Differences in egg nutrient availability, development, and nutrient metabolism of broiler and layer embryos

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ABSTRACT Selection for production traits of broilers and layers leads to physiological differences, which may already be present during incubation. This study aimed to investigate the influence of strain (broiler vs layer) on egg nutrient availability, embryonic development and nutrient metabolism. A total of 480 eggs with an egg weight range of 62.0 to 64.0 g from Lohmann Brown Lite and Ross 308 breeder flocks of 41 or 42 weeks of age were selected in two batches of 120 eggs per batch per strain. For each batch, 30 eggs per strain were used to determine egg composition, including nutrient and energy content, and 90 eggs per strain were separately incubated in one of two climate respiration chambers at an eggshell temperature of 37.8°C. The results showed that broiler eggs had a higher ratio of yolk: albumen with 2.41 g more yolk and 1.48 g less albumen than layers. The yolk energy content of broiler eggs was 46.32 kJ higher than that of layer eggs, whereas total energy content of broiler eggs was 47.85 kJ higher compared to layer eggs. Yolk-free body mass at incubation day 16 and chick weight and length at hatch were higher in broilers compared to layers. Respiration quotient of broiler embryos was higher than layer embryos during incubation day 8 to incubation day 10. A 0.24 g lower residual yolk at the hatch of broiler embryos than for the layer embryos indicated that broiler embryos used more yolk and had a higher energy utilization and energy deposition in yolk-free body mass. Heat production of broiler embryos was higher than that of layer embryos from incubation day 12 to incubation day 18, but efficiency of converting egg energy used by embryos to form yolk-free body mass was similar. In conclusion, broiler and layer embryos have different embryonic development patterns, which affect energy utilization and embryonic heat production. However, the embryos are equal in efficiency of converting the energy used to yolk-free body mass.

Key words: layers and broilers, nutrient availability, embryonic development, energy utilization, heat production

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INTRODUCTION

Broiler and layer strains are selected intensively for different production purposes, the former especially for growth and meat yield, and the latter mainly for egg production. This selection has led to physiological differences between both strains during incubation as well as the post-hatch period (Jones et al., 1986; Muramatsu et al., 1990; Cooke et al., 2003; Ohta et al., 2004; Janke et al., 2004). Cooke et al. (2003) reported that broilers grew faster than layers at all ages during 4 to 20 weeks post-hatch. During incubation, it has been found that broiler and layer embryos show differences in yolk utilization (Sato et al., 2006; Everaert et al., 2008), yolk-free body mass (**YFBM**; Everaert et al., 2008), heat production (**HP**; Janke et al., 2004), and incubation duration (Janke et al., 2004; Everaert et al., 2008).

However, it has to be emphasized that in all the above studies, eggs of layers and broilers were incubated at the same incubator temperature. Lourens et al. (2005) demonstrated that temperature experienced by embryos or eggshell temperature (**EST**) rather than incubator temperature has a significant effect on embryonic development and nutrient utilization, and this was confirmed by Molenaar et al. (2010a). At the same incubator temperature, variation in embryonic HP significantly affects EST (Meijerhof and Van Beek, 1993; French, 1997). It was shown in studies with broiler eggs that embryonic HP during incubation was influenced by the size of the egg (Lourens et al., 2006) and age of the breeder flock (Nangsuay et al., 2013), which is probably the result of a difference in egg composition and, thus, egg energy content. This suggests that embryos of eggs from different origins, which have different egg composition and energy content, could have different EST

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and develop differently when kept at equal incubator temperatures.

At the same breeder age, Everaert et al. (2008) reported that broiler eggs were about 10 g heavier than layer eggs. Ho et al. (2011) showed that the eggs of broilers contained more yolk, and less albumen and eggshell compared to layer eggs. As yolk is the main nutritional source, it is possible that broiler and layer embryos differ in using nutrients for development and growth, which might be reflected in the respiratory quotient (**RQ**; Romanoff, 1967), hepatic glycogen as well as blood plasma levels of metabolites (Molenaar et al., 2011).

Based on this information, we hypothesized that embryos of broilers and layers differ in energy utilization and, consequently, in embryonic HP, even when they are incubated at the same EST. The objective of this study was to investigate the influence of strain, "broilers versus layers," on egg nutrient availability, embryonic development, and nutrient metabolism.

MATERIALS AND METHODS

In two subsequent batches, layer and broiler hatching eggs of the same egg weight range and breeder flock age were incubated at an EST of 37.8°C. This EST was shown to give good embryonic development during incubation of broiler (Lourens et al., 2005) and layer eggs (Molenaar et al., 2010b). To minimize potential confounding factors, such as breeder age, egg size, and EST, eggs of the same breeder age, and egg weight range were incubated separately at the same EST of 37.8°C. The experimental protocol was approved by the Animal Care and Use Committee of Wageningen University, the Netherlands.

