PHYSIOLOGY AND REPRODUCTION

Both the rooster line and incubation temperature affect embryonic metabolism and hatchling quality in laying hen crossbreds

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ABSTRACT Effects of 3 eggshell temperatures (EST; 36.7. 37.8, and 38.9°C) in 2 genetic laying hen crossbreds (AB and BB; same hen line, different rooster line) on embryonic metabolism and hatchling quality were investigated. EST were applied from day 14.5 of incubation (E14.5) until hatching. The experiment consisted of 6 consecutive batches with eggs weighing between 59 and 61 g. Heat production was determined continuously from E14.5 onward. In fresh eggs, yolk weight tended to be higher ($\Delta = 0.28$ g; $P = 0.08$) in the AB crossbred than in the BB crossbred. At E14.5 and E18.5, yolk-free body mass (YFBM) and residual yolk (RY) weight did not differ between genetic crossbred and EST. Hatching time after the start of incubation was not affected by genetic crossbred, but was longer in the 36.7°C (517 h) than in the 38.9°C (505 h), with 37.8°C in between (506 h). At 6 h after hatching, no differences between crossbreds were found for chicken quality parameters, such as chicken weight, chicken length, RY, YFBM, and organ weights, but heart weight was higher in the 36.7°C EST than in the other 2 EST ($\Delta = 0.24$ to 0.30% of YFBM, $P = 0.005$). Intestinal weight was higher at 36.7°C EST than at 38.9°C EST ($\Delta = 0.79$% of YFBM; $P = 0.02$), with 37.8°C EST in between. Heat production between E14.5 and E18.5 was higher in the AB crossbred than in the BB crossbred ($\Delta = 2.61$%, $P < 0.001$) and regardless of crossbred higher at an EST of 38.9°C than at other 2 EST ($\Delta = 3.59$% on average; $P < 0.001$). Hatchling quality determined at pulling (E21.5) was not affected by EST, but AB chickens were lighter ($\Delta = 0.46$ g; $P = 0.03$), had less red hocks ($\Delta = 0.03$; $P = 0.02$), more red beaks ($\Delta = 0.10$; $P < 0.001$), and a higher (worse) navel score ($\Delta = 0.11$; $P < 0.001$) than BB chickens. It can be concluded that not only incubation temperature, but also the rooster line appears to play a role in layer crossbred embryo metabolism and hatchling quality.

Key words: incubation temperature, laying hens, embryo development, genetic crossbred, heat production

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INTRODUCTION

During incubation, the main factors driving embryonic metabolism and development are nutrient availability, oxygen availability, and embryo temperature (Nangsuay et al., 2016a). Both nutrient and oxygen availability are expected to be particularly affected by the hen line (O'Sullivan et al., 1991; Nangsuay et al., 2011) and not by the rooster line. However, in wild birds, male attractiveness has been shown to affect egg size, clutch size, and yolk androgen level (Cunningham and Russell, 2000; Rutstein et al., 2004; Uller et al., 2005; Gilbert et al., 2012), which at least suggest that the rooster might play a role in offspring development. Moreover, the roosters' genetic contribution to the offspring may affect embryonic development, which may affect metabolism during incubation. In poultry, the role of the rooster in embryonic development and metabolism is hardly investigated. Christensen et al. (2000) demonstrated that in turkeys the sire family did not influence egg weight and eggshell conductance, but affected body weight (BW), organ development,

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and blood metabolite concentration of the pullets at hatching. Whether a potential role of the rooster is also present for laying hen chickens is to our knowledge unknown. However, this knowledge might be of importance, because egg size, egg composition, and genetic make-up of the chicken determine embryonic metabolic rate and thus heat production (Lourens et al., 2006; Nangsuay et al., 2013). To obtain optimal embryo development, the embryo temperature needs to be constant throughout incubation (Lourens et al., 2005) and thus the incubator temperature needs to be adjusted to the embryonic metabolic heat production. It can be speculated that in case the rooster might affect embryonic development, via affecting egg size, egg composition, or genetic make-up of the embryo, incubator temperature needs to be adjusted to genetic background of the rooster.

The aim of this study was to investigate potential interactive effects of genetic background of the rooster line and incubation temperature on laying hen embryonic metabolism and development and on hatchling quality.

MATERIAL AND METHODS

Experimental Design

The experiment consisted of a 2 × 3 factorial arrangement with 2 laying hen genetic crossbreds (**AB** and **BB**) that originated from the same hen line (B), but different rooster lines (A or B) and 3 eggshell temperatures (**EST**; 36.7, 37.8, and 38.9°C), applied from day 14.5 of incubation onward. Six consecutive batches of eggs were used with both genetic crossbreds in each batch, whereas EST varied among batches. Each genetic crossbred × EST combination was performed twice. The experimental protocol was approved by the Central Committee Animal Experiments, the Hague, the Netherlands (number 2016.W-0081.001).

