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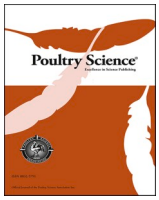

Use of in ovo transponder telemetry to determine the effects of a reduction in temperature initiated on day twelve of incubation on the subsequent body temperature and somatic characteristics of Ross 708 broiler chicks

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ABSTRACT

The effects of a reduction in incubation temperature, made to accommodate higher levels of embryonic heat production, on the post hatch body temperature and somatic characteristics of Ross 708 broilers were determined. Incubation temperature treatments (TRT) were a standard (STRT, 37.5 °C) and a lower (LTRT, 35.6°C) TRT provided between 12 and 21 d of incubation (DOI). All eggs were incubated at 37.5 °C between 0 and 12 DOI. Temperature transponders implanted in the air cell of each egg at 12 DOI were extracted and inserted subcutaneously into the neck of the corresponding hatchling to record chick body temperature (CBT) through 21 d of grow out (DOG). After placement, multiple CBT and litter temperature (LT) readings were recorded daily between 1 and 21 DOG, and BW was determined at placement (0 DOG), and BW, body length (BL), and BW to length ratio (BWTLR) were determined on 7, 14, and 21 DOG. Thirteen daily mean CBT readings in the STRT were significantly higher than those in the LTRT between 1 and 21 DOG. Nevertheless, there was no significant correlation between LT and CBT, and when hatch time (HT) and BW were accounted for, embryo temperature (ET) and CBT were not significantly correlated. At 0 and 7 DOG, no significant differences in BW were observed between the STRT and LTRT within either sex; however, BW was greatest in males belonging to the STRT at 14 ($\bar{x}$ = 483.1 g) and 21 ($\bar{x}$ = 1,033.8 g) DOG. Across DOG and sex, BL was significantly longer in the STRT than in the LTRT, and at 14 and 21 DOG, BWTLR was greater in the STRT than in the LTRT. The LTRT subsequently lowered CBT and negatively affected chick BW, BL, and BWTLR. In conclusion, CBT is not directly associated with ET, but the reductions in CBT and various performance variables in Ross 708 broilers in response to the LTRT is a result of its adverse effects on chick HT and BW.

Introduction

Incubation temperature is an important factor that can affect hatchability (Lourens et al., 2005; Almeida et al., 2016; Lindsey et al., 2023), overall chick growth (Geers et al., 1983; Wilson, 1991; Lourens et al., 2005; Hulet, 2007; Van der Pol et al., 2014), and time of hatch (**HT**) (Almeida et al., 2016; Maatjens et al, 2016a; Hamidu et al., 2018; Van den Brand et al., 2019). Almeida et al. (2016) concluded that the manipulation of incubation temperature is a potential tool for managing chick quality. This is of particular significance since embryogenesis encompasses between 30 and 40 % of the life of a broiler (Hulet, 2007). Janisch et al. (2015) also found that manipulating incubator air temperature affects the muscles as well as other tissues of broilers, with a subsequent effect on post hatch growth. Genes associated with prenatal and postnatal muscle deposition have been shown to be up-regulated in response to an air temperature of 38.0 °C (Clark et al., 2016). Modern broilers grow more rapidly, generate more metabolic heat, and are larger genetically than those of the past (Havenstein et al., 1994; Collins et al., 2014). Likewise, current commercial broiler embryos experience an increased metabolism (Hamidu et al., 2018) and would subsequently

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produce higher amounts of metabolic heat. French (1997) and Romanini et al. (2013) have discussed that eggshell temperature (**EST**), as an estimator of embryo temperature (**ET**), often exceeds incubator air temperature due to the amount of heat produced by eggs during incubation.

Currently, the standard temperature for incubating broiler hatching eggs is approximately 37.5°C (Barott, 1937; Lundy, 1969; Almeida et al., 2016; Hamidu et al., 2018). However, Hamidu et al. (2018) have concluded that an incubation temperature of 37.5 °C could result in the overheating of broiler embryos. Maatjens et al. (2017) found that EST readings of 35.6 °C and 36.7 °C, in Ross 308 hatching eggs from 15 d of incubation (**DOI**) through hatch, were beneficial to embryo physiology. Maatjens et al., (2016b) further reported that although not reflected in first wk Ross 308 broiler performance, an incubation temperature of 35.6 °C over the last wk of incubation, resulted in equal or greater chick quality and organ weights compared to those incubated at 36.7 °C over the same period of time. More specifically, the quality of chicks at placement, when expressed in terms of body length (**BL**) and incidence of closed or healed navels, were greater when they hatched from eggs having an EST of 35.6 °C.

