Impact of incubation temperature profile on chick quality, bone, and immune system during the late period of incubation of Cobb 500 broiler strain

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ABSTRACT The present study was conducted to examine the impact of incubation temperature profile on embryonic growth and chick quality post-hatch. Hatching eggs (n = 405) were incubated in a Jamesway PS-5000 single-stage incubator at 37.5°C and 56% RH until embryonic day 14 (**ED14**). At ED14, 135 eggs each were transferred into 3 identical G.Q.F. MFG. CO incubators, and each set to one of the following incubation temperatures: 36.5°C, 37.0°C, and 37.5°C. Data on eggshell temperature (**EST**) and embryo quality were collected from ED15 to ED20. At hatch, chick quality and leg bone qualities were assessed. Blood collected from chicks was used to assess hematological and immunological parameters. The remainder of the chicks was reared on standard broiler feed for 8 wk to measure growth performance. Data were analyzed using the SAS Proc. GLM at $P \leq 0.05$. The daily EST was higher at 37.5°C incubation temperature compared to 36.5°C and 37.0°C during ED15 to ED21. Chicks of 37.5°C had early external pipping and hatching times compared to 36.5°C. There were no significant differences in external chick quality parameters. The chick leg bone Ca and P levels increased with increasing incubation temperature at day old, 4 wk, and 8 wk. Besides mean corpuscular hemoglobin and concentration, which were higher at 37.5°C compared to 36.5°C and 37.0°C, all blood parameters measured were not different. Bone mineral levels may not be the same as bone development. Therefore, appropriate incubation and nutritional strategies are needed to increase bone development, and broiler growth to reduce leg problems.

Key words: incubation temperature, eggshell temperature, embryo length, chick length, bone development

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INTRODUCTION

The temperature of the incubator is one of the most important parameters for embryonic development, hatchability, and subsequent broiler performance during the incubation period (Leksrisompong et al., 2007; Willemsen et al., 2010; Molenaar et al., 2011a). Managing the embryo or eggshell temperature is more important than controlling the incubator temperature since embryos with rapid growth rates appear to be sensitive to temperature variations (Hamidu et al., 2007; Molenaar et al., 2010). Eggs containing living embryos absorb heat from the incubator's surrounding air during the first stage of incubation, and the embryo temperature is lower than the incubator temperature (Leksrisompong et al., 2007; Pulikanti et al., 2012). In contrast, due to increased heat production from developing embryos, optimal incubation temperature ranges are difficult to achieve during the second stage of the incubation period, especially after embryonic day (**ED**) 9 (Lourens et al., 2007; Meijerhof, 2009).

Furthermore, higher incubation temperatures decrease the development of the crop, gizzard, proventriculus, liver, and gut in embryos (Maatjens et al., 2014), with the heart being the organ that is most consistently affected by increased temperatures (Wineland et al., 2000; Leksrisompong et al., 2007). Higher eggshell temperatures (**EST**) (39.5°C) after ED 14 reduced relative heart weight in broilers by 17 to 31% (Wineland et al., 2000; Leksrisompong et al., 2007). Higher incubation temperature may also have a role in metabolic problems linked to cardiovascular growth such as ascites (Molenaar et al., 2011a). Beyond 14 d of incubation, there is evidence that lower or higher incubation temperatures affect embryonic metabolism and chick quality (Hamidu et al., 2018). Few studies, however, have been conducted to compare the effects of a short period of increased incubation temperature against embryonic

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development and subsequent broiler performance, especially in the third period of incubation. The objectives of this study were to determine the effect of variation in incubation temperature on embryonic development and chick quality, and calcium and phosphorous levels in the bone of hatched chicks. Second, to determine the carry-over effect of varying incubation temperature during embryogenesis on broiler growth and calcium and phosphorous levels in the bones.