Hatching Eggs and Storage

A total of 5,760 hatching eggs were collected from 2 different genetic laying hen flocks with the same hen line (B), but different rooster lines (A or B). This resulted in AB and BB laying hen crossbred chickens. The used hens originated from the same flock. Both breeder flocks were housed at one farm and had the same age (51 to 59 wk). All management conditions, including nutrition, were the same for both flocks. Eggs of both flocks were selected within the range of 59 to 61 g for all batches. Per batch, 480 eggs per genetic crossbred were stored for 8 d at 16°C at the hatchery (Hendrix Genetics, Boxmeer, the Netherlands). Thereafter, eggs were transported to the experimental accommodation of Wageningen University. Eggs of both genetic crossbreds were placed in one HT-Combi incubator (HatchTech, Veenendaal, the Netherlands) with a maximum capacity of 1,408 eggs. Eggs of both genetic crossbreds were placed on separate trays, which were alternately placed in the incubator and stored for 2 more days at an incubator temperature of 16°C.

Fresh Egg Composition and Embryonic Development

The day before the start of incubation, 15 eggs per batch per genetic crossbred were randomly sampled to determine egg weight, yolk weight, albumen weight, eggshell weight, and eggshell thickness. Eggs were weighed and then boiled for 10 min. After boiling, eggshell including membranes, and yolk were carefully separated and the yolk was weighed. The eggshell was weighed after drying for a 24 h period at room temperature. Albumen was calculated by subtracting eggshell weight and yolk weight from fresh egg weight. Eggshell thickness was measured with an electronic micrometer (IP54, Helios-Preisser, Grosuplje, Germany) at the top, middle, and blunt end of the egg and expressed as the average of these 3 areas.

Additionally, at the day before the start of incubation, a total of 10 eggs per batch per genetic crossbred were randomly selected to determine the morphological stage of embryonic development, using the method of Eyal-Giladi and Kochav (1976). The filter ring technique, as described by Gupta and Bakst (1993), was used to isolate the embryo from the yolk sac membrane. After isolation, the embryo was flushed with a buffered saline solution to remove yolk residuals. The dorsal and ventral sides of the embryo were examined with a stereo microscope (Olympus SZ61, Olympus Nederland B.V., Zoeterwoude, the Netherlands) to determine the morphological stage of embryonic development.

Incubation Until d 14.5 of Incubation

Just before incubation started, all eggs were weighed individually and numbered. At 5, randomly chosen, individual eggs per incubator, a temperature sensor (Pt-100, Sensor Data BV, Rijswijk, The Netherlands) was placed halfway between the blunt and pointed end to monitor EST. Temperature sensors were attached to the eggshell by using heat-conducting paste (Schaffner Holding AG, Luterbach, Switzerland) and a small piece of tape (Tesa BV, Almere, the Netherlands). Based on the median of the 5 temperature sensors, incubator temperature was adjusted to maintain an EST of 37.8°C until day 14.5 of incubation (**E14.5**). Relative humidity was maintained between 50 and 60%. At E14.5, 15 fertile eggs per batch per genetic crossbred were opened to determine yolk-free body mass (**YFBM**) and residual yolk (RY) weight of each embryo.

Incubation from day 14.5 of Incubation Till Hatching

At E14.5, all eggs were weighed individually to determine egg weight loss and candled thereafter. Clear eggs or eggs containing a dead embryo were opened to

determine fertility or moment of mortality as described by Lourens et al. (2006). After candling, 320 fertile eggs per batch per genetic crossbred were randomly selected. Per genetic crossbred, eggs were divided over 2 climate respiration chambers (**CRC**). Thirty-five eggs were placed in one small CRC (Lourens et al., 2006) for intensive data collection, whereas the other 285 eggs were placed in a medium-sized CRC (Verstegen et al., 1987) for more extensive data collection. Within each CRC, at 4 randomly chosen eggs a Pt-100 temperature sensor was attached as described above to monitor and maintain EST. Within each batch, EST was the same in all 4 CRC, but alternated among batches (36.7°C, 37.8°C, or 38.9°C until hatching). This resulted in 2 repetitions per crossbred × EST combination for both the intensive and extensive data collection.