Almeida et al. (2016) reported that the yolk absorption rates of Cobb 500 broiler hatchlings were altered in response to a change in incubation temperature from 39.0 °C to 36.0 °C. Nevertheless, Ross broiler embryos have a greater growth rate than that of Cobb broiler embryos (Druyan, 2010), and embryonic metabolism and subsequent daily heat production in Ross 308 embryos is higher than that of Cobb 500 embryos during the early and late stages of incubation (Hamidu et al., 2007; Nangsuay et al., 2015). Furthermore, Ross 708 broilers have higher percentages of breast meat yield and breast muscle fat than Ross 308 broilers (Bedford et al., 2016; Wang, 2017). Hamidu et al. (2018) have surmised that Ross 708 broiler embryos are more sensitive to incubation temperature than are Ross 308 broiler embryos and, therefore, experience more pronounced effects from an elevated incubation temperature than do Ross 308 broiler embryos. Since overheating broiler embryos can lead to poorer hatch and grow out quality results (Hulet, 2007), the relationship of ET and post hatch chick body temperature (**CBT**) and the subsequent effects of incubator air temperature on CBT and other post hatch physiological variables in Ross 708 broilers is worth examining.

Transponder telemetry can be employed in order to obtain a closer estimate of ET and CBT, while minimizing the confounding effects of variations in environmental temperature. Use of a transponder inserted into the air cell of an egg is considered as a more accurate means of estimating ET than EST, since the transponder lies on the inner eggshell membrane in close proximity to the embryo (Peebles et al., 2012; Olojede et al., 2017). However, Pulikanti et al. (2011a) and Peebles et al. (2012) found that 12 DOI was the earliest a transponder could be inserted into the air cell without having a negative effect on the survivability of the embryo.

In a companion study employing the same Ross 708 broiler hatching eggs and incubation procedures and temperature treatment (**TRT**) regimens as in the current study, Lindsey et al. (2023) reported the effects of TRT on ET and various egg and hatch variables. In 88 % of multiple readings recorded daily between 12 and 21 DOI, ET was significantly higher in the standard TRT (**STRT**; 37.5 °C between 0 and 21 DOI) than in the reduced TRT (**LTRT**; 37.5 °C between 0 and 12 DOI, and 35.6 °C between 12 and 21 DOI). It was also observed that percent hatch of fertile eggs containing live embryos at 12 DOI was 93.3 % in the STRT and 100 % in the LTRT, but that hatchling BW was lower, and HT occurred 14 to 19 h later in the LTRT than in the STRT. In addition to the previous research conducted by Lindsey et al. (2023), various other studies have been conducted examining the effects of incubation temperature on post hatch broiler growth (Nyuiadzi et al., 2017; Wijnen et al., 2020). However, studies are lacking that examine the relationship of ET and CBT of the same bird and the response of CBT to a change in incubation temperature.

The objective in this experiment included determining the effects of lowering incubation temperature from 37.5 °C to 35.6 °C at 12 DOI on CBT and various somatic characteristics in Ross 708 broilers through 21 d of grow out (**DOG**). A further objective included determining the potential relationship between ET and CBT using telemetric technology. A better understanding of these relationships and effects may likewise be of economic value. Because a drop in temperature by 1.0 °C can generally result in a 3 % reduction in energy consumption for heating, a decrease in incubator temperature by 1.9 °C, as in the current study, could result in an approximate 6 % energy savings in the 12 to 21 DOI time period.

Materials and methods

Egg incubation and transponder implantation

All handling and care of broiler chicks was conducted under the approval of the Mississippi State University Institutional Animal Care and Use Committee (Protocol # IACUC-20-248). As indicated in the companion study by Lindsey et al. (2023), Ross 708 broiler hatching eggs were obtained from 41-wk-old hens housed at a commercial facility. Approximately 540 of the eggs were stored under commercial conditions according to the procedures of Fatemi et al. (2020). Specifically, those conditions were 12.8 °C and 10.4 °C dry-and wet-bulb temperatures, respectively, for 24 h. The eggs were subsequently incubated between 0 and 12 DOI under standard conditions. Of those eggs, 120 were randomly selected and candled to verify live embryonation at 12 DOI. Further detailed information on egg selection (Pulikanti et al., 2011a) and incubation through 12 DOI (Lindsey et al., 2023) have been previously described.

