an increase in energy utilization requires availability of nutrients and gaseous exchange, a higher availability of nutrients in the eggs and a better gaseous exchange due to a thinner egg shell and a tendency of higher conductance might permit Ross 308 embryos to utilize more energy than Cobb 500 embryos. An increase in growth rate and therefore a larger embryo in this period might increase the energy utilization for maintenance as well. After E18, the growth rate was similar for both strains, but the energy utilization was increased at hatch for Ross 308 compared to Cobb 500 chicks, which might be explained by a higher energy requirement for maintenance. At E18 and 3 h after hatch, YFBM was approximately 0.6 g higher for Ross 308 than for Cobb 500. To calculate the requirements for maintenance, we used the maintenance requirements for broilers of 435 kJ/kg/day (CVB, 2010), as maintenance requirements for embryos are not known to the author's knowledge. An expression of maintenance requirement based on direct weight of embryos instead of body weight to the power 0.75 as normally is used for broilers, has been proposed by Vleck et al. (1980) and Mortola and Cooney (2008). The calculations suggest that the requirement for maintenance of Ross 308 embryos at E18 and at 3 h after hatch was higher than of Cobb 500 embryos in the same period with approximately 260 to 270 Joule per day or 3 mW. These values are in agreement with the observed differences in HP at E18 between Ross 308 and Cobb 500. This might explain at least part of the higher energy

usage and numerically higher embryonic HP at E18 for Ross 308 compared to Cobb 500.

From the total amount of energy used, Ross 308 embryo had approximately 8% lower E_{YFB} than Cobb 500 embryos. It is possible that the embryos of both strains have different strategies for nutrient metabolism during the period of reaching limitation of gaseous exchanges or plateau stage. Dietz et al. (1998) suggested that during the plateau phase, embryos allocate the available energy in favor for maintenance by decreasing the development, increasing synthesis efficiency, and depressing the formation of glycogen. Cobb 500 embryos had less nutrients available in the eggs than Ross 308 embryos and they might have an earlier limitation of gaseous exchanges during incubation. Tona et al. (2010) demonstrated a similar partial pressure of CO_2 ($_PCO_2$) and O_2 ($_PO_2$) in the air cell at E18 for Cobb and Ross strains, but at the IP stage the $_PO_2$ was lower, whereas $_PCO_2$ was higher in Cobb than in Ross. Under these conditions, Cobb 500 embryos might have to slow down growth rate and a strategy to use the available resources at high synthesis efficiency might take place. On the other hand, Ross 308 embryos had more nutrients available in the eggs and maybe less limitation in gaseous exchanges during the last stage of incubation. As a result, the embryonic development was able to continue. When the plateau stage is reached, Ross 308 embryos need to allocate more nutrients for maintenance due to a bigger YFBM. Furthermore, the prolonged incubation duration and the expanded time between IP and EP, and IP and hatch of 4 h for Ross 308 than for Cobb 500 might lead to a higher energy requirement for hatching activities. A higher energy lost in Ross 308 than in Cobb 500 could to a certain extent be a reflection of the differences in energy used for maintenance and for hatching activities. In agreement with the proposition of Dietz et al. (1998) we found that at 3 h after hatch Ross 308 chicks had a lower E_{YFB} accompanied with a lower glycogen storage than Cobb 500. As hepatic glycogen levels are one of the indicators for energetic status of the hatchling, the differences of glycogen levels might have an influence on the early growth (Uni et al., 2005). The differences of E_{YFB} demonstrated that embryos of different strains differ in converting energy used to YFB, however the significance for the hatchling in later life needs further research.

Our findings for energy utilization are consistent with Nangsuay et al. (2013, 2015) who demonstrated that yolk size and the availability of nutrients in the yolk can influence the amount of energy used by the embryos. The same authors proposed that embryonic HP increases with an increase of energy utilization. Although we did find a significant difference in energy lost, we found only a numerically higher HP in Ross 308 from E16 onwards. Although there was no EST control in the studies of Hamidu et al. (2007) and Tona et al. (2010), the current results of HP show a similar trend as found in those studies.

In conclusion, the current results indicate that genetic background of broiler strains influences embryonic development and nutrient metabolism during incubation. The differences occurred even with an identical EST. Taking into account the influence of genetic background on the results obtained, it can be questioned whether the optimal level of EST will be similar for Ross 308 and Cobb 500.

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