Intensive Data Collection in the Small CRC

From E14.5 onward, heat production was determined in the 2 small CRC, using indirect calorimetry (intensive part). Concentrations of oxygen and carbon dioxide were determined every 9 min in both CRC and in fresh air. Carbon dioxide concentration was measured with a non-dispersive infrared CO_2 analyzer (type Uras 3 G, Hartmann and Braun, Frankfurt, Germany). Oxygen concentration was measured with a paramagnetic oxygen analyzer (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). Air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, Dordrecht, the Netherlands). The heat production (**HP**) was calculated as: HP (kJ) = 16.18 × O_2 consumption (L) + 5.02 × CO_2 production (L) (Romijn and Lokhorst, 1961) and adjusted for embryonic mortality.

At E18.5, all eggs were candled again and weighed to determine egg weight loss. Eggs containing a dead embryo were opened to determine fertility or moment of mortality as described by Lourens et al. (2006). From E19.5 (468 h of incubation) onward, the moment of hatching was determined each 6 h, in both small CRC. Hatchlings were allowed to dry and sampled 6 h after emergence from the eggshell for body and organ development, hepatic glycogen, and blood metabolite measurements. After completing the hatching process, nonhatched eggs were opened to classify day of embryonic mortality as described by Lourens et al. (2006).

Embryo and Hatchling Measurements

At E18.5, per small CRC per batch per genetic crossbred, 15 fertile eggs were randomly selected. Eggs were opened and embryos were decapitated, where after the YFBM and RY weight were determined. The sex of embryos was determined based on the presence of testes. Subsequently, at 6 h after emergence from the eggshell, hatchlings (n = 15 chickens per crossbred per batch) were weighed and chicken length was determined from the top of the beak to the tip of the middle-toe, excluding the nail (Hill, 2001). Hatchlings were also scored for navel condition and the prevalence of red beaks or red hocks. Navel condition of the hatchlings was evaluated with a score of 0 to 2, where 0 was a closed and clean navel area, 1 a black button up to 2 mm or black string, and 2 a black button, exceeding 2 mm or an open navel area (Molenaar et al., 2010a). Prevalence of red beaks and red hocks was evaluated separately with a score of 0 or 1, where 0 was no redness on beak or hock and 1 was redness on beak or hock, regardless of the amount of redness or intensity of redness. Then hatchlings were decapitated and blood was collected for blood metabolite determination. After bleeding, chickens were opened and livers were immediately dissected, weighed, and frozen in liquid nitrogen. Livers were stored at −80°C until further analysis. The RY was removed and weighed. YFBM was calculated as BW minus RY weight. Heart, proventriculus plus gizzard, and intestines were removed and weighed. All organ weights were expressed as percentage of YFBM. Finally, sex of the hatchlings was determined according to the presence of testes.

Blood Metabolites and Hepatic Glycogen Determination

At 6 h after hatching, blood samples (n = 15 per batch per genetic crossbred) were 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 to the tube and mixed through the tube before sampling. Blood samples were centrifuged at 2000 × g for 10 min at room temperature, where after plasma was decanted and stored at −20°C until further analysis. Plasma glucose, lactate, and uric acid concentrations were determined with commercially available enzymatic photometric kits (DiaSys Diagnostic Systems International, Holzheim, Germany).

The hepatic glycogen determination was performed as described by Molenaar et al. (2010a). All procedures for hepatic glycogen determination were performed on ice (n = 15 per genetic crossbred per batch). After the addition of 1 μL of 7% $HCLO_4$/mg of wet tissue, the liver was mixed and homogenized with a glass stirring spoon. The substance was centrifuged at 2900 × g at 4°C for 15 min. Then the supernatant was decanted, cleaned with 1 mL of petroleum ether and frozen at −80°C until further analysis. The supernatant was defrosted, centrifuged, and decanted again. Hepatic glycogen was determined by the iodine binding assay, using an iodine solution containing saturated $CaCl_2$ and absorbance was measured by using microtiter plate reader at 450 nm (Dreiling et al., 1987) and hepatic bovine glycogen (Type IX, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as a standard.

Extensive Data Collection in Medium Sizes CRC

In the large CRC, at E18.5, all eggs were weighed to determine egg weight loss. The hatching process was not monitored and no embryos or chickens were decapitated and opened for determination of embryo or hatchling development. In these CRC, incubation was stopped at E21.5 and all hatchlings were pulled, counted, and weighed, where after they were scored for navel condition and prevalence of red beak and red hock as described above.