At 12 DOI, an implantable and programmable temperature transponder was inserted aseptically into the air cell of each egg, allowing it to lay on the inner eggshell membrane proximal to the embryo. The measured temperature was considered an estimate of ET (Lindsey et al., 2023). Descriptions of the transponder (± 0.20 °C accuracy) and the implantation procedure have been provided by Pulikanti et al. (2011b). As previously reported by Lindsey et al. (2023), the 120 eggs were divided into 4 groups of 30 eggs. Two replicate groups were assigned to each of the STRT and LTRT regimens. Almeida et al. (2016) reported that fertile egg hatchability and hatchling yolk-free BW were not significantly different among Cobb 500 broiler hatching eggs incubated at either 37.5 °C or 36.0 °C between 13 and 21.7 DOI. Therefore, a 35.6 °C incubation temperature between 12 and 21 DOI was considered by Lindsey et al. (2023) as a feasible setting for the LTRT. Eggs were incubated between 12 and 21 DOI in 1602 N-Thermal Air Hovabator incubators (GQF Manufacturing Co., Inc., Savannah, GA), and were turned by hand 3 times daily through 18 DOI. Mean relative humidity between 12 and 21 DOI in the 2 replicate incubators assigned to the STRT and LTRT were 58.00 % and 59.08 %, respectively. In each egg, the ET detected by the implanted transponder was recorded using a wireless probe and the associated telemetric technological software (DAS-6006/7 Smart Probe, Bio Medic Data Systems Inc., Seaford, DE). Without opening the incubator, this system allowed the individual ET readings of each egg to be directly transmitted through the wall of the incubator. Through 21 DOI, 3 ET readings, 5 h apart, were recorded daily for each egg, which took approximately 30 min to complete (Lindsey et al., 2023).

Also, using HOBO ZW series wireless data loggers with an accuracy of ± 0.21 °C (Onset Computer Corporation, Bourne, MA), incubator air temperature readings were recorded once per min between 12:00 PM and 11:59 PM at 12 DOI and between 12:00 AM and 11:59 PM from 13 to 18 DOI. Over the 12 to 18 DOI period, mean air temperature in the 2 replicate incubators assigned to the STRT were 36.9 °C and 37.4 °C, and mean air temperature in the 2 replicate incubators assigned to the LTRT were 35.8 °C and 35.7 °C. The replicate incubators belonging to the STRT had a correlation coefficient of 0.97242 and the replicate incubators belonging to the LTRT had a correlation coefficient of 0.78284.

Therefore, incubator air temperature in each TRT was maintained close to the set temperature, with the mean air temperature difference between the TRT being approximately 1.42 °C. Furthermore, there was a significant (P = 0.001) positive correlation between incubator air temperature and ET across TRT (Lindsey et al., 2023). At 18 DOI, netted material was tied loosely around each egg to capture the transponders to match their identity to the corresponding egg and hatchling.

Chick transponder implantation and grow out performance

Chicks remained in their respective incubator until all were pulled for grow out at 22.3 DOI, and were considered as viable when fully hatched, mobile, and having dry down (Lindsey et al., 2023). The transponder captured from each egg was then subcutaneously inserted into the dorsal region of the neck of the corresponding viable hatchling. Chick sex was determined by feather-sexing, and 2 or 3 male and female transponder-implanted chicks, taken from each of the 2 replicate incubators in each TRT (STRT and LTRT) group, were randomly placed in each of 6 floor pens (1 m^2; 0.048-0.056 m^2 floor space per bird). A total of 115 transponder-implanted chicks were distributed among the 6 pens, with 19 being placed in 4 pens, 18 placed in one pen, and 21 placed in another pen. Each of the 6 pens represented a complete block, with 4 or 6 male and 4 or 6 female chicks from each TRT randomized within each of the pens (complete blocks). Three pens were arranged on each side of the middle of the grow out facility to minimize temperature differences in the facility. In the grow out period, chicks were brooded and grown out under standard conditions (Zhai et al., 2016) and received ad libitum standard starter (0 to 14 DOG) and grower (14 to 21 DOG) Mississippi State University basal broiler diets, which were formulated to meet or exceed NRC (1994) recommendations (Fatemi et al., 2024).

After placement, litter temperature (**LT**) and individual bird CBT readings were recorded at 12:00 and 5:00 PM on 1 DOG; at 7:00 AM, 12:00 PM, and 5:00 PM from 2 through 20 DOG; and at 7:00 AM on 21 DOG. The mean of the individual readings on each DOG were calculated. Quadrant LT readings were recorded using a Westward 54TZ29 infrared thermometer with an accuracy of ± 1.27 °C (W. W. Grainger, Inc., 100 Grainger Parkway, Lake Forest, IL, 60045), and the averages of the 4 LT readings in each pen were calculated. The same transponder, wireless probe, and telemetric technological software used for measuring ET were again employed to detect and record the CBT of each chick. Correlation analysis between the daily means of CBT and LT was performed. Furthermore, means of the ET readings recorded on 14, 16, and 19 DOI, and means of the CBT readings on 7, 14, and 21 DOG were used in the correlation analysis between mean ET at 14, 16, and 19 DOI with mean CBT at 7, 14, and 21 DOG without (Table 1) and with (Table 2) BW and HT assigned as covariates.

