Effects of incubation temperature pattern on broiler performance

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ABSTRACT During incubation, development of embryos is affected by eggshell temperature (**EST**). A constant EST of 37.8°C has been considered so far to result in most optimal embryo development. However, it can be hypothesized that a higher EST in week 2 in combination with a lower EST in week 3 stimulates embryo development and subsequent grow-out performance. In this study, 468 eggs of a 44-week-old Ross 308 breeder flock were incubated at different incubation temperature patterns in a 2 × 2 factorial arrangement. In week 2, EST was either 37.8°C or 38.9°C, and in week 3, EST was either 37.8°C or 36.7°C. At hatch, chick quality was determined. Thereafter, 320 broilers were grown in 32 pens (8 replicates/treatment) for 6 wk. Weekly BW and ADFI were determined, and at day 40, slaughter yield from 128 broilers (4 per pen) was determined. Results showed that EST in week 2 did not interact with EST in week 3 for any variable. An EST of 38.9°C in week 2 resulted in a 1 mm longer chick length ($P < 0.001$) and 0.4 mmol/L lower blood glucose level ($P = 0.04$) at hatch than an EST of 37.8°C. Grow-out performance was not affected by EST in week 2 of incubation. An EST of 36.7°C in week 3 resulted in a 1 mm shorter chick length ($P = 0.02$), 1.0 mmol/L higher blood glucose level ($P < 0.001$), and higher relative heart ($P = 0.01$) and stomach weights ($P = 0.03$) at hatch than an EST of 37.8°C. Additionally, an EST of 36.7°C in week 3 resulted in lower BW, ADG, and ADFI on slaughter age (all $P < 0.03$) than an EST of 37.8°C. In conclusion, no interaction between EST in week 2 and 3 of incubation was found for any variable. A higher EST in week 2 had minor effects at hatching and during rearing, whereas a lower EST in week 3 seemed to result in better organ development, but resulted in lower grow-out performance.

Key words: incubation, eggshell temperature, chick quality, growth performance, broiler

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INTRODUCTION

Neonatal chick quality has been found to be related to later life performance (Fasenko and O'Dea, 2008; van de Ven et al., 2012). One of the factors affecting embryonic development and consequently neonatal chick quality is incubation temperature. Until now, a constant eggshell temperature (**EST**, reflecting embryo temperature) of 37.8°C throughout incubation is considered to result in the best embryo development and neonatal chick quality (Lourens et al., 2005).

However, Nangsuay (2016) speculated that a higher EST than 37.8°C during the second week of incubation might be beneficial for embryo development. It was shown that embryos had a higher yolk-free body mass (**YFBM**) at embryonic day (**E**)14 and E16 when EST was raised to 38.9°C from E7 onward compared with maintaining EST at 37.8°C continuously. When this higher EST of 38.9°C was maintained after E16 until hatch, differences in YFBM disappeared at E18, and at hatch, YFBM was lower for chicks incubated at a higher EST compared with the control EST of 37.8°C. When temperature is raised during incubation, embryo metabolism increases as broiler embryos act mainly as poikilotherms (French, 1997), suggesting that embryonic development will be stimulated with a higher EST than 37.8°C. For the second week of incubation, this might be true, but in the third week of incubation, a higher EST than 37.8°C has been shown to negatively affect neonatal chick quality and posthatch performance (Romanoff, 1936; Lourens et al., 2005; Hulet et al., 2007; Leksrisompong et al., 2007; Ipek et al., 2014; Maatjens et al., 2014b, 2016a). Hatchlings incubated at an EST higher than 37.8°C during late incubation showed a poorer quality, expressed by a shorter length, more residual yolk (**RY**), worse navel score, and lower weights of various organs relative to their YFBM. Moreover, a

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lower growth performance on slaughter age has been found.

The cause of this impaired embryo development and neonatal chick quality might be related to an imbalance between embryonic metabolic rate and oxygen availability in the last part of incubation (Lourens et al., 2007). It has been shown that in the second week of incubation, oxygen availability is not limited (Lourens et al., 2007), but at approximately E14, embryo metabolic rate reaches its maximum increment (Nangsuay, 2016). With a higher temperature and consequently higher metabolic rate, more oxygen is needed. However, after E14, the amount of oxygen available to the embryo is limited by the conductance of the eggshell and consequently an imbalance between metabolic rate and oxygen availability seems to occur. To balance oxygen requirement and oxygen availability in week 3 of incubation, it can be suggested that after E14, a lower EST than 37.8°C might be more optimal. Maatjens et al. (2016a,b) demonstrated that an EST of 36.7°C or even 35.6°C from E15 onward resulted in a higher YFBM and higher relative heart, intestine, liver, and bursa of Fabricius weights of neonatal chicks than an EST of 37.8°C throughout incubation.

Based on the studies of Nangsuay et al. (2016) and Maatjens et al. (2016a,b), it can be hypothesized that a combination of a higher EST in week 2 and a lower EST in week 3 of incubation may stimulate embryo development and chick quality at hatch compared with a constant EST of 37.8°C throughout incubation. Studies investigating these different EST patterns and the effects on posthatch performance are lacking. Therefore, the aim of this study was to investigate effects of different EST patterns during incubation on neonatal chick quality and growth performance of broilers.

MATERIAL AND METHODS

Experimental Design

The experiment was set up as a 2 × 2 factorial arrangement. All eggs (n = 117/treatment group) were incubated at an EST of 37.8°C until E7. From E7 until E15, eggs were incubated at an EST of either 37.8°C or an EST of 38.9°C. From E15 until hatch, eggs were incubated at an EST of either 37.8°C or an EST of 36.7°C. The experimental protocol was approved by the Governmental Commission on Animal Experiments, the Hague, the Netherlands; approval number: 2016.W-0087.001.