Statistical Analysis

All data were analyzed, using SAS (version 9.4, 2012, Cary, NC, USA). Data were checked for normality for both means and residuals. Ordinal data was analyzed with the Mixed procedure. For data about fresh eggs and eggs and embryos before E14.5, the following model was used: Y = μ + crossbred + e [model 1], where Y = the dependent variable, μ = the overall mean, crossbred = effect of genetic crossbred (AB, BB), e = residual error. Ordinal data after E18.5 was analyzed with the following model: Y = μ + crossbred + EST + crossbred x EST + e [model 2], where Y = the dependent variable, μ = the overall mean, crossbred = effect of genetic crossbred (AB, BB), EST = effect of eggshell temperature (36.7, 37.8, 38.9°C), crossbred × EST = interaction between genetic crossbred and eggshell temperature, e = residual error. EST nested within batch was used as a random effect and egg weight at day 0 was used as a covariate. Preliminary analyses demonstrated no effect of sex at the moment of hatching on any of the determined variables ($P > 0.10$) and consequently sex was not included in the model. Hatchability, navel score, beak score, and hock score were analyzed with the Glimmix procedure, using model 2.

For all analyses till E18.5, the egg or embryo was used as the experimental unit. At hatching and for heat production, the CRC was used as the experimental unit. Data are expressed as LSmeans ± SEM and data are corrected for multiple comparisons, using Bonferroni.

RESULTS

Fresh Eggs

Morphological stage of embryos in fresh eggs according to Eyal-Giladi and Kochav (1976) did not differ between both genetic crossbreds (Table 1). Additionally, egg weight, albumen weight, eggshell weight, and eggshell thickness did not differ between genetic crossbreds. Yolk weight tended to by higher in the AB crossbred compared to the BB crossbred (Δ = 0.28 g; P = 0.08).

Table 1. Effects of genetic laying hen crossbred on egg weight and egg components before incubation.

	Genetic crossbred		SEM	*P*-value
	AB	BB		
n	90	90		
Morphological stage[1]	9.62	9.70	0.09	0.34
Egg weight, g	60.74	60.90	0.65	0.27
Yolk weight, g[2]	15.93	15.65	0.11	0.08
Albumen weight, g[2]	38.87	39.06	0.11	0.21
Shell weight, g[2]	6.02	6.11	0.04	0.17
Shell thickness, mm[2,3]	0.403	0.408	0.019	0.25

[1]According to Eyal-Giladi and Kochav (1976); n = 62 for AB and 64 for BB.
[2]Corrected for egg weight at day 0.
[3]Average of sharp end, equator and blunt end.

Table 2. Effects of genetic laying hen crossbred on egg characteristics and embryonic development at day 14.5 of incubation (E14.5).

	Genetic crossbred		SEM	*P*-value
	AB	BB		
n	91	92		
Egg weight d 0, g	61.28	61.39	0.65	0.56
Egg weight E14.5, g[1]	55.68	55.90	0.14	0.29
Egg weight loss E14.5, g	5.67	5.47	0.14	0.32
Egg weight loss E14.5, %	9.25	8.91	0.24	0.31
Residual yolk weight E14.5, g[1]	13.11	13.11	0.21	0.99
Yolk-free body mass E14.5, g[1]	13.23	12.96	0.31	0.11

[1]Corrected for egg weight at day 0.

Eggs and Embryos at day 14.5 and 18.5 of Incubation (Intensive Part)

At E14.5, when no EST was applied yet, egg weight (loss), YFBM, and RY weight did not differ between genetic crossbreds (Table 2). Embryos used for measurements at E18.5 did not differ in egg weight at E14.5 or E18.5, nor in egg weight loss between E14.5 and E18.5 (Table 3). Furthermore, no interactions or effect of genetic crossbred or EST were found on YFBM or RY weight. No interaction between genetic crossbred and EST was found for heat production between E14.5 and E18.5. Embryos of the AB crossbred produced on average 3.16 mW/egg (2.61%; $P < 0.001$) more heat than embryos of the BB crossbred (Figure 1A). Additionally, embryos incubated at 38.9°C produced more heat than embryos incubated at both other EST (Δ = 4.34 mW/egg on average, 3.59%; $P < 0.001$; Figure 1B).

Hatching and Hatchling Characteristics (Intensive Part)

Eggs of both genetic crossbreds did not differ in hatching time. Eggs exposed to an EST of 38.9°C hatched 12 h earlier than eggs exposed to an EST of 36.7°C (P = 0.03), with eggs exposed to an EST of 37.8°C in between and not different from other 2 EST (Table 4). At 6 h after hatching, chicken weight showed

Table 3. Effects of genetic laying hen crossbred (CB) and eggshell temperature (EST) from day 14.5 of incubation onward on embryo characteristics at day 18.5 of incubation (E18.5; intensive part).