MATERIALS AND METHODS

Experimental Site

The study was conducted at the Olympio Hatchery, Department of Animal Science, Kwame Nkrumah University of Science and Technology (**KNUST**), Kumasi-Ghana. All experiments were conducted according to the Guidelines and Procedures for Animal Research Ethics Committee (**AREC**) of the Kwame Nkrumah University of Science and Technology, Kumasi-Ghana, Quality Assurance, and Planning Unit (KNUST POLICY 0016) (AREC, 2018).

Experimental Design

A total of 405 freshly laid eggs from Cobb 500 breeders of the same age (56 wk) were randomly collected at a commercial breeder farm (Topman Farms, Kumasi). The eggs were selected at random at the beginning of the study (d 1 of incubation), however, data collection started at d 15 of incubation and difference in egg weight considered during data analysis. When eggs were initially collected they were stored in a cold room at temperature of 18°C at a relative humidity of 75% for 24 hours. Afterwards eggs were pre-warm for 6 h in a room temperature before setting. All the eggs were initially incubated in 5,040 egg capacity single-stage Jamesway incubator (PS-5000) at a temperature of 37.5°C and 56% until the 14th day (ED14). The eggs were set along with other similarly aged eggs, however, the experimental eggs were closer to incubator fan to ensure there were no secondary heat and ventilation from the other eggs to the experimental eggs. Afterward, the 135 trial eggs each were transferred into three identical 300 egg capacity G.Q.F. MFG. CO. incubators (Model GQF1502, Wichita, Kansas) and set at either 36.5°C, 37.0°C, or 37.5°C incubation temperatures in a completely random design and 65% RH during ED14 to ED21. The thermostat operating range of the incubator is between 60 and 103°F with electrical specifications including 110-120 VAC, 50/60 Hz, 325 Watts. Temperature and humidity inside the incubators were recorded with a LogTag HAXO-8 humidity and temperature recorders (sensor specifications: Temperature Arrange −20°C to 60°C Temperature Accuracy ± 0.2°C, Humidity Arrange 0 to 100% RH, Humidity Accuracy ±2% RH).

Eggshell and Embryo Parameters Measured

Eggshell Temperature The eggshell temperatures from 10 eggs were measured 4 times daily (morning, afternoon, evening, and night) at 6-h intervals from ED15 to ED20 days. The temperature of the eggshells was measured at the equator of the eggs using the Vicks Thermometer (model V971 CFN- CAN; temperature range is 89.6°F to 109.2°F, accuracy within ±0.2°F). The following conditions were checked during the measurement of the eggshell temperatures. Eggs were sampled from various positions of the setter. The eggshell temperatures were measured from eggs based on their position in three horizontal racks in the incubators (Figure 1). At least 3 eggs each were randomly targeted at each time during measurement on the first rack to the third rack. Eggs were selected from back, middle, and front position of the rack in all the in the incubators. The last egg to make it to10 in each of the incubators was allocated randomly from the middle or second rack from the bottom or top of the incubator.

Five eggs from each treatment from 15 to 20 d of incubation were selected and broken to determine the egg and the embryo characteristics such as egg weight, embryo weight, embryo length, embryo temperature, femur and tibia length and width, and eggshell thickness. While all 10 eggs for EST were selected specifically based on position in the incubators as described above, the 5 eggs broken to measure embryo parameters were randomly selected soon after taking the EST