Individual chick BW was determined at placement (0 DOG), and both individual chick BW and BL were determined on 7, 14, and 21 DOG. Chick BL was measured by the same method described by Hill (2001), Joseph et al. (2006), and van den Brand et al. (2019), and is considered as an estimator of the initial quality (Molenaar et al., 2008) and potential performance (Tona et al., 2005) of chicks. Chick BW to length ratio (**BWTLR;** BW/BL) was also calculated (Fatemi et al., 2023), which represents body mass per unit of body length, and is an estimator of body mass index. The post hatch variable measurements that were used were restricted to those birds that were viable throughout the entire course of the study. Upon euthanizing all chicks by carbon dioxide asphyxiation at 21 DOI, their sex was confirmed by necropsy, and the confirmed sex was used in all statistical analyses.

Table 1
Partial correlation coefficients [r (Prob > |r|)] between mean embryo temperature at 14, 16 and 19 d of incubation (**DOI**) and mean chick body temperature at 7, 14, and 21 d of grow out (**DOG**) without hatch time and chick BW assigned as covariates[1]

Embryo Temperature	Chick Body Temperature[2]		
DOI	DOG		
	7	14	21
14	0.06111	0.21686	0.21382
	(0.5221)	(0.0216)	(0.0236)
16	0.09111	0.23558	0.21752
	(0.3372)	(0.0120)	(0.0207)
19	0.04675	0.16578	0.23077
	(0.6229)	(0.0793)	(0.0139)

[1] Level of significance was set at P ≤ 0.05.
[2] The same transponder implanted in the egg to record embryo temperature was inserted subcutaneously in the corresponding chick that hatched from that egg to record its body temperature.

Table 2
Partial correlation coefficients [r (Prob > |r|)] between mean embryo temperature at 14, 16 and 19 d of incubation (**DOI**) and mean chick body temperature at 7, 14, and 21 d of grow out (**DOG**) with hatch time and chick BW assigned as covariates[1]

Embryo Temperature	Chick Body Temperature[2]		
DOI	DOG		
	7	14	21
14	0.03036	0.02422	0.10266
	(0.7540)	(0.8026)	(0.2881)
16	0.06679	0.01421	0.08931
	(0.4881)	(0.8828)	(0.3535)
19	0.01763	-0.00085	0.09783
	(0.8549)	(0.9930)	(0.3093)

[1] Level of significance was set at P ≤ 0.05.
[2] The same transponder implanted in the egg to record embryo temperature was inserted subcutaneously in the corresponding chick that hatched from that egg to record its body temperature.

Statistical analysis

An RCB experimental design was used, with individual bird considered as the experimental unit. All data analysis procedures were conducted using SAS software (version 9.4, SAS© Institute, 2013). A repeated measures ANOVA of Proc MIXED was used in the analysis of the post hatch variables. In the experimental model, DOG, TRT, and sex were fixed effects, whereas pen, replicate incubator within TRT, and pen × TRT and pen × replicate incubator interactions within TRT served as random effects. The main and interactive effects of DOG, TRT, and sex on CBT, BW, BL, and BWTLR were analyzed. In addition, an analysis of potential LT main and interactive (LT × TRT) influences on CBT were conducted. Least squares means were separated by least significant difference (Steel and Torrie, 1980). With and without chick BW and HT being assigned as covariates, partial correlation analysis, using MANOVA of Proc GLM, was conducted between ET at 14, 16, and 19 DOI and CBT at 7, 14, and 21 DOG. Furthermore, using the same procedure, partial correlation analysis was conducted between CBT and LT across DOG and TRT, and within STRT and LTRT across DOG. Differences were considered significant at P ≤ 0.05.

Results

Abbreviations and their meanings used in the results and the discussion sections and tables

BL (body length), BWTLR (BW to length ratio), CBT (chick body temperature), DOG (d of grow out), DOI (d of incubation), EST (eggshell temperature), ET (embryo temperature), HT (hatch time), LT (litter temperature), LTRT (lower incubation temperature), STRT (standard incubation temperature), and TRT (treatment).

Chick body temperature and litter temperature

Using daily mean CBT values, it was observed that there was no significant effect (P = 0.8077) due to sex on CBT. However, there were significant main effects of DOG (P < 0.0001) and TRT (P = 0.0045) on CBT. Furthermore, there was a significant DOG × TRT interaction (P < 0.0001) for CBT (Table 3). In the STRT and LTRT, the lowest and highest CBT readings occurred at 1 and 18 DOG, respectively. Conversely, in the STRT, the CBT at 18 DOG was significantly higher than those between 1 and 15 DOG, and at 21 DOG; whereas, in the LTRT, the CBT at 18 DOG was significantly higher than those between 1 and 8, 10 and 15, and 19 and 21 DOG, and at 17 DOG. The differences in CBT between the STRT and LTRT also varied over DOG, with the CBT readings in the STRT being significantly higher than those in the LTRT at 1, 6, 10-13, and 15-21 DOG (CBT in the STRT was higher than that in the LTRT on 13 out of 21 total DOG) (Table 3).