Hatching Eggs and Incubation

Good quality hatching eggs weighing 62.0 to 64.0 g from Lohmann Brown Lite and Ross 308 breeder flocks at 41 or 42 wk were obtained from two commercial hatcheries (Verbeek B.V., Lunteren, The Netherlands and Probroed & Sloot, Groenlo, The Netherlands). A total of 480 eggs were obtained in two batches, (120 eggs per batch per strain). For each batch, 30 eggs per strain were used to determine egg composition while the other eggs were stored at 18 to 20°C and a relative humidity of 50 to 60% for 4 or 5 days before incubation. Ninety eggs per strain per batch were incubated separately in one of two climate respiration chambers (**CRC**; Lourens et al., 2006). Five eggs per CRC were equipped with a temperature sensor attached to the equator of the egg with 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 throughout the incubation period to maintain EST at 37.8°C. Because of logistic reasons, egg candling and transfer were performed at incubation day (**E**) 13 and 16, respectively. At E16, the eggshell sensors were removed and the eggs were transferred to hatching baskets. Thereafter, the hatching baskets were returned to the same CRC and remained there throughout the hatching period. The temperature of the CRC was fixed at the last temperature before removal of eggshell sensors, and the EST was allowed to change during the remaining incubation period. Relative humidity was maintained at 50% in both CRCs and the concentration of oxygen (O_2) and carbon dioxide (CO_2) were at normal levels of approximately 20.9 and 0.4%. The O_2 and CO_2 concentrations during E0 to E18 were measured in 9-minute intervals in both chambers and in fresh air. Carbon dioxide concentration was measured with a non-dispersive infrared CO_2 analyzer (type Uras 3G, Hartmann and Braun, Frankfurt, Germany). Oxygen concentration was measured with a paramagnetic oxygen analyzer (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). The exact air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, The Netherlands).

Eggs were candled at E13 of incubation, and infertile eggs and eggs containing dead embryos were removed. Infertile eggs and eggs containing dead embryos were opened and macroscopically inspected for fertility or moment of death (Lourens et al., 2006). Non-hatched eggs were opened as well and evaluated in the same way. The embryonic HP during E0 to E18 was calculated from oxygen consumption and carbon dioxide production according to Romijn and Lokhorst (1961) and adjusted for fertility and day of embryo mortality. The respiratory quotient (**RQ**) during E8 to E18 was calculated as the ratio of carbon dioxide production and oxygen consumption (litre per embryo per day).

Hatching Egg and Hatchling Measurements

A total of 30 fresh eggs per batch per strain were boiled, and albumen and yolk from each egg were separated and weighed. The eggshell was dried for 24 h at room temperature and weighed. Albumen and yolk were stored at −20°C for further analysis.

All eggs were weighed at the start of incubation (E0). At E16 of incubation, eggs were reweighed to determine egg weight loss. Twenty eggs per strain per batch were removed from the CRC at E16. Eggs were opened and the embryo and residual yolk **(RSY)** removed from the egg and weighed. Starting at E19 of incubation, eggs were checked every 3 h for hatching time. The incubation duration was defined as the time between setting of the egg in the incubator and the moment that chicks emerged from the shell. Percentage of hatch of fertile eggs was calculated by using the number of fertile eggs at E16 and the number of hatched chicks.

The hatchlings remained in the CRC for 6 h and were then removed. Chick weight and chick length from the top of the beak to the tip of the middle toe,

excluding the nail (Hill, 2001), were determined. Thereafter, chicks were killed by decapitation, blood was collected, and the liver was removed, weighed, and immediately stored in liquid nitrogen. The RSY weight was determined and YFBM was calculated as chick weight minus RSY weight. The RSY and YFBM were stored at −20°C for further analysis. Heart, stomach (gizzard plus proventriculus), intestine, and Bursa of Fabricius of hatchlings were weighed after defrosting the YFBM in a plastic bag in a water bath at 37°C for 15 min.

Chemical Analysis

To obtain a sufficient number of samples for the chemical analyses, albumen and yolk from 2 eggs were pooled (n = 30 per strain). The YFBM and RSY of chicks at 6 h after hatch were selected equally distributed across the hatching period. The RSY of 2 chicks were pooled to obtain enough amount of samples (n = 30 per strain), and 30 YFBM samples per strain (n = 30) were used for the analyses. Proximate analyses were performed for dry matter (**DM**; ISO 6496, 1999), crude protein (**CP**; ISO 5983-2, 2005), and gross energy (**GE**; ISO 9831, 1998) in albumen and yolk from fresh eggs and RSY and YFBM of chicks at 6 h after hatch. Albumen, yolk, and RSY were dried in a freeze dryer before analyses of DM, CP, and GE. For YFBM analysis, one YFBM (without liver) was placed in 150 mL of water and autoclaved for 2 h at 120°C. Thereafter, YFBM in water 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 then dried in a freeze dryer to determine GE.