Incubation

Eggs of a 44-week-old Ross 308 broiler breeder flock were stored for 2 D at a storage temperature of 20°C at a commercial hatchery (Lagerwey BV, Lunteren, the Netherlands). Ten egg trays with 150 eggs each were bulk-weighed to determine the average egg weight from this batch of eggs. Thereafter, eggs were weighed individually, and 468 first-grade eggs within 3 g of the average egg weight were selected and divided into 3 weight classes: 62.0 to 62.9 g, 63.0 to 63.9 g, and 64.0 to 64.9 g (156 eggs/weight class). Eggs were transported for approximately 30 min to the animal research facility of the Wageningen University (Wageningen, the Netherlands), where eggs of each weight class were equally divided over the 4 treatment groups to exclude potential effects of initial egg weight on chick quality variables (e.g., body weight).

Before the start of incubation, all eggs were placed in 1 incubator. Eggs were warmed linearly in 14 h from storage temperature (20°C) to an EST of 37.8°C. The moment the eggs reached an EST of 37.8°C was considered to be the start of incubation (E0), and this EST was maintained until E7. The EST was monitored by 4 EST sensors (NTC Thermistors: type DC 95; Thermometrics, Somerset, UK), which were placed at the equator of the eggshell of 4 individual eggs, using silicone heat sink compound (Type 340; Dow Corning, Midland, MI) and a small piece of duct-tape (approx. 1.5 × 1.5 cm). Incubator air temperature was continuously adjusted, based on the median temperature of the 4 EST sensors to maintain an EST of 37.8°C.

At E7, all eggs were candled, and infertile eggs were removed. Thereafter, eggs of each weight class were equally divided over 4 incubators (2 replicates/treatment group). Each incubator had 4 EST sensors, which were attached to 4 individual eggs as described above. Eggshell temperature in each incubator was determined continuously, and incubator air temperature was adjusted automatically when necessary based on the median of the 4 EST sensors, as described above. From E7 until E15, 2 incubators were maintained at an EST of 37.8°C, whereas the other 2 incubators were maintained at an EST of 38.9°C.

At E15, all eggs were candled again, and eggs containing a dead embryo were removed. Eggs containing a viable embryo were redistributed over the same 4 incubators (2 replicates/treatment group). Two incubators were then maintained at an EST of 37.8°C, whereas the other 2 incubators were maintained at an EST of 36.7°C. Eggshell temperature control in all incubators was performed as described above.

At E18, all eggs were candled again, and eggs containing a viable embryo were transferred to hatching baskets and placed back in the same incubators. After 454 h of incubation (E18h22), the incubator air temperatures were fixed at their current setting, and EST was allowed to change.

Throughout incubation, relative humidity was maintained between 50 and 65%, and CO_2 levels were < 3,500 ppm for all treatments. Eggs were turned every hour by an angle of 90° from the start of incubation until E18.

Hatch

From 468 h of incubation onwards (E19h12), every 6 h, the 4 incubators were opened to check whether chicks had hatched. Hatched chicks were marked with

a permanent marker on the head. Six h later, these marked chicks were collected, feather sexed, and chick quality was scored as described in the section "data collection". Per treatment, every eighth chick that hatched was decapitated, and organs were sampled as described below. Remaining chicks received a leg ring and were transferred to another incubator where they were housed in hatcher baskets in which they had ad libitum access to feed and water. As a result, each chick had access to feed and water within 12 h after emergence from the eggshell. In this incubator, continuous light was provided, and temperature and relative humidity were maintained at 33.2°C and 50%, respectively. All chicks remained in the incubator until the last chick had hatched, which was at 519 h after the start of incubation (E21h15).

Grow-out

After all chicks had hatched, 40 male and 40 female first-grade chicks per treatment were transported to the grow-out facility, which was located in a neighboring building at 20 m distance. Chicks were divided over 32 floor pens in 2 adjacent similar broiler houses (8 replicate pens per treatment). Within each house, pens were divided over 4 blocks, and within each block, treatments were randomly assigned. Each pen contained 5 male and 5 female broilers, and from each sex, 1 broiler hatched early in the hatch window, 3 broilers were mid hatchers, and 1 broiler hatched late (defined as 12/76/12% of the total hatch window for early, mid, and late hatchers, respectively). Pen size was 1 × 2 m, and each pen contained 1 Valènta feeding pan (VDL Agrotech, Eindhoven, the Netherlands), 5 drinking nipples with drip cups, and 1 perch. The floor was covered with 1 cm thick layer of wood shavings. No extra wood shavings were added until slaughter age. Temperature and humidity were set according to the Ross 308 management guide. The moment that chicks were placed in the grow-out facility was regarded as day 1. The first 3 D after placement, 24 h of light was provided and thereafter a lighting schedule of 16 h of light: 8 h of darkness. Starter pellet feed (ME broiler = 2,850 kcal/kg, CP = 220 g/kg, digestible lysine = 12.52 g/kg) with a diameter of 2.6 mm was provided from day 0 to day 10, grower pellet feed (ME broiler = 2,952 kcal/kg, CP = 209.7 g/kg, digestible Lysine = 11.81 g/kg) with a diameter of 3.2 mm was provided from day 10 to day 28, and finisher pellet feed (ME broiler = 2,999 kcal/kg, CP = 199.8 g/kg, digestible Lysine = 11.14 g/kg) with a diameter of 3.2 mm was provided from day 28 until slaughter age. Diets were made by Research Diet Services (RDS, Wijk bij Duurstede, the Netherlands) according to the guidelines of the Federation Dutch Animal Feed chain (CVB, 2016). Both on day 41 and on day 42, 64 broilers were slaughtered (16 broilers/treatment/day). Per pen, 2 male and 2 female broilers were randomly selected from the mid hatchers, electrically stunned, and decapitated. Broilers were not feed or water restricted before slaughter.