	n	Egg weight d 14.5, g[1]	Egg weight E18.5, g[1]	Egg weight loss E14.5 - 18.5, g	Egg weight loss E14.5 - 18.5%	RY, g[1,2]	YFBM, g[1,2]
CB							
AB	90	55.04	53.62	1.42	2.59	11.12	27.46
BB	89	54.87	53.40	1.47	2.70	11.13	27.47
SEM		0.15	0.17	0.03	0.07	0.31	0.64
EST (°C)							
36.7	60	54.82	53.46	1.35	2.49	10.76	27.25
37.8	60	54.98	53.53	1.47	2.66	11.23	27.13
38.9	59	55.06	53.54	1.51	2.77	11.43	28.01
SEM		0.21	0.22	0.04	0.09	0.51	1.08
CB × EST							
AB × 36.7	30	54.94	53.61	1.33	2.44	10.57	27.63
AB × 37.8	30	54.99	53.55	1.45	2.64	11.10	26.91
AB × 38.9	30	55.19	53.72	1.47	2.68	11.68	27.84
BB × 36.7	30	54.71	53.33	1.37	2.54	10.84	26.86
BB × 37.8	30	54.98	53.61	1.49	2.69	11.36	27.36
BB × 38.9	29	54.93	53.36	1.55	2.86	11.19	28.19
SEM		0.26	0.30	0.06	0.12	0.54	1.11
P-values							
CB		0.35	0.33	0.32	0.27	0.97	0.98
EST		0.73	0.97	0.15	0.21	0.63	0.84
CB × EST		0.83	0.85	0.93	0.86	0.25	0.13

[1]Corrected for egg weight at day 0.
[2]RY = residual yolk; YFBM = yolk-free body mass.

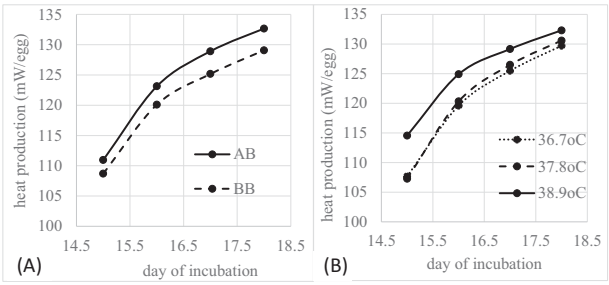

Figure 1. Effects of genetic laying hen crossbred (1A; $P < 0.001$) and eggshell temperature (EST) from day 14.5 of incubation onward (1B; $P < 0.001$) on embryonic heat production between day 14.5 and 18.5 of incubation (intensive part).

an interaction between genetic crossbred and EST, but this effect disappeared after correction for Bonferroni. No other interactions between genetic crossbred and EST were found on chicken characteristics and furthermore, no effects of genetic crossbred were found. An EST of 36.7°C resulted in a lower RY weight (Δ = 0.92 g on average; $P = 0.05$) and higher heart weight ($\Delta = 0.27\%$ of YFBM on average, $P = 0.005$) than other 2 EST. Intestine weight was higher in the 36.7°C EST compared to the 38.9°C EST (Δ = 0.79% of YFBM; $P = 0.02$), with an EST of 37.8°C in between and not different from both other EST.

At 6 h after hatching, the lactate concentration showed an interaction between genetic crossbred and EST (Table 5). In de BB crossbred, no effect of EST was found, but in the AB crossbred lactate concentration was higher in the 36.7°C EST than in both other EST ($\Delta = 1.36$ mmol/l on average; $P = 0.007$). Glycogen, uric acid, and hepatic glycogen did not differ between genetic crossbreds or EST.

Egg Characteristics at E18.5 and Chicken Characteristics at Pulling (Extensive Part)

No interaction between genetic crossbred and EST was found for egg weight at E18.5, egg weight loss between E0 and E18.5, or egg weight loss between E14.5 and E18.5 (Table 6). Additionally, no effect of genetic crossbred was found. Absolute egg weight loss between E14.5 and E18.5 was higher at 37.8 and 38.9°C EST than at 36.7°C (Δ = 0.18 g on average; $P = 0.04$). Relative egg weight loss was higher at 38.9°C than at 36.7°C EST ($\Delta = 0.38\%$; $P = 0.03$), with 37.8°C EST in between and not different from both other EST.

No interaction between genetic crossbred and EST was found on hatchling quality parameters at pulling and neither an effect of EST was found (Table 7). Hatchlings from the AB crossbred were lighter at pulling (Δ = 0.46 g; $P = 0.03$), had less red hocks (Δ = 0.03; $P = 0.02$), more read beaks (Δ = 0.10; $P < 0.001$), and a higher (worse) navel score ($\Delta = 0.11$; $P < 0.001$) than hatchlings from the BB crossbred.