Nutrient Utilization

Energy content of protein in albumen and yolk of fresh eggs and RSY and YFBM of chicks at 6 h after hatch was calculated using an energy density for protein of 16.8 MJ/kg of DM (International System of Units, 1998).

Protein-free energy (kJ; albumen, yolk, RSY, YFBM) was calculated by;

$$= \text{Energy content (kJ)} - \text{Protein energy (kJ)}$$

Energy utilization (kJ; total, protein and protein-free) was calculated by;

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

Energy loss (kJ) was calculated by;

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

Efficiency of converting energy used to YFBM ($\mathbf{E_{YFB}}$,%; total, protein and protein-free) was calculated by;

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

Plasma Metabolite and Liver Glycogen Determination

After decapitation of the chicks at 6 h after hatch, 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 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 stored at −20°C for further analyses. Plasma glucose, lactate, and uric acid concentrations were determined with commercially available enzymatic photometric kits (DiaSys Diagnostic Systems International, Holzheim, Germany).

After freezing in liquid nitrogen, livers were stored at −80°C until analysis of hepatic glycogen, as described by Molenaar et al. (2010b). All procedures for hepatic glycogen determination were carried out on ice.

Statistical Analyses

For statistical analyses of DM, CP, and energy variables of albumen, yolk, albumen + yolk, and RSY, each experimental unit was a combination of samples from 2 eggs or 2 RSY of 2 hatchlings. An egg or a chicken was used as the experimental unit for other variables, except for HP and RQ, for which the CRC was used as the experimental unit. Distributions of the means and residuals were examined to verify model assumptions. The HP per day from E0 to E18 and RQ from E8 to E18 were analysed using the MIXED procedure of the 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 or RQ, μ is the overall mean, A_i is the strain (i = broiler or layer), B_j is the batch (j = 1 or 2), C_k is the incubation day (k = E0 to E18 for HP and E8 to E18 for RQ), $A_i \times C_k$ is the interaction between the strain and incubation day and $e_{ijkl,}$ is the error term. Hatching of fertile eggs was analyzed using the logistic regression analysis (PROC LOGISTIC) whereas all other variables were analyzed with the GLM procedure of the SAS 9.2 software package (SAS Institute, 2009). Strain, batch, and their interaction were included as class variables. In none of the analyses was the interaction between strain and batch significant ($P > 0.05$), and thereafter this interaction was excluded from the model. Least square means were compared using Bonferroni adjustments for multiple comparisons. Values

Table 1. Egg compositions of broiler and layer hatching eggs.

Variables[1]	Broiler	Layer	SEM	*P*-Value
Egg wt. (g)	63.17	62.98	0.11	0.23
Yolk wt. (g)	19.52	17.11	0.15	<0.001
Albumen wt. (g)	37.99	39.47	0.15	<0.001
Shell wt. (g)	5.65	6.40	0.06	<0.001
Yolk: Albumen	0.52	0.43	0.01	<0.001

[1]n = 60 per strain.

Table 2. Dry matter (DM: %) and crude protein (CP: as % of DM) in albumen and yolk of fresh eggs and RSY and YFBM of chicks at 6 h after hatch.

Variables[1]	Broiler	Layer	SEM	*P*-Value
Albumen				
DM	12.91	12.32	0.10	<0.001
CP	87.64	87.99	0.16	0.111
Yolk				
DM	48.17	47.16	0.11	<0.001
CP	32.09	32.44	0.14	0.087
RSY				
DM	49.77	47.41	0.27	<0.001
CP	50.33	56.19	0.59	<0.001
YFBM[2]				
DM	19.64	17.44	0.15	<0.001
CP	56.02	48.46	0.85	<0.001

[1]n = 30 per strain.
[2]YFBM without liver.

Table 3. Energy content (kJ) in hatching egg (albumen, yolk, albumen + yolk) and chicks at 6 h after hatch (RSY, YFBM and RSY + YFBM) of broiler and layers.

Variables[1]	Broiler	Layer	SEM	*P*-value
Albumen				
Protein	68.94	68.64	0.61	0.727
Protein-free[3]	38.85	37.63	0.35	0.017
Total	107.79	106.27	0.89	0.232
Yolk				
Protein	50.77	43.98	0.47	<0.001
Protein-free[3]	263.15	223.61	1.98	<0.001
Total	313.91	267.59	2.35	<0.001
Albumen + yolk				
Protein	119.71	112.62	0.63	<0.001
Protein-free[3]	301.99	261.24	1.83	<0.001
Total	421.71	373.86	2.11	<0.001
RSY				
Protein	27.28	29.93	0.70	0.010
Protein-free[3]	59.69	52.67	1.45	0.001
Total	86.97	82.59	1.93	0.115
YFBM[2]				
Protein	83.72	72.49	0.83	<0.001
Protein-free[3]	97.65	84.97	1.65	<0.001
Total	181.37	157.50	1.94	<0.001
RSY + YFBM[2]				
Protein	111.27	102.42	0.96	<0.001
Protein-free[3]	156.57	137.81	2.06	<0.001
Total	267.85	240.09	2.33	<0.001

[1]n = 30 per strain.
[2]YFBM without liver.
[3]Protein-free = total − protein.

are expressed as LS means. In all cases, a difference was considered significant at $P \leq 0.05$.