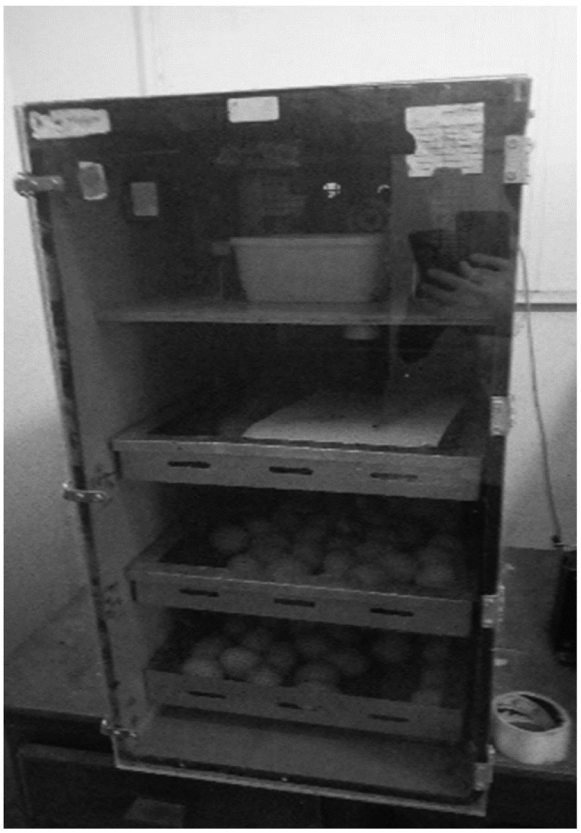

Figure 1. A 300 egg capacity G.Q.F. MFG. CO. incubator with eggs set up in three horizontal rack.

measurement without necessarily considering them being used earlier to measure the EST.

Egg Weight Egg weight was measured using an Ohaus Scout Pro portable precision electric balance (model SP602AM; 165 mm × 142 mm; 2,000 g, resolution: 0.1 g).

Embryo Weight The embryos were separated from the yolk, the excess moisture extracted, and then weighed. The embryo was then stretched gently for other measurements to be taken (Hamidu et al., 2011; Willemsen et al., 2011).

Embryo Length The embryo length was measured with a divider from the tip of the beak to the tip of the middle toes and then placed on a meter rule to determine the length (Browne, 2006).

Embryo Temperature Embryo temperature which is also known as the rectal temperature was measured with the Microlife Vet-Temp thermometer (Model VT 1831; Temperature range is between 34°C and 42°C), which was placed in the rectum of the embryo to determine the temperature.

Femur and Tibia Length The lengths of the femur and tibia were determined with the aid of the Vernier caliper. Each tibia and femur bone was placed in boiling water for 15 min to facilitate tissue removal after which the width was taken with a caliper and transferred to a ruler while a ruler was used to measure the length of each bone.

Eggshell Thickness The eggshell thicknesses (mm) of the 5 eggs broken above comprising the eggshell itself and shell membrane following air drying after 24 h in ambient temperature were measured using a micrometer screw gauge (model H-2780; measurement range 0 to 25 mm, resolution 0.001 mm, accuracy ± 1 μm) at the equator of the egg.

External Piping and Hatching Times From the 19th d, external pipping and hatching times were assessed by checking the remaining eggs at every 6 h interval (6 am, 6 pm, 12 pm, and 12 am) in each of the three different incubation temperatures. To avoid confusion about which chicks were pipping or hatching the eggs or chicks were marked after every checking to help different them from others that may pipp or hatch at the next checking 6 h afterward.

Post-hatch Chick Quality Assessment

On the 21st d, 5 hatched chicks from each treatment were removed at random to assess: chick weight, chick length, shank length, yolk sac disappearance of dissected chicks, calcium and phosphorus contents of the chick bone, hematological, and immunological properties.

Chick Weight Five chicks from each treatment were placed on the Ohaus External chemical balance and their weights were recorded (model SP602AM; 165 mm × 142 mm; 2,000 g; resolution 0.1 g).

Chick Length The total length of the chick was determined from the tip of the beak to the end of the middle toe. Precautionary measures were implemented in order not to cause injury to the chick during the measurement of a chick's length.

Shank Length The shank length was measured from the tip of the shank to the mid-portion between the feet with a Vernier caliper.

Yolk Sac Disappearance The yolk sac disappearance was determined in the five chicks which were used in the measurement of chick length and shank length. Their yolk sacs were surgically removed and weighed. The wet yolk sac and yolk free body were oven-dried for 4 d at a temperature of 65°C and then re-weighed to determine the dry yolk sac, dry yolk free body mass, and actual weight of the yolk.