There was no significant difference (P = 0.9812) in LT between the TRT groups. Moreover, across DOG and TRT, there was no significant correlation between CBT and LT (r = -0.00641, Prob > |r| = 0.6126). Also, across DOG, no significant correlations were observed between CBT and LT within the STRT (r = 0.01633, Prob > |r| = 0.3659) and the LTRT (r = -0.02229, Prob > |r| = 0.2008). In addition, there was no significant LT main effect (P = 0.4479) or LT × TRT interactive effect (P = 0.1187) on CBT.

Correlation of corresponding embryo and chick body temperatures

When chick HT and BW were not accounted for in the correlation analysis between CBT at 7, 14, and 21 DOG with ET at 14, 16, and 19 DOI, there were significant correlations between CBT at 14 DOG with ET at 14 and 16 DOI, and between CBT at 21 DOG with ET at 14, 16, and 19 DOI (Table 1). However, when chick HT and BW were accounted for in the correlation analysis (HT and BW were assigned as covariates), there were no significant correlations between CBT and ET at any of the time periods (Table 2).

Chick BW, BL, and BWTLR

No significant TRT difference in chick mortality occurred, as only one mortality was observed throughout the post hatch trial. There were significant main effects due to DOG (P ≤ 0.0001), TRT (P = 0.0373), and sex (P ≤ 0.0001), and significant DOG × TRT (P ≤ 0.0001), DOG × sex (P ≤ 0.0001), and TRT × sex (P = 0.0440) interactions for BW. Across sex, chick BW was decreased by 67.7 and 144.9 g at 14 and 21 DOG, respectively, due to the LTRT. Nevertheless, there was a significant (P = 0.0172) DOG × TRT × sex interaction for BW (Table 4). At 14 and 21 DOG, bird BW was greatest in males belonging to the STRT, which was significantly greater than males in the LTRT and females in the STRT. However, the BW of the males in the LTRT and the females in the STRT did not differ significantly. Also, at 21 DOG, the BW of LTRT males and STRT females was significantly greater than that of females in the LTRT, but at 14 DOG, STRT and LTRT females did not differ significantly. Conversely, at both 0 and 7 DOG, there were no significant differences in mean BW between any TRT-sex combination group.

There were significant main effects due to DOG (P ≤ 0.0001), TRT (P = 0.0007), and sex (P = 0.0037) for BL. Across DOG and sex, BL in the STRT (32.66 ± 0.18 cm) was significantly longer than that in the LTRT (32.02 ± 0.17 cm). However, there was a significant DOG × sex (P = 0.0074) interaction for BL, with the BL of male chicks being significantly longer than that of females at 14 and 21 DOG (Table 5).

There were significant main effects due to DOG (P ≤ 0.0001), TRT (P = 0.0499), and sex (P ≤ 0.0001) for BWTLR. However, there was a significant DOG × sex interaction (P ≤ 0.0001) for BWTLR, with the BWTLR of male chicks being significantly greater than that of females at 14 and 21 DOG (Table 5). There was also a significant DOG × TRT interaction (P = 0.0513) for BWTLR, with the BWTLR of chicks in the STRT being significantly greater than that of those in the LTRT at 14 and 21 DOG (Table 6).

Discussion

As broilers continue to be genetically selected for larger size, it is possible that the embryos are being overheated during incubation. This is of particular concern when incubating modern commercial broiler hatching eggs containing embryos that can generate greater amounts of

Table 3
Mean chick body temperature (**CBT**) between 1 and 21 d of grow out (**DOG**). The treatment (**TRT**) groups were a standard temperature TRT (**STRT**, 37.5 °C) provided between 0 and 21 d of incubation (**DOI**), and a reduced temperature TRT (**LTRT,** 35.6 °C) provided between 12 and 21 DOI. Eggs in the LTRT were incubated at the STRT between 0 and 12 DOI[1]