Data Collection

Each egg was weighed before the start of incubation and at transfer to the hatching baskets (E18) to calculate egg weight loss. Clear eggs and eggs containing a dead embryo at candling at E7, E15, and E18 were opened and scored for fertility or moment of mortality (Lourens et al., 2006). Hatchability was calculated as the number of hatched chicks divided by the number of eggs that contained a viable embryo at E7, right before treatments started. Hatch moment was calculated as the number of hours from start of incubation to emergence from the eggshell. Hatch window was calculated per treatment as the difference in hatch moment between the first and last chick per treatment. Neonatal chick quality was evaluated by determining chick weight, chick length from beak-tip to toe-tip, and navel quality score (Reijrink et al., 2009) as 1 (completely closed and clean), 2 (discolored and opened to a maximum of 2 mm), and 3 (discolored and opened to more than 2 mm). Neonatal chicks were classified as second-grade chicks if any abnormality (e.g., crossed beak, blindness, exposed brains, 4 legs, not incorporated yolk) was observed. All other neonatal chicks were classified as first-grade chicks.

For the neonatal chicks that were decapitated, blood glucose was determined immediately after decapitation, using a blood glucose analyzer (Contour TS, Bayer AG, Leverkusen, Germany). Thereafter, chicks were opened, and RY, heart, liver, intestines, and stomach were removed and weighed on a two-decimal scale. Stomach included the proventriculus, the intermediate zone, the ventriculus (gizzard), and the pylorus. Bursa of Fabricius and spleen were weighed on an analytical four-decimal scale (A200S, Sartorius GmbH, Göttingen, Germany). The gizzard was opened, and erosions were scored as 0 (no erosions), 1 (single erosion), 2 (2 or 3 erosions), and 3 (>3 erosions). Erosions were defined as a dark discoloration of the koilin layer (Gjevre et al., 2013). YFBM was calculated as neonatal chick weight minus RY weight.

During grow-out, all broilers (n = 320) were weighed individually at placement day 7, 14, 21, 28, 35, and 40. Feed intake was also determined per pen on those weighing days. Feed conversion ratio (**FCR**) was calculated by dividing the amount of feed consumed by body weight gain in that particular period. Feed conversion ratio over the total grow-out period was corrected for body weight at day 40 (**FCRc**) by adding body weight at day 40 as a covariate to the model (see section statistics).

Temperature preference was determined at day 1 and day 7 for half of the pens. Two male and 2 female broilers from the same pen were placed together in the middle of a wooden box (160 × 60 × 50 cm) with a Plexiglas lid and wood shavings at the bottom, and 2 infrared lights (250 Watt) on 1 side of the box (creating a linear temperature gradient from 20°C to 50°C). Twenty-four temperature sensors (Hobo UX100-011, Onset, Bourne, MA) were equally distributed in the box at broiler height, which continuously monitored the actual temperature.

Ten min after placement of the broilers in the box, the location of each broiler was noted, and the ambient temperature of each location was determined, based on temperature of the sensor log data (protocol adapted from Walstra et al. (2010)).

All broilers selected to be slaughtered (n = 128) were weighed individually before slaughter (day 41 or day 42). After stunning, cutting, and bleeding, the head was removed (cut closest to upper cervical vertebra), and toes were removed (cut at tarsal joint), and thereafter, the carcass was manually skinned and eviscerated. Heart, liver, intestines (incl. intestinal content), lungs, and stomach (incl. proventriculus, intermediate part, ventriculus, pylorus, and feed content) were weighed. Legs (cut at hip joint), wings (cut at shoulder joint), and breast meat were removed and weighed (all without skin). The remaining carcass was weighed. The carcass yield was calculated for the eviscerated carcass (legs + wings + breast meat + remaining carcass) as a percentage of live body weight. The cut-up yields and relative organ weights were calculated as a percentage of the eviscerated carcass.

Statistical Analyses

All data were analyzed using the statistical software package SAS (version 9.4, SAS Institute 2010). The basic model used for all data was

$$Y_{ij} = \mu + ESTwk2_i + ESTwk3_j + ESTwk2 \times ESTwk3_{ij} + e_{ij},$$

where, Y_{ij} = the dependent variable, μ = the overall mean, $ESTwk2_i$ = EST in week 2 ($_i$ = 38.9 or 37.8°C), $ESTwk3_j$ = EST in week 3 ($_j$ = 37.8 or 36.7°C), $ESTwk2 \times ESTwk3_{ij}$ = the interaction between EST week 2 and EST week 3, and e_{ij} = the error term.

For all incubation variables, egg was considered the experimental unit, and the egg tray number in which the egg was positioned was added as a random factor. For neonatal chick quality variables, hatch moment, and the temperature preference test, broiler was considered the experimental unit, and sex was added to the model as a fixed factor. For chick length and navel quality score, the person that scored the neonatal chick (3 individuals) was also added to the model as a fixed factor.

For posthatch performance variables and slaughter variables, pen was considered the experimental unit, the model described above was used, and block (1–8) was added as a random factor. Preliminary analysis showed that adding hatch moment (averaged per pen) to the model as a covariate had no significant effect for any performance variable, so it was excluded from the model. BW, ADG, ADFI, and FCR (all averaged/pen) were analyzed both per week and per total grow-out period (day 1–40), and FCRc was analyzed for the total grow-out period by adding body weight at day 40 as a covariate to the model.

All variables were analyzed with the PROC MIXED procedure in SAS, using the model described above, except for embryo mortality data, gizzard erosion score, and navel quality score. Embryo mortality data were analyzed with the PROC GLIMMIX procedure, using the model described above, including a binomial log logit link function. Gizzard erosion score and navel quality score were also analyzed with the PROC GLIMMIX procedure, using the model described above, including a multinomial log logit link function. In PROC MIXED procedure, the model assumptions were verified by inspection of residual plots. All data were normal distributed, except for RY. Residual yolk was log-transformed, and the LSMeans and the SEM of the untransformed data was used and given in the results section, whereas the *P*-value of the transformed data was given. Tukey adjustments for multiple comparisons were used to compare least square means (LSMeans). *P*-values ≤ 0.05 were considered to be significant.

RESULTS

Incubation

No interaction between EST week 2 and EST week 3 was found ($P \geq 0.52$) for any of the incubation variables, which includes egg weight loss, embryonic mortality (data not shown), hatchability (described below), and hatch moment (Table 1). Duration of the hatch window was 42 h for 37.8°C EST continuously, 36 h for 37.8°C × 36.7°C EST for week 2 × week 3, respectively, and 30 h for both 38.9°C × 37.8°C EST and 38.9°C × 36.7°C EST for week 2 × week 3, respectively.