Hematological and Immunological Properties

Two chicks were euthanized using a sterilized knife and the blood samples were collected into EDTA tubes and analyzed using an auto-analyzer to measure the following blood parameters, erythrocytes (red blood cells), and leukocytes (white blood cells). The differential count (white blood cells) was also analyzed for monocyte percentage, neutrophil percentage, lymphocyte percentage, basophil percentage, and eosinophil percentage.

Growth Performance

All hatched chicks left after those dissected for chick quality analysis comprising of 82 chicks from 36.5°C, 86 chicks from 37.0°C and 89 chicks from 37.5°C incubation temperatures (total = 257) were split into 4 replicates per treatment and reared under field conditions for 8 wk using recommended feed formula meeting the nutritional requirement of broilers (NRC, 1994). Chicks from the 3 treatments were randomly allocated into pens and replicated 3 times (36.5°C: pen 1 = 19 chicks, pen = 21 chicks, pen 3 = 21 chicks, pen 4 = 21 chicks; 37.0°C: pen 1 = 21 chicks, pen = 21 chicks, pen 3 = 22 chicks, pen 4 = 22 chicks; 37.5°C: pen 1 = 21 chicks, pen = 23 chicks, pen 3 = 23 chicks, pen 4 = 22 chicks). The chicks were given the same standard broiler diet (Table 1). Feed and water were provided ad libitum. Medications and vaccinations were administered orally according to the schedule of the Ghana Veterinary Service Directorate. Feed intake, weight gain, and feed conversion ratio were recorded.

Calcium and Phosphorus Determination

The calcium and phosphorous contents of the bones were determined at hatch, 4 wk, and 8 wk of age using 2 birds from each replicate. At each stage, 2 chicks from a replicate were selected and euthanized by cervical dislocation. The left tibia bone was removed and cleaned of flesh. The calcium and phosphorus contents were determined by dry-ashing the bone sample at 550°C in a furnace and dissolving the ash in 10% HCl followed by

Table 1. Composition of standard broiler diet.

Ingredient, air dry basis	Amount (g/kg)
Maize	630.0
Wheat bran	60.0
Soya bean meal	160.0
Fishmeal	130.0
*Vitamin mineral premix	10.0
Salt	5.0
Oyster shell	5.0
Total	100.0
Analyzed chemical composition	
Moisture	136.0
Crude fat	39.0
Crude fiber	28.4
Total ash	55.0
Crude protein	229.9
Nitrogen free extract	511.7
Metabolizable energy (kcal/kg)	2,960.56
Calcium	8.23
Phosphorous	28.74

*Vitamin mineral premix per kg of diet: Fe 100 mg, Mn 110 mg, Cu 20 mg, Zn 100 mg, Se 0.2 mg, Co 0.6 mgsanoquin 0.6 mg, retinol 2,000 mg, cholecalciferol 25 mg, tocopherol 23,000, menadione 1.33 mg, cobalamin 0.03 mg, thiamin 0.83 mg, riboflavin 2 mg, folic acid 0.33 mg, biotin 0.03 mg, pantothenic acid 3.75 mg, niacin 23.3 mg and pyridoxine 1.33 mg.

filtering. The EDTA titration method and the Atomic absorption spectrometer (**AAS**) were used to determine the calcium and phosphorous contents of the bones (Moss, 1961; Paul et al., 2014)

Statistical Analysis

The data obtained were analyzed as one-way ANOVA using the Proc. GLM procedure of SAS 9.4 (SAS Institute Inc., 2012). The means were separated using Duncan multiple range test at $P < 0.05$. The statistical model used to analyze all egg, embryo and chick reponses relied on the fixed effect of incubation temperature. The statistical model used was: $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where: Y_{ij} = effect measured, μ = overall mean, α_i = main effect of incubation temperature, and ε_{ij} = residual error term. The experimental units were individual eggs (n = 10 for EST and n = 5 for embryo parameters), hatch chicks (n = 5), and broiler chicks (n = 4 pens as replicates). Regression analysis was performed between incubation temperature, eggshell temperature, and embryo temperature to determine their relationship.