DOG	Chick body temperature (°C)					
	STRT			LTRT		
	Mean	SEM	N	Mean	SEM	N
1	40.44^{A,m}	0.027	56	40.23^{B,k}	0.027	60
2	40.58^{i}	0.025	84	40.49^{j}	0.025	90
3	40.79^{i}	0.026	84	40.79^{fgh}	0.025	90
4	40.65^{k}	0.026	84	40.64^{i}	0.025	90
5	40.73^{jk}	0.026	84	40.67^{i}	0.025	90
6	40.77^{A,ij}	0.026	84	40.64^{B,i}	0.025	90
7	40.85^{h}	0.026	84	40.77^{gh}	0.025	90
8	40.85^{h}	0.026	84	40.77^{gh}	0.025	90
9	41.00^{bcde}	0.026	84	40.92^{ab}	0.025	90
10	40.95^{A,efg}	0.026	84	40.83^{B,def}	0.025	90
11	40.86^{A,h}	0.026	84	40.75^{B,h}	0.025	90
12	40.91^{A,gh}	0.026	84	40.77^{B,gh}	0.025	90
13	40.98^{A,cdef}	0.026	84	40.81^{B,efg}	0.025	90
14	40.94^{fg}	0.026	84	40.86^{cde}	0.025	90
15	40.96^{A,defg}	0.026	84	40.81^{B,efg}	0.025	90
16	41.05^{A,ab}	0.026	84	40.93^{B,ab}	0.025	90
17	41.02^{A,abc}	0.026	84	40.88^{B,bc}	0.025	90
18	41.06^{A,a}	0.026	84	40.94^{B,a}	0.025	90
19	41.01^{A,abcd}	0.026	84	40.87^{B,bcd}	0.025	90
20	41.03^{A,abc}	0.026	84	40.84^{B,cdef}	0.025	90
21	40.92^{A,fgh}	0.032	28	40.79^{B,efgh}	0.031	30

^{A,B} Means within a DOG row, and
$^{a–m}$ means within a TRT column with no common superscript differ significantly (P < 0.05).
[1] Significant (P < 0.0001) DOG × TRT interaction.

Table 4
Effects of standard (**STRT**; 37.5 °C between 0 and 21 d) and reduced (**LTRT**; 37.5 °C between 0 and 12 d, and 35.6 °C between 12 and 21 d) incubation temperature treatments (**TRT**), and sex (male and female) on mean BW at 0, 7, 14, and 21 d of grow out (**DOG**)

Treatment	BW[1] (g)	SEM	N
0 DOG-STRT-Female	48.2^{h}	14.4	23
0 DOG-STRT-Male	51.4^{h}	13.1	32
0 DOG- LTRT-Female	48.6^{h}	13.5	30
0 DOG- LTRT-Male	41.7^{h}	13.7	29
7DOG-STRT-Female	147.3^{g}	14.4	23
7DOG-STRT-Male	155.5^{g}	13.1	32
7DOG- LTRT-Female	133.8^{g}	13.3	30
7DOG- LTRT-Male	133.3^{g}	13.5	29
14DOG-STRT-Female	440.6^{ef}	14.4	23
14DOG-STRT-Male	483.1^{d}	13.1	32
14DOG- LTRT-Female	412.1^{f}	13.3	30
14DOG- LTRT-Male	443.9^{e}	13.5	29
21DOG-STRT-Female	899.7^{b}	14.4	23
21DOG-STRT-Male	1,033.8^{a}	13.1	32
21DOG- LTRT-Female	866.6^{c}	13.3	30
21DOG- LTRT-Male	922.0^{b}	13.5	29

$^{a–h}$ TRT means with no common superscript are significantly (P < 0.05) different.
[1] Significant (P = 0.0172) DOG × TRT × sex interaction.

Table 5
Effects of sex (male and female) on mean body length (**BL**) and BW to length ratio (**BWTLR**) at 7, 14, and 21 d of grow out (**DOG**)

	BL (cm)[1]		BWTLR (g/cm)[2]		
Treatment	Mean	SEM	Mean	SEM	N
7DOG-Female	24.4[e]	0.20	5.75[e]	0.35	53
7DOG-Male	24.6[e]	0.19	5.85[e]	0.34	61
14DOG-Female	32.7[d]	0.20	13.03[d]	0.35	53
14DOG-Male	33.3[c]	0.19	13.92[c]	0.34	61
21DOG-Female	39.1[b]	0.20	22.57[b]	0.35	53
21DOG-Male	40.0[a]	0.19	24.45[a]	0.34	61

[a–e] TRT means within a variable column with no common superscript are significantly ($P < 0.05$) different.
[1] Significant ($P = 0.0074$) DOG × sex interaction.
[2] Significant ($P < 0.0001$) DOG × sex interaction.

Table 6
Effects of standard (**STRT**; 37.5 °C between 0 and 21 d) and reduced (**LTRT**; 37.5 °C between 0 and 12 d, and 35.6 °C between 12 and 21 d) incubation temperature treatments (**TRT**) on mean BW to length ratio (**BWTLR**) at 7, 14, and 21 d of grow out (**DOG**).