A higher EST of 38.9°C in week 2 resulted in a higher egg weight loss compared with a constant EST of 37.8°C (10.3 vs. 9.7% ± 0.1 for 38.9°C and 37.8°C, respectively; $P < 0.001$). Hatch moment was on average 5 h earlier when EST in week 2 was raised to 38.9°C compared with a constant EST of 37.8°C ($P < 0.001$; Table 1). Eggshell temperature in week 2 had no effect on hatchability (95.7 vs. 97.7% ± 0.4 for 38.9°C and 37.8°C, respectively; $P = 0.32$).

A lower EST of 36.7°C in week 3 resulted in a 8 h later hatch moment ($P < 0.001$; Table 1) compared with an constant EST of 37.8°C, but it had no effect on egg weight loss (9.9 vs. 10.1% ± 0.1 for 36.7 and 37.8°C, respectively; $P = 0.17$) and on hatchability (97.2 vs. 96.5% ± 0.4 for 37.8°C and 36.7°C, respectively; $P = 0.67$).

Hatch moment was on average 3 h earlier for female than for male chicks (495 vs. 498 h ± 0.4, respectively; $P < 0.001$).

Chick Quality at Hatch

No interaction between EST week 2 and EST week 3 was found for any of the chick quality variables at hatch (Table 1; $P \geq 0.12$). A higher EST of 38.9°C in week 2 resulted in a 1 mm longer chick length ($P < 0.001$) and 0.4 mmol/L lower blood glucose level ($P = 0.04$) compared with a constant EST of 37.8°C. Eggshell temperature in week 2 had no effect on BW, RY, YFBM,

Table 1. Effect of 2 eggshell temperatures (EST; 37.8, 38.9°C) applied during week 2 and 2 EST (36.7°C, 37.8°C) during week 3 of incubation on hatch moment, neonatal chick quality, and relative organ weights at the moment of hatch.

Item	Hatch[1] moment (h)	BW[1] (g)	RY[2] (g)	YFBM[2] (g)	Chick[1] length (cm)	Navel[1] score (1–3)	Blood glucose[2] (mmol/L)	Heart[2] (%)[3]	Liver[2] (%)[3]	Bursa[2] (%)[3]	Spleen[2] (%)[3]	Intestines[2] (%)[3]	Stomach[2] (%)[3]	Giz.er.sc.[2] (0–3)
EST week 2														
37.8°C	499[a]	46.04	5.76	40.17	19.4[b]	1.7	11.4[a]	0.84	2.63	0.0551	0.0280	4.62	5.57	1.9
38.9°C	494[b]	45.85	5.43	40.22	19.5[a]	1.7	11.0[b]	0.78	2.69	0.0505	0.0323	4.79	5.60	2.0
SEM	0.4	0.09	0.25	0.33	0.03	0.05	0.2	0.02	0.05	0.0066	0.0032	0.17	0.12	0.2
EST week 3														
36.7°C	501[a]	45.93	5.49	40.20	19.4[b]	1.7	11.7[a]	0.85[a]	2.70	0.0618	0.0342	4.80	5.78[a]	1.9
37.8°C	493[b]	45.95	5.71	40.19	19.5[a]	1.7	10.7[b]	0.77[b]	2.62	0.0437	0.0260	4.61	5.39[b]	2.0
SEM	0.4	0.09	0.25	0.33	0.03	0.05	0.2	0.02	0.05	0.0066	0.0032	0.17	0.12	0.2
EST week 2 × week 3														
37.8°C × 36.7°C	503	46.05	5.81	40.01	19.3	1.7	11.9	0.89	2.69	0.0646	0.0303	4.62	5.73	2.1
37.8°C × 37.8°C	496	46.02	5.72	40.32	19.4	1.6	11.0	0.79	2.57	0.0454	0.0256	4.62	5.41	1.8
38.9°C × 36.7°C	498	45.82	5.17	40.39	19.4	1.7	11.6	0.81	2.71	0.0589	0.0382	4.98	5.83	1.9
38.9°C × 37.8°C	491	45.88	5.70	40.05	19.5	1.7	10.4	0.75	2.68	0.0420	0.0264	4.60	5.37	2.1
SEM	0.6	0.13	0.35	0.47	0.04	0.07	0.2	0.03	0.08	0.0093	0.0045	0.24	0.17	0.3
P-value														
week 2	<0.001	0.14	0.39	0.90	<0.001	0.57	0.04	0.07	0.41	0.63	0.34	0.47	0.86	0.96
week 3	<0.001	0.87	0.44	0.98	0.02	0.36	<0.001	0.008	0.33	0.06	0.08	0.41	0.03	0.86
week 2 × week 3	0.90	0.71	0.33	0.50	0.93	0.68	0.71	0.55	0.59	0.91	0.44	0.45	0.70	0.12

[a-b]Least squares means within a column and factor lacking a common superscript differ ($P \leq 0.05$).

[1]n = 103, n = 104, n = 111, n = 108 for treatment groups 37.8°C × 37.8°C, 38.9°C × 37.8°C, 37.8°C × 36.7°C, 38.9°C × 36.7°C, respectively; determined within 12 h after emergence from the eggshell.

[2]RY = residual yolk, YFBM = yolk-free body mass, mmol/L = millimole/L, Giz.er.sc. = gizzard erosion score: n = 13, n = 12, n = 13, n = 12 for treatment groups 37.8°C × 37.8°C, 38.9°C × 37.8°C, 37.8°C × 36.7°C, 38.9°C × 36.7°C, respectively, determined between 6 and 12 h after hatching. Stomach includes the proventriculus, intermediate part, pylorus, and ventriculus.

[3]Weight relative to YFBM.

navel quality score, any organ weights relative to YFBM, or gizzard erosions ($P \geq 0.07$).