RESULTS

Effect of Incubation Temperature on Egg Weight and Eggshell Temperature

The results of the study (Table 2) indicated that incubation temperatures had no significant effect on the egg weight before breakout during ED15 to ED20 ($P > 0.05$). The egg weights measured daily did appears suggest any confounding effect of egg weight on daily embryo parameters because of the lack of significant differences. The EST increased significantly with increasing incubation temperature in all the days of incubation irrespective of the time of day (midnight, morning, afternoon, and evening). There was moderately stronger positive linear relationship between incubation temperature and eggshell temperature ($y = 0.9508x + 2.8678$; $R^2 = 0.64$; Figure 2).

Table 2. Effect of different incubation temperatures on egg weight (g) depreciation during the late period of incubation of Cobb 500 broiler breeder eggs.

	Incubation temperature (°C)				
Days	36.5°C	37°C	37.5 °C	SEM	*P*-value
15	61.31	57.82	60.04	2.269	0.5606
16	57.58	60.08	60.47	1.728	0.4623
17	58.28	58.79	59.14	1.698	0.9371
18	57.64	57.41	57.53	2.013	0.9965
19	57.65	59.81	58.76	1.506	0.6125
20	60.95	57.96	60.57	1.276	0.2368

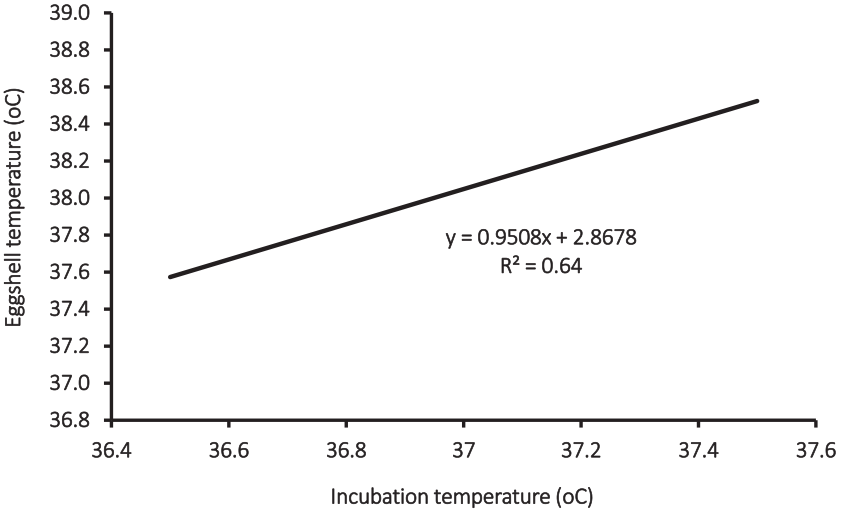

Figure 2. Relationship between incubation temperature and eggshell temperature during incubation of Cobb 500 broiler eggs from 15 to 20 d of incubation.