DISCUSSION

This study aimed to investigate potential interactive effects between genetic background of the rooster and incubation temperature on laying hen embryonic

Table 4. Effects of genetic laying hen crossbred (CB) and eggshell temperature (EST) from day 14.5 of incubation onward on hatching time and chicken characteristics at 6 h after hatching[1] (intensive part).

	n	Hatching time, h	Chicken length, cm	Chicken weight, g	RY, g[2]	YFBM, g[2]	Liver, % YFBM[2]	Heart, % YFBM[2]	Stomach, % YFBM[2]	Intestines, % YFBM[2]
CB										
AB	89	509	17.7	42.19	5.92	36.28	2.34	0.83	6.83	4.00
BB	90	510	17.8	42.26	6.07	36.19	2.31	0.83	6.71	4.12
SEM		1	0.1	0.08	0.12	0.11	0.03	0.01	0.11	0.06
EST (°C)										
36.7	60	517[a]	17.8	41.88	5.41[b]	36.47	2.43	1.01[a]	6.95	4.47[a]
37.8	60	506[a,b]	17.6	42.61	6.31[a]	36.30	2.31	0.77[b]	6.64	4.02[a,b]
38.9	59	505[b]	17.7	42.19	6.27[a]	35.92	2.24	0.71[b]	6.72	3.68[b]
SEM		2	0.1	0.17	0.18	0.16	0.04	0.03	0.16	0.09
CB × EST										
AB × 36.7	30	515	17.8	42.04	5.51	36.54	2.44	1.00	6.99	4.36
AB × 37.8	30	507	17.5	42.35	6.06	36.29	2.33	0.75	6.80	3.96
AB × 38.9	29	504	17.6	42.19	6.19	36.00	2.25	0.74	6.69	3.67
BB × 36.7	30	520	17.8	41.73	5.32	36.40	2.41	1.02	6.90	4.57
BB × 37.8	30	505	17.7	42.87	6.56	36.31	2.28	0.79	6.48	4.09
BB × 38.9	30	506	17.8	42.19	6.34	35.84	2.22	0.68	6.76	3.69
SEM		3	0.1	0.18	0.24	0.21	0.05	0.03	0.21	0.12
P-values										
CB		0.26	0.32	0.28	0.47	0.63	0.44	0.91	0.52	0.33
EST		0.03	0.55	0.13	0.05	0.14	0.07	0.005	0.42	0.02
CB × EST		0.26	0.55	0.04[3]	0.44	0.90	0.97	0.19	0.64	0.73

[1]All variables corrected for egg weight at day 0.
[2]RY = residual yolk; YFBM = yolk-free body mass.
[3]No differences among treatments after correction for Bonferroni.
[a,b]LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

Table 5. Effects of genetic laying hen crossbred (CB) and eggshell temperature (EST) from day 14.5 of incubation onward on blood characteristics and hepatic glycogen at 6 h after hatching (intensive part).

	n	Glucose, mmol/l	Lactate, mmol/l	Uric acid, mmol/l	Glycogen, mg/liver
CB					
AB	89	10.52	3.41	0.30	23.15
BB	90	10.46	3.75	0.32	24.76
SEM		0.15	0.16	0.01	0.99
EST (°C)					
36.7	60	10.56	4.14	0.32	26.24
37.8	60	10.49	3.29	0.27	24.79
38.9	59	10.43	3.31	0.34	20.83
SEM		0.25	0.23	0.02	1.28
CB × EST					
AB × 36.7	30	10.46	4.31[a]	0.33	26.16
AB × 37.8	30	10.59	3.09[b,c]	0.27	23.16
AB × 38.9	29	10.52	2.81[c]	0.31	20.12
BB × 36.7	30	10.66	3.97[a,b]	0.31	26.31
BB × 37.8	30	10.39	3.48[a–c]	0.28	26.42
BB × 38.9	30	10.34	3.81[a,b]	0.37	21.55
SEM		0.27	0.27	0.02	1.72
P-values					
CB		0.65	0.05	0.49	0.23
EST		0.94	0.13	0.12	0.12
CB × EST		0.33	0.007	0.06	0.64

[a–c]LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

metabolism and development and on hatchling quality. Results of this study demonstrated that hardly any interaction was found between genetic crossbred of the chicken and incubation temperature, but that both the genetic crossbred and the incubation temperature affect the embryonic metabolism and chicken quality at hatching.