A lower EST of 36.7°C in week 3 resulted in a 1 mm shorter chick length ($P = 0.02$), 1.0 mmol/L higher blood glucose level, 0.08% higher relative heart weight ($P = 0.008$), and a 0.39% higher relative stomach weight ($P = 0.03$) compared with a constant EST of 37.8°C. Eggshell temperature in week 3 had no effect on BW, RY, YFBM, navel quality score, relative liver, bursa, spleen, intestine weight, and gizzard erosions ($P \geq 0.06$).

Chick length was longer for male (19.4 cm) than for female chicks (19.2 cm; ± 0.03; $P = 0.002$). Navel quality score was higher (worse) for female (score 1.8) than for male chicks (score 1.6; $P = 0.03$). Gizzard erosion score was higher (worse) for male (score 2.6) than for female chicks (score 1.6; $P < 0.001$).

Temperature Preference

Temperature preference of broilers at day 1 and day 7 did not show an interaction between EST in week 2 and EST in week 3 and was also not affected by a main effect of EST in week 2 or EST in week 3 (Table 2; $P \geq 0.09$). Temperature preference did not differ between male and female broilers at day 1 ($P = 0.81$; ± 0.6) or day 7 ($P = 0.81$; ± 0.2).

Broiler Performance

No interaction between EST week 2 and EST week 3 was found for any of the performance variables ($P \geq 0.12$), which includes BW, ADG, ADFI, FCR, and FCRc. A higher EST of 38.9°C in week 2 compared with a constant EST of 37.8°C resulted in a 3 g higher BW at day 1 (Table 3; $P < 0.001$), but BW at later ages was not affected by EST in week 2 (Table 3; $P \geq 0.16$). A higher EST of 38.9°C in week 2 compared with a constant EST of 37.8°C resulted in 3 g lower ADG in week 5 (Table 4; $P = 0.05$), but it had no effect on ADG at other ages (Table 4; $P \geq 0.21$). ADFI was not affected at any age by EST in week 2 ($P \geq 0.14$). A higher EST of 38.9°C in week 2 compared with a constant EST of 37.8°C resulted in a 0.06 lower (better) FCR in week 6 (Table 4; $P = 0.04$), but FCR was not affected at other ages (Table 4; $P \geq 0.12$). Over the total grow-out period (day 1–40), EST in week 2 had no effect on ADG, ADFI, FCR, and FCRc (Table 4; $P \geq 0.22$).

A lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C resulted in a lower BW at all ages (Table 3; $P \leq 0.02$). A lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C resulted in a lower ADG during week 1, week 2, and week 4 (Table 4; $P \leq 0.03$) but comparable ADG in week 3, week 5, and week 6 (Table 4; $P \geq 0.09$). ADFI was lower in week 1 and week 3 for a lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C (Table 4; $P < 0.02$), but ADFI at other ages was not affected by EST in week 3 (Table 4; $P \geq 0.06$). A lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C resulted in a 0.02 lower (better) FCR in week 1 (Table 4; $P = 0.04$), but in a higher (worse) FCR in week 2 and week 4 (Table 4; $P \leq 0.03$). In week 3, week 5, and week 6, FCR was not affected by EST week 3 (Table 4; $P \geq 0.20$). Over the total grow-out period (day 1–40), a lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C resulted in a 3 g lower ADG (Table 4; $P = 0.02$) and a 3 g lower ADFI (Table 4; $P = 0.04$), whereas no effects were found for FCR and FCRc (Table 4; both $P = 0.94$).

Table 2. Effect of 2 eggshell temperatures (EST; 37.8°C, 38.9°C) applied during week 2 and 2 EST (36.7°C, 37.8°C) during week 3 of incubation on broiler temperature preference (°C) at day 1 and day 7 posthatch.

Item	n[1]	Day 1	Day 7
EST week 2			
37.8°C	32	33.9	27.4
38.9°C	32	33.4	27.2
SEM		0.6	0.2
EST week 3			
36.7°C	32	34.4	27.4
37.8°C	32	33.0	27.2
SEM		0.6	0.2
EST week 2 × EST week 3			
37.8°C × 36.7°C	16	35.1	27.3
37.8°C × 37.8°C	16	32.7	27.6
38.9°C × 36.7°C	16	33.6	27.5
38.9°C × 37.8°C	16	33.2	26.8
SEM		0.8	0.3
P-value			
week 2		0.53	0.32
week 3		0.09	0.37
week 2 × week 3		0.26	0.09

[1]Number of broilers, 50:50 sex ratio.

Slaughter

No interaction between EST week 2 and EST week 3 was found for any of the slaughter variables (Table 5; $P \geq 0.05$). A higher EST of 38.9°C in week 2 resulted in higher relative stomach weight compared with a constant EST of 37.8°C (Table 5; Δ = 0.2%; $P = 0.009$). Eggshell temperature in week 2 had no effect on carcass yield, cut up yields, or relative heart, liver, lungs, or intestines weight (Table 5; $P \geq 0.09$).

A lower EST of 36.7°C in week 3 resulted in a lower relative breast meat yield compared with a constant EST of 37.8°C (Table 5; Δ = 0.7%; $P = 0.05$). Relative intestine weight was higher after a lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C (Table 5; Δ = 0.4%; $P = 0.02$).

DISCUSSION

It was hypothesized that an incubation pattern consisting of a higher EST of 38.9°C in the second week of incubation in combination with a lower EST of 36.7°C in the last week of incubation would result in most optimal embryo development, neonatal chick quality, and subsequent broiler performance during grow-out compared with a constant EST of 37.8°C throughout incubation. This study showed that this incubation temperature pattern had no synergistic stimulating effect

Table 3. Effect of 2 eggshell temperatures (EST; 37.8°C, 38.9°C) applied during week 2 and 2 EST (36.7°C, 37.8°C) during week 3 of incubation on later life broiler body weight (g).