RESULTS

Egg Compositions

Compositions of broiler and layer eggs are described in Table 1. By selecting eggs with the same egg weight range, broiler, and layer eggs had similar average egg weight. Broiler eggs had a higher ratio of yolk: albumen ($P < 0.001$) with 2.41 g more yolk ($P < 0.001$) and 1.48 g less albumen ($P < 0.001$) than layer eggs. Eggshell weight of broiler eggs was 0.75 g less than that of layer eggs ($P < 0.001$).

Nutrient Content of Eggs, YFBM, and RSY

The percentage of DM was higher in the albumen ($\Delta = 0.59\%$; $P < 0.001$) and yolk ($\Delta = 1.01\%$; $P < 0.001$) of broiler eggs compared to layer eggs (Table 2). The percentage of CP in DM of the albumen and yolk of broiler and layer eggs was similar. The RSY of broiler chicks at 6 h after hatch had a higher percentage of DM ($\Delta = 2.36\%$; $P < 0.001$), but a lower percentage of CP in DM ($\Delta = 5.86\%$; $P < 0.001$) compared to layer chicks. The YFBM of broiler chicks at 6 h after hatch had a higher percentage of DM ($\Delta = 2.20\%$) and CP in DM ($\Delta = 7.56\%$) than that of layer chicks (both $P < 0.001$).

The amount of energy in the albumen and yolk of fresh eggs and RSY and YFBM of chicks at 6 h after hatch is shown in Table 3. The albumen of broiler and layer eggs was similar in the amount of energy from protein, but the amount of protein-free energy was higher in albumen of broilers than layers ($\Delta = 1.22$ kJ; $P = 0.017$). Total amount of energy in albumen of broiler and layer eggs did not differ. The yolk of broiler eggs had a higher amount of energy from protein ($\Delta = 6.79$ kJ) and protein-free energy ($\Delta = 39.54$ kJ) than that of layer eggs (both $P < 0.001$). As a result, the total amount of energy in the yolk of broiler eggs was 46.32 kJ higher than that of layer eggs ($P < 0.001$). The albumen + yolk of broiler eggs contained more energy from protein ($\Delta = 7.09$ kJ) and protein-free energy ($\Delta = 40.75$ kJ) compared to layer eggs (all $P < 0.001$). Consequently, the total amount of energy in albumen + yolk of broiler eggs was 47.85 kJ higher than that of layer eggs ($P < 0.001$).

The RSY of broiler chicks had a lower amount of energy from protein ($\Delta = 2.65$ kJ; $P = 0.010$) but a higher amount of protein-free energy ($\Delta = 7.02$ kJ; $P = 0.001$) compared to the RSY of layer chicks. The total amount of energy in the RSY of broiler and layer chicks did not differ. The YFBM of broiler chicks contained more energy from protein ($\Delta = 11.23$ kJ), protein-free energy ($\Delta = 12.68$ kJ) and total energy content ($\Delta = 23.87$ kJ) than the YFBM of layer chicks (all $P < 0.001$). The RSY + YFBM of broiler chicks contained more energy from protein ($\Delta = 8.85$ kJ), protein-free energy ($\Delta = 18.76$ kJ), and total energy ($\Delta = 27.76$ kJ) than RSY + YFBM of layer chicks (all $P < 0.001$).

Table 4. Yolk-free body mass (YFBM, g) and residual yolk (RSY, g) at E16 and at 6 h after hatch, chick weight (g) and chick length (cm) at 6 h after hatch and incubation duration (h) of broiler and layer chick.

Variables[1]	Broiler	Layer	SEM	*P*-Value
Weight loss E16 (%)	8.15	7.67	0.21	0.113
YFBM (g)				
E16	20.88	16.86	0.27	<0.001
Hatch	40.39	38.77	0.14	<0.001
RSY (g)				
E16	13.22	12.41	0.28	0.046
Hatch	6.14	6.38	0.09	0.046
Chick weight (g)	46.48	45.10	0.12	<0.001
Chick length (cm)	19.42	18.19	0.04	<0.001
Hatch of fertile egg (%)	98.35	95.16	na	0.967
Incubation duration (h)	492.37	498.13	0.88	<0.001

[1]at E16 n = 40 per strain; at hatch n = 119 and 118 for broiler and layer.