The paternal effect on embryonic metabolism and development in poultry is hardly described in literature. Christensen et al. (2000) demonstrated a clear role of

Table 6. Effects of genetic laying hen crossbred (CB) and eggshell temperature (EST) on egg weight (loss) from day 14.5 of incubation (E14.5) onward (extensive part).

	n	Egg weight E18.5, g[1]	Egg weight loss E0–18.5, g	Egg weight loss E0–18.5, %	Egg weight loss E14.5–18.5, g	Egg weight loss E14.5–18.5, %
CB						
AB	1,905	53.74	7.03	11.57	1.51	2.75
BB	1,903	53.83	6.94	11.42	1.49	2.72
SEM		0.09	0.09	0.15	0.02	0.04
EST (°C)						
36.7	1,278	53.85	6.92	11.51	1.38[b]	2.54[b]
37.8	1,242	53.75	7.01	11.29	1.54[a]	2.73[a,b]
38.9	1,288	53.75	7.01	11.69	1.59[a]	2.92[a]
SEM		0.15	0.14	0.25	0.03	0.05
CB × EST						
AB × 36.7	640	53.84	6.93	11.53	1.37	2.53
AB × 37.8	620	53.72	7.05	11.35	1.56	2.78
AB × 38.9	645	53.66	7.10	11.83	1.59	2.93
BB × 36.7	638	53.86	6.91	11.49	1.38	2.55
BB × 37.8	622	53.79	6.98	11.23	1.51	2.68
BB × 38.9	643	53.85	6.92	11.55	1.58	2.92
SEM		0.15	0.15	0.26	0.04	0.07
P-values						
CB		0.08	0.08	0.11	0.65	0.61
EST		0.87	0.86	0.59	0.04	0.03
CB × EST		0.43	0.42	0.53	0.73	0.70

[1]Corrected for egg weight at day 0.
[a,b]LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

Table 7. Effects of genetic laying hen crossbred (CB) and eggshell temperature (EST) from day 14.5 of incubation onward on chicken characteristics at pulling (extensive part).

	n	Body weight, g[1]	Red hocks[2]	Red beaks[2]	Navel score[3]	Sex[4]
CB						
AB	1,503	41.31[b]	0.49[b]	0.27[a]	1.12[a]	0.50
BB	1,438	41.77[a]	0.52[a]	0.17[b]	1.01[b]	–
SEM		0.23	–	–	–	
EST (°C)						
36.7	933	41.88	0.62	0.33	1.08	0.52
37.8	994	41.89	0.51	0.22	1.06	0.49
38.9	1,014	40.85	0.40	0.11	1.07	0.50
SEM		0.44	–	–	–	–
CB × EST						
AB × 36.7	494	41.72	0.60	0.40	1.13	0.52
AB × 37.8	505	41.71	0.47	0.25	1.13	0.49
AB × 38.9	504	40.51	0.40	0.15	1.12	0.50
BB × 36.7	439	42.05	0.64	0.26	1.01	–
BB × 37.8	489	42.08	0.54	0.19	0.99	–
BB × 38.9	510	41.19	0.41	0.07	1.03	–
SEM		0.44	–	–	–	–
P-values						
CB		0.03	0.02	<0.001	<0.001	–
EST		0.27	0.45	0.07	0.26	0.72
CB × EST		0.34	0.47	0.06	0.83	–

[1]Corrected for average egg weight at day 0.
[2]Score with 0 = absence; 1 = present.
[3]Score with 0 = clean; 1 = small button; 2 = large button; analyzed as binary variable 0 + 1 vs. 2.
[4]Percentage males; only determined in the AB crossbred by feather sexing.
[a,b]LSmeans within a column and factor lacking a common superscript differ ($P \leq 0.05$).

the sire on embryonic turkey organ development and glycogen content in the heart, liver, and pipping muscle. This might suggest that the role of the sire is of more importance for poultry embryonic development and offspring quality than generally is assumed (O'Sullivan et al., 1991; Nangsuay et al., 2011). In wild birds, the role of the male on offspring development is much more investigated than in poultry. Male attractiveness and the role in reproductive investment of the female has been studied in several species, such as the mallard duck (Cunningham and Russell, 2000), zebra finches (Rutstein et al., 2004; Gilbert et al., 2012), and Chinese quails (Uller et al., 2005). Reproductive investment of the female can be reflected in clutch size, egg size, or androgen levels in the yolk (Gil et al., 1999; Horváthová et al., 2012). Whether male attractiveness also influences

egg composition (yolk: albumen ratio) and whether it plays a role in domestic poultry species is to our knowledge not described in literature.