Item	n[1]	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 40
EST week 2								
37.8°C	16	53[b]	188	513	1,034	1,706	2,507	3,086
38.9°C	16	56[a]	190	511	1,042	1,707	2,486	3,053
SEM		0.3	2	4	8	13	18	24
EST week 3								
36.7°C	16	53[b]	184[b]	501[b]	1,020[b]	1,677[b]	2,454[b]	3,023[b]
37.8°C	16	56[a]	193[a]	523[a]	1,056[a]	1,735[a]	2,539[a]	3,116[a]
SEM		0.3	2	4	8	13	18	24
EST week 2 x EST week 3								
37.8°C × 36.7°C	8	51	183	499	1,010	1,663	2,454	3,040
37.8°C × 37.8°C	8	56	192	527	1,057	1,748	2,560	3,132
38.9°C × 36.7°C	8	54	186	504	1,029	1,691	2,453	3,006
38.9°C × 37.8°C	8	57	194	518	1,054	1,722	2,518	3,100
SEM		0.4	2	5	12	18	25	35
P-value								
week 2		<0.001	0.16	0.74	0.51	0.96	0.40	0.35
week 3		<0.001	<0.001	<0.001	0.006	0.005	0.003	0.02
week 2 × week 3		0.15	0.79	0.18	0.35	0.16	0.42	0.99

[a-b]Least squares means within a column and factor lacking a common superscript differ ($P \leq 0.05$).
[1]Number of pens, each containing 10 broilers, 50:50 sex ratio.

on neonatal chick quality or on broiler grow-out performance compared with a constant EST of 37.8°C in the second and third week of incubation, as there was no interaction between EST in week 2 and EST in week 3 on any of the neonatal chick quality and grow-out variables.

Increasing the EST in week 2 of incubation to 38.9°C compared with maintaining a constant EST of 37.8°C affected embryo development. A smaller hatch window (12 h) and earlier hatch moment (5 h) after a higher incubation temperature of 38.9°C EST in week 2 compared with a constant EST of 37.8°C suggests that embryo development was accelerated. On the one hand, this accelerated embryo development seemed beneficial, because chick length at hatch was 1 mm longer when EST was increased in week 2 of incubation. It is known that in week 2 of incubation, ossification of the long leg bones occurs and that temperature has an effect on ossification speed (Rommel et al., 2001; Mackie et al., 2008). A faster ossification may have resulted in longer bones and consequently a slightly longer chick. Studies have shown that chick length at hatch is positively correlated to broiler BW at slaughter age (Wolanski et al., 2004; Molenaar et al., 2008; Petek et al., 2010), although the relationship was not very strong (max r = 0.60), and other studies did not confirm this (Willemsen et al., 2008). On the other hand, this accelerated embryo development seemed to be accompanied with a lower blood glucose level at hatch. Lower blood glucose levels at hatch might be explained by 1) less hepatic glycogen was available to the embryo to turn into blood glucose. Perhaps hepatic glycogen storage by the embryo was impaired in week 2 because of the higher EST. Studies showed that a higher EST than 37.8°C resulted in lower hepatic glycogen stores (Willemsen et al., 2010; Molenaar et al., 2011b; Maatjens et al., 2014a), but all these studies were performed with a high temperature until the end of incubation or 2) more glucose was utilized by the embryo, for instance during the hatching process. Blood glucose serves as the main source for adenosine triphosphate to provide energy (Freeman, 1965), especially between external pipping and hatch, as this is an energy demanding process (De Oliveira et al., 2008). The duration between external pipping and hatch may have taken longer for chicks incubated at a higher EST of 38.9°C in week 2, for instance because these chicks were slightly longer or because egg weight loss during incubation was higher in this treatment group.

Eggshell temperature in week 2 had no effect on broiler grow-out performance or slaughter yield. A higher EST of 38.9°C in week 2 only increased broiler BW at day 1, but this was most likely because of a difference in hatching moment. Body weight and YFBM at hatch were similar between both groups, but after hatch, all chicks had immediate access to feed and water and broilers incubated at 38.9°C EST in week 2 hatched on average 5 h earlier and therefore had on average 5 h longer between hatch and moment of weighing at day1 in which they could eat and grow.

Lowering the EST in week 3 of incubation to 36.7°C compared with maintaining a constant EST of 37.8°C was hypothesized to optimize the balance between metabolic rate and available oxygen at embryo level (Nangsuay et al., 2016) and thereby improving nutrient oxidation for embryonic development (Maatjens et al., 2016a, 2017). A lower EST of 36.7°C in the last week of incubation resulted on the one hand in a higher relative heart and stomach weight and higher blood glucose level at hatch, which suggest that oxygen availability and embryonic development was indeed stimulated. On the other hand, a shorter chick length was found, which may suggest that embryonic development was retarded. The higher blood glucose level at hatch that was found might be explained by a lower metabolic rate that will probably occur when EST is lowered in

Table 4. Effect of 2 eggshell temperatures (EST; 37.8°C, 38.9°C) applied during week 2 and 2 EST (36.7°C, 37.8°C) during week 3 of incubation on ADG, ADFI, and feed conversion ratio (FCR) of broilers per week and over the total grow-out period (day 1–40).