Table 5. Internal organ weight in absolute values (g) and relative to YFBM (%) of broiler and layer chick at 6 h after hatch.

Variables[1]	Broiler	Layer	SEM	*P*-Value
Heart wt. (g)	0.267	0.223	0.003	<0.001
Heart: YFBM (%)	0.661	0.574	0.009	<0.001
Liver wt. (g)	0.906	0.816	0.009	<0.001
Liver: YFBM (%)	2.246	2.107	0.023	<0.001
Stomach wt. (g)	2.289	2.202	0.018	0.001
Stomach: YFBM (%)	5.669	5.687	0.045	0.777
Intestinal wt. (g)	1.692	1.285	0.020	<0.001
Intestinal: YFBM (%)	4.189	3.317	0.049	<0.001
Bursa (g)	0.040	0.036	0.001	0.018
Bursa: YFBM (%)	0.100	0.093	0.003	0.152

[1]n = 119 and 118 for broiler and layer.

Developmental and Physiological Status

Egg weight loss at E16 and percentage of hatch of fertile eggs did not differ between broiler and layer chicks (Table 4). The YFBM of broiler embryos at E16 and chicks at 6 h after hatch were 4.02 and 1.62 g heavier, respectively, than those of layer embryos (both $P < 0.001$). At 6 h after hatch, broiler chicks were 1.4 g heavier and 1.2 cm longer than layer chicks (both $P < 0.001$). The RSY weight of broiler embryos at E16 was 0.81 g higher ($P = 0.046$) than that of layer embryos, but broiler chicks at 6 h after hatch had 0.24 g less RSY ($P = 0.046$) than layer chicks. Broiler chicks hatched 5.76 h earlier than layer chicks ($P < 0.001$).

Weights in absolute values and as a percentage of YFBM for heart ($\Delta = 0.044$g, 0.087%), liver ($\Delta = 0.090$ g, 0.035%), and intestines ($\Delta = 0.407$ g, 0.872%) were higher in broiler chicks than in layer chicks (Table 5; all $P < 0.001$). Broiler chicks had a heavier stomach ($\Delta = 0.087$ g; $P = 0.001$) and bursa ($\Delta = 0.004$ g; $P = 0.018$) than layer chicks, but these two values did not differ as a percentage of YFBM.

The hepatic glycogen at 6 h after hatch was higher in layer chicks than in broiler chicks ($\Delta = 12.75$ mg/g; $P < 0.001$; Table 6). The level of lactate, uric acid, and glucose did not differ between broiler and layer chicks.

Table 6. Hepatic glycogen (mg/mg), lactate, uric acid, and glucose (mmol/L) of broiler and layer chick at 6 h after hatch.

Variables[1]	Broiler	Layer	SEM	*P*-Value
Hepatic glycogen (mg/g)	15.17	27.92	2.18	<0.001
Lactate (mmol/L)	2.27	2.50	0.10	0.119
Uric acid (mmol/L)	0.23	0.27	0.02	0.167
Glucose (mmol/L)	10.94	11.25	0.13	0.090

[1]n = 30 per strain.

Table 7. Energy utilization (kJ), energy lost (kJ), and E_{YFB} (%) of broiler and layer chicks at 6 h after hatch.

Variables[1]	Broiler	Layer	SEM	*P*-Value
Energy utilization				
Protein	92.85	82.69	0.95	<0.001
Protein-free[2]	241.04	208.89	2.32	<0.001
Total	333.89	291.58	2.85	<0.001
Energy lost	153.10	134.08	2.99	<0.001
E_{YFB}				
Protein[3]	90.76	87.93	1.36	0.147
Protein-free[4]	40.18	40.70	0.81	0.651
Total	54.20	54.11	0.72	0.926

[1]n = 30 per strain.

[2]Protein-free = total – protein.

[3]E_{YFB} protein = (protein energy in YFB/ protein energy used by embryos) × 100.

[4]E_{YFB} protein-free = (protein-free energy in YFB/ protein-free energy used by embryos) × 100.

Nutrient Metabolism

Broiler embryos used more energy from protein ($\Delta = 10.11$ kJ) and protein-free energy ($\Delta = 32.15$ kJ) compared to layer embryos (Table 7; both $P < 0.001$). As a result, total amount of energy used by broiler embryos was 42 kJ higher than that of layer embryos ($P < 0.001$). Energy loss during incubation was 19.02 kJ higher in broiler embryos than in layers ($P = 0.006$). The E_{YFB} for protein energy and protein-free energy, and overall E_{YFB} of broiler and layer embryos did not differ.