Treatment	BWTLR[1] (g/cm)	SEM	N
7DOG-STRT	6.08[c]	0.35	55
7DOG-LTRT	5.51[e]	0.34	59
14DOG-STRT	13.87[c]	0.35	55
14DOG-LTRT	13.08[d]	0.34	59
21DOG-STRT	24.21[a]	0.35	55
21DOG-LTRT	22.81[b]	0.34	59

[a–e] TRT means with no common superscript are significantly ($P < 0.05$) different.
[1] Significant ($P = 0.0513$) DOG × TRT interaction.

metabolic heat. The concentration of water, as a byproduct of lipid metabolism during embryogenesis, increases in broiler hatching eggs, which subsequently results in a greater rate of water loss from the egg (Ar, 1991). In the companion report by Lindsey et al. (2023), in which percent egg weight loss was determined between 13 and 15, 15 and 17, and 13 and 17 DOI, it was noted that mean percent egg weight losses between 13 and 17 DOI were 2.4 % and 2.0 % in the STRT and LTRT, respectively. Across TRT, percent egg weight loss and ET were significantly positively correlated between 15 and 17 DOI. Moreover, between 13 and 15 DOI and 13 and 17 DOI, percent egg weight loss and ET were significantly positively correlated in the STRT, but they were not correlated in the LTRT in any of the 3 DOI intervals. The lower percent egg weight loss and the lack of a significant correlation between percent egg weight loss and ET in the LTRT would suggest a lower metabolic rate with a lower nutrient and oxygen demand in LTRT embryos. The research findings of Maatjens et al. (2017) support this contention by showing that glycogen liver reserves in Ross 308 hatching eggs with an EST of 35.6 °C were higher than those with an EST of 36.7, 37.8, or 38.9 °C. Hamidu et al. (2018) also reported that Ross 708 embryos incubated at 37.0 or 37.5 °C between 15 and 21.5 DOI consumed more oxygen from 16 to 17 DOI than did those incubated at 36.0 or 36.5 °C.

Almeida et al. (2016) also found that manipulating incubation temperature during Cobb-500 broiler embryogenesis had a direct effect on their subsequent abdominal and cervical adiposity. More specifically, it was noted that a significantly smaller area of cervical adipocytes occurred in the hatchlings from eggs incubated at 39.0 °C in comparison to those incubated at 37.5 °C. This would suggest that the effects of the STRT on lipid catabolism in the embryo could likewise extend to the Ross 708 hatchling, which would justify conducting research to determine tissue lipid profiles in the broilers hatched from eggs subject to the STRT and LTRT regimens.

The HT of the LTRT eggs in the current study were reported by Lindsey et al. (2023) to be delayed between 14 and 19 h later in comparison to those in the STRT. This is in agreement with the results of Almeida et al. (2016), who observed that Cobb 500 broiler hatching eggs incubated at 36.0 °C between 13 and 21.7 DOI had a significantly greater HT than those eggs incubated at either 37.5 or 39.0 °C. A later HT can cause a chick to have a lower BW than those that hatched earlier. This is supported by the report by Ipek et al. (2015), who found that when Cobb 500 broiler hatching egg EST was decreased from 38.0 °C to 35.0 °C between 0 and 18 DOI, that broiler BW through 6 wk post hatch decreased significantly. Consequently, a smaller chick would also be expected to produce a lower volume of heat than a larger chick.

Romjin and Lokhorst (1955) indicated that by 7 DOG, CBT in a classical chicken breed becomes more consistent. However, although the CBT readings of the Ross 708 broilers in both TRT of this experiment were the same at 7 and 8 DOG, variable fluctuations were observed before and after those times. More notable was the finding that the current CBT readings were significantly greater in the STRT than in the LTRT in 13 of the 21 DOG. Lourens et al. (2005) found that when chicks of an unidentified strain were incubated at 36.7 °C for the first wk of incubation, they had a lower rectal temperature through 7 DOG than those incubated at 37.8 °C and 38.9 °C, and that the highest rectal temperatures at hatch were found in chicks incubated at 37.8 °C for the entire duration of incubation. It was also noted in the Ross 708 broiler hatching eggs of the present study that ET in in the second and third wk of incubation was significantly correlated with CBT in the second and third wk of grow out. In addition to differences in the timing of the DOI readings in this study and that of Lourens et al. (2005), possible differences in broiler strain may have skewed the reported results of the 2 studies concerning the relationship between ET and CBT. However, the overall results of both studies clearly indicate that without consideration of the influences of BW and HT, ET and CBT are correlated in broiler hatching eggs. The results of this study and that of Lourens et al. (2005) have commonality, when chick BW and HT are not accounted for as potential confounding factors. Nevertheless, when the influences of BW and HT were accounted for in the current study, there was no significant correlation between the ET and CBT of the Ross 708 broilers. Although multiple factors can influence CBT, based on the results of the current study, it may be inferred that BW and HT have major influences on CBT.