		ADG							ADFI							FCR							
		Week							Week							Week							
Item	n[1]	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total	corr[2]
EST week 2																							
37.8°C	16	22	46	74	96	114[a]	114	78	24	59	98	146	178	201	117	1.08	1.28	1.33	1.53	1.56	1.77[a]	1.51	1.51
38.9°C	16	22	46	76	95	111[b]	114	77	25	58	99	146	174	194	115	1.10	1.26	1.31	1.53	1.58	1.71[b]	1.50	1.50
SEM		0.2	0.5	0.9	0.9	1.5	2.1	0.6	0.2	0.6	1.2	1.4	1.8	2.9	1.0	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
EST week 3																							
36.7°C	16	22[b]	45[b]	74	94[b]	111	112	76[b]	24[b]	58	96[b]	145	174	194	115[b]	1.08[b]	1.28[a]	1.30	1.55[a]	1.57	1.73	1.51	1.51
37.8°C	16	23[a]	47[a]	76	97[a]	114	116	79[a]	25[a]	59	101[a]	147	178	202	118[a]	1.10[a]	1.25[b]	1.33	1.51[b]	1.56	1.75	1.51	1.51
SEM		0.2	0.5	0.9	0.9	1.5	2.1	0.7	0.2	0.6	1.2	1.4	1.8	2.9	1.0	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
EST week 2 x EST week 3																							
37.8°C × 36.7°C	8	22	45	73	93	113	113	77	24	58	96	144	175	198	115	1.07	1.29	1.32	1.55	1.55	1.77	1.50	1.50
37.8°C × 37.8°C	8	23	48	76	99	116	115	79	25	60	101	149	181	203	119	1.10	1.26	1.33	1.51	1.57	1.78	1.51	1.52
38.9°C × 36.7°C	8	22	46	75	95	109	112	76	24	58	97	146	173	189	114	1.09	1.27	1.29	1.55	1.59	1.69	1.51	1.51
38.9°C × 37.8°C	8	23	47	77	95	112	117	78	25	59	101	145	175	200	117	1.11	1.24	1.32	1.52	1.56	1.73	1.50	1.50
SEM		0.3	0.7	1.2	1.2	1.9	2.8	0.9	0.3	0.8	1.7	1.9	2.6	4.1	1.4	0.01	0.01	0.02	0.01	0.02	0.03	0.01	0.01
P-value																							
week 2		0.48	0.79	0.21	0.45	0.05	0.79	0.30	0.21	0.34	0.73	0.66	0.15	0.14	0.22	0.12	0.15	0.29	0.65	0.23	0.04	0.52	0.50
week 3		0.005	0.007	0.10	0.03	0.09	0.21	0.02	<0.001	0.15	0.02	0.34	0.14	0.06	0.04	0.04	0.009	0.20	0.03	0.70	0.44	0.94	0.94
week 2 × week 3		0.48	0.19	0.55	0.08	0.92	0.62	0.95	0.78	0.42	0.68	0.14	0.38	0.44	0.44	0.80	0.79	0.79	0.65	0.12	0.68	0.12	0.13

[a-b]Least squares means within a column and factor lacking a common superscript differ ($P \leq 0.05$).

[1]Number of pens, each containing 10 broilers, 50:50 sex ratio.

[2]FCRcorrected for BW at day 40.

Table 5. Effect of 2 eggshell temperatures (EST; 37.8°C, 38.9°C) applied during week 2 and 2 EST (36.7°C, 37.8°C) during week 3 of incubation on broiler slaughter (day 41/day 42) variables.

Item	n[1]	Carcass yield[2]	Breast[3]	Legs[3]	Wings[3]	Heart[3]	Liver[3]	Lungs[3]	Intestines[3]	Stomach[3]
EST week 2										
37.8°C	16	65.8	38.8	29.6	8.6	0.7	3.2	0.5	7.9	1.7[b]
38.9°C	16	65.9	39.2	29.5	8.6	0.7	3.1	0.6	7.9	1.9[a]
SEM		0.3	0.3	0.2	0.2	0.01	0.04	0.03	0.1	0.04
EST week 3										
36.7°C	16	65.5	38.6[b]	29.6	8.6	0.7	3.2	0.6	8.1[a]	1.8
37.8°C	16	66.2	39.3[a]	29.5	8.6	0.7	3.2	0.6	7.7[b]	1.8
SEM		0.3	0.3	0.2	0.2	0.01	0.04	0.03	0.1	0.04
EST week 2 × week 3										
37.8°C × 36.7°C	8	65.8	38.7	29.4	8.4	0.7	3.2	0.5	8.1	1.8
37.8°C × 37.8°C	8	65.8	38.8	29.7	8.8	0.7	3.2	0.5	7.7	1.7
38.9°C × 36.7°C	8	65.2	38.5	29.7	8.8	0.7	3.2	0.6	8.1	1.8
38.9°C × 37.8°C	8	66.6	39.8	29.3	8.4	0.7	3.1	0.6	7.6	2.0
SEM		0.4	0.4	0.3	0.2	0.02	0.06	0.03	0.2	0.1
P-values										
week 2		0.80	0.21	0.71	0.95	0.76	0.34	0.09	0.79	0.009
week 3		0.08	0.05	0.93	0.95	0.14	0.70	0.85	0.02	0.66
week 2 × week 3		0.08	0.08	0.23	0.04[4]	0.37	0.99	0.55	0.85	0.09

[a-b]Least squares means within a column and factor lacking a common superscript differ ($P \leq 0.05$).
[1]Number of pens, each containing 10 broilers, 50:50 sex ratio.
[2]% relative to live body weight.
[3]% relative to carcass weight. Stomach includes proventriculus, intermediate part, pylorus, and ventriculus.
[4]After Tukey adjustment no significant differences appeared.

week 3. A lower metabolic rate results in a lower demand for oxygen, and if the demand does not exceed the available amount of oxygen, which is limited during late incubation because of eggshell conductance, the embryo will probably use mostly fat as energy resource. In case oxygen availability is insufficient, for instance when metabolic rate is increased, the embryo might also use glucose via glycolysis as an energy resource, which is an anaerobic process. The higher relative heart and stomach weights that were found in the current study when EST was lower in week 3 might also be caused by an improved balance between metabolic rate and available oxygen and thereby improved resources available for development of these organs. This is supported by a study from Wineland et al. (2001) who were the first to show that a lower incubation temperature during late incubation resulted in a higher heart weight. Molenaar et al. (2011b) showed that increasing oxygen levels to 25% from E7 to E19 of incubation resulted in a higher YFBM when EST was 38.9°C in that period, but relative organ weights were not affected. This indicates that oxygen was available to the embryo, but that organ development was not affected by a higher oxygen availability. Probably the higher relative heart and stomach weights that were found in the current study were not the result of a higher oxygen availability but were the result of a difference in incubation duration. The total incubation duration was 8 h longer for embryos in the lower EST treatment group, and therefore, these embryos had 8 h more time within the egg to develop. As the maturation of organs mainly takes place during late incubation, this prolonged incubation duration might specifically have affected relative organ weights and for instance not the length of the chick.