Respiration quotient (liter CO2 produced/liter O2 consumed, RQ) during E8 to E10 was higher in broiler embryos than in layer embryos (all $P < 0.05$; Figure 1). The RQ level at E8 was 0.75 for broilers and 0.74 for layer embryos and thereafter RQ of both strains decreased to an equal level of 0.71 at E12. From E12 to E18, RQ was approximately 0.71 for both strains. In both strains, HP increased between E8 and E18 of incubation and reached a plateau stage at about E16 (Figure 2). Broiler embryos had higher HP than layer embryos from E12 to E18 (E12 to E18; $P < 0.05$).

DISCUSSION

This study aimed to investigate the influence of chicken strain, "broilers vs. layers," on egg nutrient availability, embryonic development, and nutrient metabolism during incubation. To eliminate possible confounding effects, hatching eggs were obtained from

Day	8	9	10	11	12	13	14	15	16	17	18
Age	*	*	*	-	-	-	-	-	-	-	-

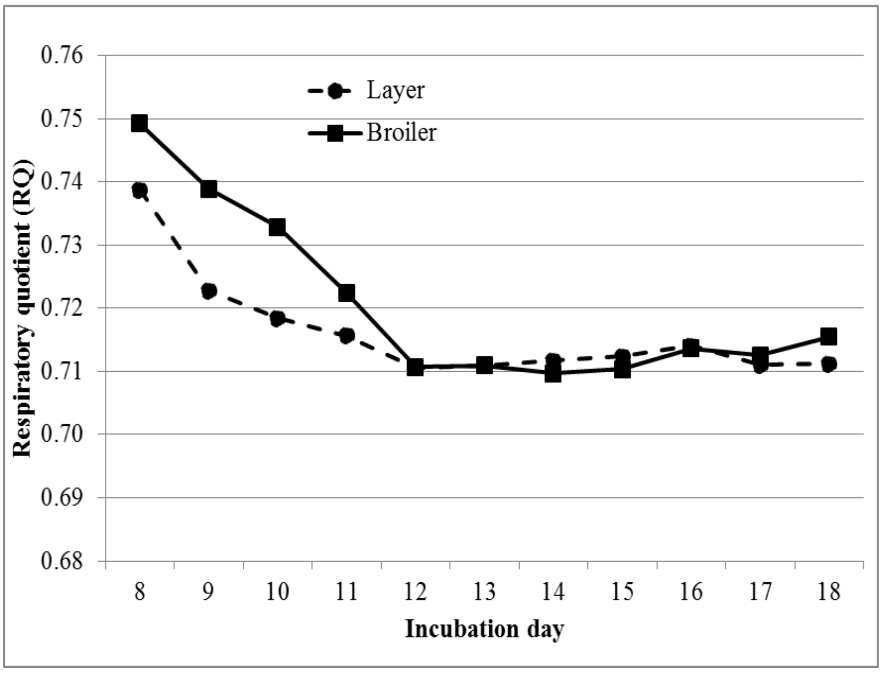

Figure 1. Respiratory quotient (RQ) of broiler and layer from E8 to E18 of incubation. The legend indicates the level of significance ($^{*}P \leq 0.05$).

the same breeder age and egg weight range and incubated at the same EST.

Egg Compositions and Energy Content

The results clearly demonstrate the effect of genetic strain on egg composition and amount of nutrients in the eggs. At similar egg weight, broiler eggs had a higher ratio of yolk: albumen and less eggshell weight than layer eggs. The higher energy content in broiler eggs compared to layer eggs was due to a combination of a higher DM of albumen and yolk and especially a larger yolk size. A major contribution of yolk size to amount of energy in the yolk and, consequently, in the egg is consistent with the study of Nangsuay et al. (2013). The current study indicates that despite an equal percentage of CP in yolk DM, a larger yolk size of 2.4 g led to an approximately 15% higher protein energy in the yolk of broiler eggs compared to layers. Furthermore, an equal amount of energy in the albumen clearly demonstrated that the higher energy content in broiler eggs was due to both protein and protein-free energy in the yolk. Since the yolk contained a negligible amount of energy from carbohydrates (Lourens et al., 2006; Nangsuay et al., 2013), the higher amount of protein-free energy in the yolk probably indicated a higher amount of energy derived from lipid sources. This means that at the start of incubation, broiler eggs had more energy from both protein and lipid sources, which could contribute to a higher growth rate.

Embryonic Development and Nutrient Metabolism

Broiler and layer embryos had a different growth pattern during incubation. Broiler embryos developed and grew faster than layer embryos, as expressed by the embryo development at E16 and chick quality parameters at 6 h after hatch. These results are in agreement with Ohta et al. (2004) who studied embryonic growth in broiler and layer eggs of similar egg weight and reported a higher growth rate of broiler embryos compared to layer embryos at E14 and E19. The current results show that a higher growth rate of broiler compared to layer embryos was accompanied by a higher weight of heart, liver, and intestines in absolute values and relative to YFBM. These findings clearly demonstrate that genetic background has an influence on embryonic development and growth pattern. Moreover, this result indicates that broilers, which have been selected for high growth rate post-hatch, have already developed heavier digestive and supply organs during the embryonic stages.