Lowering incubation temperature from 37.5 °C to 35.6 °C at 12 DOI subsequently lowered CBT and negatively affected chick BW, BL, and BWTLR. Across DOG and sex, BW was significantly greater in the STRT than in the LTRT. These results are in agreement with those of Maatjens et al. (2016a), who found that in comparison to EST readings of 36.7, 37.8, or 38.9 °C between 19 DOI until hatch, an EST reading of 35.6 °C resulted in a lower yolk-free body mass in Ross 308 hatchlings. Nevertheless, the current differences in BW became manifested later in the 21 DOG period. Male and female chick BW in the STRT was noticeably heavier than those chicks in the LTRT at 14 and 21 DOG. Ipek (2016), who incubated Cobb-500 broiler hatching eggs at different temperatures from 10 to 18 DOI, also found that chicks from eggs incubated at temperatures from 33.3 to 36.7 °C had a significantly lower BW than chicks incubated from 37.8 to 38.2 °C by 42 DOG. Although hatchlings in the STRT hatched approximately 14 to 19 h sooner, and therefore, remained in their respective incubators longer than those in the LTRT, this had no significant impact on post hatch chick BW through 7 DOG. The initial BW results in this study are in closer accordance with the results of Hamidu et al. (2018), who found no differences in the initial BW of Ross 708 chicks incubated at 36.0, 36.5, 37.0, or 37.5 °C. Although Maatjens et al. (2016b) observed that Ross 308 broiler BW at 7 DOG was higher when they hatched from eggs having an EST of 36.7 °C than at either 35.6, 37.8, or 38.9 °C, it was also found that the organ weights and quality of the Ross 308 broiler chicks that hatched from eggs having an EST of 35.6 °C was equal to or higher than those that hatched from eggs having an EST of 36.7 °C. In addition to an increased hepatic glycogen concentration, Maatjens et al. (2017) also attributed the beneficial effects of the 35.6 °C EST on the physiology of the embryos to a higher oxygen availability associated with their lower metabolic rate.

Sokale et al. (2021) has noted that after a reduction in growth performance in broilers through 28 DOG in response to a peak in intestinal coccidia cycling, subsequent compensatory growth occurred by 35 DOG, and Geers et al. (1983) and Bruzual et al. (2000) observed compensatory growth in chickens and broilers, respectively, after being subjected to changes in incubational conditions. More specifically, Geers et al. (1983) observed post hatch compensatory growth in Rhode Island Red chickens after being incubated at a lower incubation temperature between 1 and 10 DOI, and Bruzual et al. (2000) observed that if brooded at a sufficiently high temperature, broiler chicks can experience acceptable livability and growth despite being hatched from eggs that were incubated at 43, 53, or 63 % between 16 and 21.67 DOI. Because compensatory growth may continue past 21 DOG, a subsequent experiment with a longer grow out period may, therefore, be necessary in order to observe later possible differences in BW between chicks incubated at 35.6 °C or 37.5 °C from 12 DOI until hatch.

Across DOG and sex, BL and BWTLR of the Ross 708 broilers were greater in the STRT than in the LTRT. In contrast, van den Brand et al. (2019) observed no significant effects on the 6 h post hatch BL of layer chicks that were hatched from eggs having an EST of either 36.7, 37.8, or 38.9 °C from 14.5 DOI to hatch. Hamidu et al. (2018) also observed that BL was not different in Ross 308 broilers hatched from eggs incubated at 36.0, 36.5, 37.0, or 37.5 °C, and that it was significantly greater when Ross 708 hatching eggs were incubated at 37.0 rather than 37.5 °C. However, similar to the results of the current study, Wijnen et al. (2020) showed that an EST of 38.9 °C resulted in a significantly longer BL of Ross 308 broilers on wk 2 and 3 of grow out than did an EST of 37.8 °C. There are no earlier studies that have been conducted to determine the effects of incubation temperature on the post hatch BWTLR or body mass index of broilers. Nonetheless, in broilers, the BL and BWTLR results in response to TRT in this study followed the same pattern as BW.

It is concluded that CBT is not directly associated with ET in Ross 708 broilers, and that although implementation of the LTRT, in which incubation temperature was lowered from 37.5 to 35.6 °C between 12 and 21 DOI, may lead to some physiological benefits during embryogenesis, the observed reductions in post hatch CBT and performance in response to the LTRT were related to a delay in chick HT and associated adverse effects on their BW. Although a reduction in incubational energy costs may be realized by employing the LTRT, its subsequent adverse effects on HT and BW may outweigh its benefits. The effects of incubational temperature on the developing embryo also depend on broiler strain, the stage of incubation, and the length and magnitude of exposure (Wilson, 1991). With this in mind, commercial hatchery managers should also consider these factors before instituting certain incubational temperature regimens. A small temperature reduction for a shorter duration may provide acceptable results. Nevertheless, because embryos tend to be more susceptible to suboptimal temperatures during early incubation (Romanoff, 1936), it is recommended that any reductions in incubational temperature be implemented during the later stages of incubation. Increased incubator size and replication in an environment that better reflects those in a commercial hatchery, would also better account for potential environmental and management influences encountered in industrial facilities. Therefore, using the general concepts applied in this study, further applied research is required to better establish the effects of these TRT in scopes and settings that reflect those in a commercial hatchery.

Declaration of competing interest

None

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