The higher blood glucose level and the higher relative stomach weight and heart weight that were found at hatch after a lower EST in week 3 suggest that some parts of embryonic development were stimulated after a lower EST in week 3 compared with a constant EST of 37.8°C. Some chick quality characteristics seemed improved, and as a result, a higher broiler grow-out performance would be expected. After all, a worse chick quality at hatch that was found after a higher EST of 38.9°C in week 3 was found to decrease performance upon slaughter age (Hulet et al., 2007; Leksrisompong et al., 2007; Ipek et al., 2014; Maatjens et al., 2016b), so it can be speculated that a better chick quality at hatch that was found in the current study after a lower EST of 36.7°C in week 3 could improve performance upon slaughter age. For instance, the higher blood glucose level at hatch indicates that more energy was available during the start of life and the higher relative stomach weight could indicate an improved development of the digestive tract. Furthermore, concerning the higher relative heart weight, it can be suggested that during grow-out with a high growth rate, a larger heart might improve provision of oxygen and nutrients to the tissues, and consequently, broiler grow-out performance might be improved (Molenaar et al., 2011a; Sozcu and Ipek, 2015; Druyan et al., 2018). However, grow-out performance at later ages showed opposite results. It seems that improved chick quality characteristics at hatch after a lower EST in week 3 are only short term. For instance, FCR was improved in week 1, but not at later ages for broilers incubated at a lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C. Maybe the higher relative stomach and heart weight at hatch after a lower EST in week 3 of incubation were not the result of an 8 h longer incubation duration and maturation of organs as stated before but of a breakdown of organ muscles in the constant EST treatment group. The breakdown of own body muscles is

hypothesized to be a short-term solution to gain glycogen via gluconeogenesis in anaerobic circumstances (Maatjens et al., 2017), and it is known that oxygen is limited in week 3 of incubation. Muscle break down during incubation was probably recovered quickly after hatch and probably therefore the higher relative organ weights at hatch after a lower EST in week 3 did not stimulate broiler performance during grow-out. In the current study, relative organ weights at slaughter age did not differ anymore between incubation temperatures, and Maatjens et al. (2016b) also found that differences in relative heart weights that were found at hatch after different incubation temperatures disappeared within 1 wk after hatching. Nevertheless, no indicators of fewer muscle degradation were found in the blood of chicks at hatch that were incubated at lower EST of 36.7°C during late incubation compared with a constant EST of 37.8°C, because levels of lactate and uric acid were similar (Maatjens et al., 2017). Therefore, muscle degradation seems not very likely, and it remains unknown why broiler performance during grow-out was not higher, whereas some chick quality characteristics at hatch were improved after a lower EST of 36.7°C in week 3 compared with a constant EST of 37.8°C. It can be speculated that broilers prefer different conditions in the growing house after different incubation temperatures, but this was not illustrated in the temperature preference test of the current study.

A lower EST in week 3 of 36.7°C negatively affected broiler grow-out performance on slaughter age. Broiler BW was lower at all ages, and ADG and ADFI were lower over the total grow-out period when EST in week 3 of incubation was lowered to 36.7°C compared with maintaining EST at 37.8°C. Most likely, grow-out performance was lower, because the onset of growth was delayed because of longer incubation duration and thus a later moment of hatch and a shorter timespan with access to exogenous feed and water in the 36.7°C EST treatment group. Chicks from the 36.7°C EST in week 3 treatment group hatched on average 8 h later compared with chicks from the 37.8°C EST treatment. All broilers had access to ad libitum feed and water within 12 h after hatch and were placed in the growing houses at the same time point, indicating that broilers incubated at an EST of 36.7°C had on average 8 h less to grow between hatch and placement compared with broilers incubated at an EST of 37.8°C. This indeed could have had an effect, as BW at hatch was similar for both EST week 3 treatment groups, whereas it was significantly lower at day 1 for the 36.7°C EST treatment group than for the 37.8°C EST treatment group. Moreover, extrapolation of BW data also suggests that onset of growth was delayed after incubation at 36.7°C EST in week 3, without affecting growth competence. Fitting a polynomial curve to the body weight data of the 36.7°C EST treatment ($R^2 > 0.99$) and extrapolating it to 40 D and 8 h (40.333 D) results in a predicted BW of 3,105 g. This only differs 11 g from the actual average BW of the constant 37.8°C EST treatment (3,116 g). Additionally, not only the average incubation time differed between the 36.7°C EST treatment in week 3 and the constant 37.8°C EST treatment in week 3, but also the duration of the hatch window. The hatch window of the constant 37.8°C EST treatment was 42 h, whereas the hatch window of the 36.7°C EST treatment was only 30 h. As a result, some broilers from the constant 37.8°C EST treatment, especially the early hatchers, were even more ahead in growth than the average 8 h compared with broilers from to the 36.7°C EST treatment. Besides, FCR over the total grow-out period and slaughter yields were not different between broilers incubated at a lower EST of 36.7°C EST in week 3 and broilers incubated at a constant EST of 37.8°C, which suggests that growth efficiency was not affected and that a delayed onset of growth was more likely to be the explanation in growth performance differences between EST in week 3.

In conclusion, the hypothesis that a higher EST of 38.9°C in week 2 of incubation in combination with a lower EST of 36.7°C EST in week 3 of incubation would stimulate embryo development and grow-out performance compared with a constant EST of 37.8°C could not be accepted. Eggshell temperature in week 2 did not interact with EST in week 3 for any of the neonatal chick quality or grow-out variables. A higher EST of 38.9°C in week 2 compared with a constant EST of 37.8°C had little effect on neonatal chick quality, and grow-out performance was not affected. A lower EST 36.7°C in week 3 compared with incubation at a constant EST of 37.8°C seemed to stimulate neonatal chick quality in terms of some relative organ weights, but grow-out performance was not higher. Thus, so far there is no good reason to deviate from the standard of incubating eggs at a constant EST of 37.8°C throughout incubation.

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Conflict of Interest Statement: Authors H. J. Wijnen, I. A. M. van Roovert-Reijrink, and C. W. van der Pol are employed by company HatchTech. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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