A higher embryonic growth rate of broilers compared to layers can be related to two possible synergistic effects of strain-specific production traits. These traits are genetic growth potential and egg composition

Day	9	10	11	12	13	14	15	16	17	18
Age	-	-	-	*	*	*	*	*	*	*

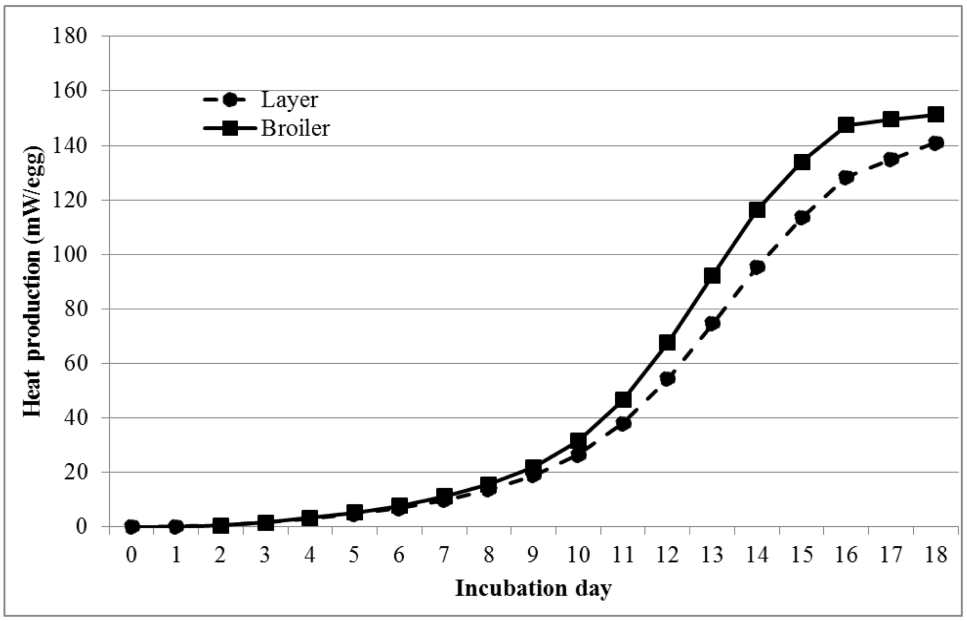

Figure 2. Heat production (HP; mW/egg) of broiler and layer embryo from E0 to E18. The legend indicates the level of significance ($^{*}P \leq 0.05$).

differences. It could be inferred from the current results that the genetic growth potential of the broilers has an influence on growth of developing embryos. To ensure sufficient energy supply for growth, broiler embryos will require more nutrients and oxygen than layer embryos. The differences in nutrients and oxygen requirements might be fulfilled by the egg compositions, especially by the eggshell characteristics and yolk content. The lower shell weight of broiler eggs found in the current study might improve shell conductance (Wolanski et al., 2007) and lead to more oxygen availability. A combination of more oxygen and a higher nutrient availability due to especially yolk size suggests that broiler embryos have more energy stored and, therefore, have more capacity to metabolize. Furthermore, it is possible that a larger yolk size of broiler eggs compared to layer eggs has an influence on yolk absorption capacity (Yadgary et al., 2013) and vitelline vascularization (Adair et al., 1990). This could mean that broiler embryos have a higher capacity to absorb and transport yolk nutrients to embryonic tissues. These synergetic effects of genetic growth potential and egg compositions might be mechanisms underlying a higher embryonic development and growth of broiler embryos compared to layer embryos.

To obtain energy, embryos consume oxygen and produce carbon dioxide and heat (Etches, 1996), and this can be measured. The RQ, which is the ratio of carbon dioxide production to oxygen consumption, can be an indicator of the nutritional sources used for metabolism. Romanoff (1967) related an RQ during early development of the embryos mainly to protein metabolism and to a lesser extent carbohydrate metabolism. Fiske and Boyden (1926) proposed that approximately 96% of the proteins absorbed during the first 13 days of incubation will be retained in embryonic tissues including membranes. It is possible that the higher RQ of broiler embryos compared to layer embryos during E8 to E10 is an indication of higher protein utilization. Although we cannot specify the usages during each embryonic stage, the current results show that broiler embryos use more energy from protein than layer embryos. These findings are in agreement with Ohta et al. (2004) who reported that growth and accumulation of protein in broiler embryos are higher than those in layer embryos.