Behavioral observations of the used rooster lines (A or B) in the current study, demonstrated that the A rooster appears to be more actively directed to the female than the B rooster (not quantified). It can be speculated that A rooster is more sexually attractive to the hen than the B rooster, which might explain the higher investment of the hen in the yolk size at the same egg size. This tendency of a larger yolk size probably explains for a large part of the higher embryonic heat production of the AB crossbred embryos than the BB crossbred embryos. Nangsuay et al. (2013) demonstrated a strong relationship in broiler chicken embryos between yolk size at start of incubation and energy utilization (and thus heat production) during incubation. Based on this relationship, it can be speculated that the tendency for a larger yolk in the AB crossbred than in the BB crossbred can explain the difference in embryonic heat production.

Although the yolk size tended to be higher in the AB crossbred, YFBM and RY at hatching did not differ between crossbreds and neither did organ weights, metabolites, and liver glycogen. However, chicken quality at pulling differed between both crossbreds, as demonstrated in differences in red hocks, red beaks, and navel score, but the differences appeared to be small and not consistent. A poorer navel score is associated with a larger RY at hatching (e.g., Nangsuay et al., 2016a), but in the current study this was not shown. It appears that besides RY size other (genetic) factors determine navel score at hatching or pulling.

The second factor included in this study was the incubation temperature, expressed as EST. In broiler studies, it has been demonstrated that EST strongly influences embryonic development, expressed in organ development, nutrient utilization, heat production, hatching moment, and/or chicken quality (e.g., Lourens et al., 2005; Molenaar et al., 2010b; Nangsuay et al., 2015, 2016b, 2017; Maatjens et al., 2016). In the current study, with laying hen crossbreds, it appears that effects of EST are less pronounced than in broiler chickens. Comparable effects to broilers of particularly a low EST are found on hatching time, RY, heart weight, and intestine weights, but effects on hepatic glycogen and chicken quality (red hocks, red beaks, navel score) were absent. When the current data of laying hen crossbreds are compared with data of broiler chickens, it becomes clear that the metabolic rate of broiler chicken embryos is higher than that of layer chicken embryos, which is also demonstrated by Janke et al. (2004) and Nangsuay et al. (2015). In the current study, heat production at E18.5 was approximately 130 mW per egg, whereas in broiler embryos this is approximately 150 mW per egg (Lourens et al., 2006, 2007; Molenaar et al., 2010b; Nangsuay et al., 2015, 2016b). In broiler chicken embryos, it has been demonstrated that particularly in late incubation there is a dis-balance between metabolic rate and oxygen availability (Nangsuay et al., 2017). Oxygen availability is determined by eggshell conductance and metabolic rate is largely determined by EST. Because eggshell conductance of an egg is fixed, the negative effects of a dis-balance between metabolic rate and oxygen availability on embryo development and chicken quality can only be reduced or solved by decreasing the EST (Maatjens et al., 2014, 2016). It can be hypothesized that oxygen availability in relation to metabolic rate is not really limited in laying hen crossbreds compared to broiler chickens, meaning that laying hen crossbred embryos run later or not in an oxygen limitation stage than broiler chicken embryos. This is supported by the results in the current study in three ways: 1) in broiler chicken embryos, the heat production reaches a plateau phase at approximately E16 to E18 (e.g., Lourens et al., 2005; Molenaar et al., 2010b; Nangsuay et al., 2013, 2016b, 2017), but no real plateau seems to be reached in the current study with laying hen crossbred embryos. The heat production in laying hen crossbred embryos still increased between E16 and E18, although it levelled off, as demonstrated as well by Nangsuay et al. (2015); 2) in the current study, embryos incubated at an EST of 38.9°C still had a higher heat production at E18.5 than embryos incubated at an EST of 36.7°C and 37.8°C. In broiler chickens, Nangsuay et al. (2017) showed that embryonic heat production was (numerically) higher at an EST of 38.9°C compared to an EST of 37.8°C till E16, but thereafter it was lower. 3) During oxygen shortage, glycogen and protein reserves are used to fuel embryo development, resulting in lower liver glycogen reserves and lower YFBM in broilers (e.g., Molenaar et al., 2011; Nangsuay et al., 2016a). This was not found in the current study. All three observations seem to suggest that broiler chicken embryos run into oxygen shortage earlier during the incubation period than layer chicken embryos, which is probably due to the smaller fresh yolk size and consequently the lower embryonic heat production. This can explain why effects of higher EST appears to be more pronounced in broilers than in layers.

Based on these results, it appears that laying hen crossbred embryos are less sensitive to higher incubation temperatures during the last week of incubation than broiler embryos, which might be related to a better balance between metabolic rate and oxygen availability.

It can be concluded, that in laying hen crossbreds both the rooster line and the incubation temperature play a role in the metabolic rate and hatchling quality. Furthermore, it appears that effects of incubation temperature on metabolic rate of laying hen crossbred embryos is smaller than that of broiler chicken embryos.

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