A similar RQ at 0.71 of broiler and layer embryos from E12 onward indicates a shift to lipid metabolism as a main energy supply of both strains, which is known to occur in the second half of incubation (Romanoff 1967; Noble and Cocchi, 1990). A higher yolk utilization, which is exhibited in less RSY at hatch and higher utilization of protein-free energy in the yolk, may indicate that broiler embryos have used more lipid sources from the eggs than layer embryos. These findings are in agreement with Sato et al. (2006) who reported that broiler embryos absorb yolk as a lipid source and use more lipids than layer embryos. As a result of both energy used from protein and protein-free energy, the total amount of energy used by broiler embryos was higher than that used by layer embryos. These findings indicate that broiler embryos had a higher metabolic rate than layer embryos. A higher energy loss results in more production of metabolic byproducts by broiler embryos

due to a higher metabolic rate, which is reflected in the higher HP from E12 to E18 of broiler embryos compared to layer embryos.

Although broiler and layer embryos differed in nutrient metabolism, they were equal in their efficiency to convert energy used from protein, protein-free energy, and total energy used to YFBM. A higher deposition of energy into YFBM of broiler chicks as a result of more energy used might be a reflection of the genetic difference, creating hatchlings with a higher growth rate in the grow-out period. Higher protein-free energy in the RSY of broiler chicks than in layer chicks suggests that broiler chicks have probably more lipids available post-hatch. However, protein available in the RSY was lower in broilers than layers as indicated in less protein energy. These findings may suggest different available resources for protein and lipids of broiler and layer chicks post-hatch.

The higher growth and metabolic rate of broiler embryos could be the reason for a shorter incubation duration and less glycogen in the liver of broiler chicks at hatch. A higher metabolic rate requires more O_2 consumption and results in a higher CO_2 production. This might drive broiler embryos to reach oxygen availability limitations or to have critically high CO_2 levels in the air cell earlier than layer embryos. Janke et al. (2004) and Everaert et al. (2008) reported that O_2 consumption in broiler embryos was higher than in layer embryos. Everaert et al. (2008) suggested that a higher CO_2 partial pressure ($\mathbf{PCO_2}$) in the air cell caused an early internal pipping for broiler embryos compared to layer embryos. It is possible that a higher PCO_2 in the air cell triggers the hatching process of broiler embryos (Visschedijk, 1968) to start approximately 6 h earlier than layer embryos in the current study. Our finding for incubation duration is similar to that of Janke et al. (2004) who reported that broiler chicks hatched about 24 h earlier than layer chicks. In contrast to our results, the study of Everaert et al. (2008) found no differences in incubation duration of broiler and layer embryos, although the moment of internal and external pipping of broilers was about 5 h earlier than layers. Variation of incubation duration found in these studies might be caused by differences in breeder age, egg weight, or EST of embryos. The results of the current study demonstrate that at the same breeder age, egg weight and EST, the broiler embryos have approximately 6 h shorter incubation duration than layer embryos, probably due to a higher growth rate.

Layer chicks hatched with more hepatic glycogen than broiler chicks, whereas the levels of glucose, lactate, and uric acid did not differ. Glycogen synthesis and depletion can influence differences in hepatic glycogen of the chicks at hatch. Because hatching is a high energy demanding process, which occurs during the period that O_2 availability is limited, glycolysis of glucose becomes the main energy supply to meet the requirements of the embryo (Freeman, 1969). Glycogen depletion from the liver is an important source for glucose glycolysis. A difference in O_2 consumption of broiler and layer embryos might alter the magnitude of O_2 limitation and, as a consequence, the requirement for glucose during hatching process might be different. Everaert et al. (2008) demonstrated that layer embryos had relatively more O_2 availability than broiler embryos, as shown in a higher blood PO_2 at internal and external pipping. This means that during the hatching process, layer embryos require or have less glycogen depletion from the liver than broiler embryos. Moreover, as glycogenic amino acids and glycerol from metabolic processes are major precursors for glycogen synthesis (Sunny and Bequette, 2011; Molenaar et al., 2013), it is possible that a lower nutrient metabolism of layer embryos enables more precursors available for glycogen synthesis.

In conclusion, the genetic background of, "broilers vs. layers," influences embryonic development and nutrient metabolism during incubation. Differences in especially the yolk size lead to a higher energy availability in the broiler eggs than the layer eggs. During incubation, the broiler embryos grew faster and used more energy than the layer embryos. However, broiler and layer embryos were equal in E_{YFB}. As a result of more energy used, broiler embryos deposited more energy into YFBM and had a higher embryonic HP than layer embryos.

For practical implications, the results of the current study indicate that we should be aware of the possible influence of the selection traits on the physiological development of the embryos. Eggs of meat type chickens yield embryos with a higher growth and metabolic rate than the embryos derived from egg type chickens. As a result, these embryos produce different amounts of heat during incubation. This can affect the EST of the embryos and subsequent performance as presented in several studies. To obtain an optimal EST for the embryos, it is important to incorporate the differences of egg origins into the applied incubation temperature profile.

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