Effect of Incubation Temperature on Embryo Development Characteristics

The incubation temperature variation affected the embryo temperature at ED15, ED18, and ED20 (Table 3). The embryo temperatures were higher in 37.0°C and 37.5°C incubation temperatures compared to 36.5°C incubation temperatures. There were no differences in embryo temperature on ED16, ED17, and ED19 between incubation temperatures. The higher embryo temperature appears to follow the EST trend as the incubation temperature increases. There was also a moderately strong positive non-linear relationship between eggshell temperature and embryo temperature (Figure 3). The embryo temperatures increased with increasing incubation temperature. At ED15, ED19, and ED20, the embryo temperatures were higher at 37.0°C and 37.5°C compared to 36.5°C (Table 4). The incubation temperature did not have a significant effect on the wet embryo weights from ED15 to ED20 (Table 5). Similarly, the dry embryo weight was not different between the treatments except at ED18, where incubation temperature of 37.0°C resulted in heaver embryos compared to 36.5°C and 37.5°C, recording dry embryo weights of 25.79 g, 21.72 g, and 17.59 g, respectively ($P < 0.0001$). No significant difference in the dry embryo weights occurred between incubation temperatures on ED15 to ED17 and ED20 (Table 5).

Embryo length was longer at 37.0°C incubation temperature compared to 37.5°C at ED18 (Table 6). There was no difference in embryo lengths between 37.0°C and 36.5°C on d 18. On ED19, the embryos were longer at 37°C compared to 36.5°C. Embryo length at 37.5°C was neither different from 36.6°C nor 37°C at ED19. Table 4 shows that from d 15 to d 20 there was no significant difference in the eggshell thickness across incubation temperatures except at ED16. At ED16, the eggshell thickness was higher in 37.0°C incubation temperature compared to 36.5°C. There was no difference in the eggshell thickness between 37.5°C and either 36.5°C or 37.0°C (Table 7).

Effect of Incubation Temperature on Hatching and Chick Quality Characteristics

The pipping time was shorter at 37.5°C compared to the eggs set under 36.5°C and 37.5°C (Table 8). However, chicks from 37.5°C emerged from the eggs earlier than 36.5°C. There was no difference in the hatching time between 36.5°C and 37°C and between 37°C and 37.5°C. The chick quality parameters including chick weight, chick length, shank length, yolk free body-weight, and residual yolk weight were not different between treatments (Table 9). The blood biochemical parameters showed no significant differences except the mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, which was all higher at 37.0°C incubation temperature compared to 36.5°C and 37.0°C (Table 10).

Table 3. Effect of different incubation temperatures on the eggshell temperature of Cobb 500 strain during the late period of incubation.

		Eggshell temperature (°C)				
Day of incubation	Incubation temperature (°C)	Midnight	Morning	Afternoon	Evening	Average
15	36.5	37.13^{b}	36.85^{c}	36.24^{b}	37.95^{c}	37.04^{b}
	37	36.00^{c}	37.32^{b}	37.29^{a}	38.40^{b}	37.25^{b}
	37.5	38.14^{a}	38.06^{a}	37.47^{a}	38.84^{a}	38.13^{a}
	SEM	0.258	0.124	0.078	0.102	0.131
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001
16	36.5	37.48^{b}	37.57^{c}	38.02^{c}	38.04^{c}	37.78^{c}
	37	37.91^{b}	37.99^{b}	38.43^{b}	38.69^{b}	38.26^{b}
	37.5	38.74^{a}	38.57^{a}	38.82^{a}	39.15^{a}	38.82^{a}
	SEM	0.172	0.121	0.090	0.147	0.078
	P-value	0.0001	0.0001	0.0001	0.0001	0.0001
17	36.5	37.37^{b}	37.24^{b}	37.75^{b}	38.10^{b}	37.62^{c}
	37	37.90^{ab}	37.59^{b}	38.39^{a}	38.37^{ab}	38.06^{b}
	37.5	38.44^{a}	38.30^{a}	38.83^{a}	38.84^{a}	38.61^{a}
	SEM	0.214	0.154	0.169	0.175	0.099
	P-value	0.0050	0.0001	0.0004	0.0165	0.0001
18	36.5	37.65^{b}	37.27^{b}	38.19^{b}	38.30	37.85^{c}
	37	38.33^{a}	37.60^{b}	38.47^{b}	38.38	38.20^{b}
	37.5	38.54^{a}	38.48^{a}	38.99^{a}	38.91	38.73^{a}
	SEM	0.159	0.197	0.156	0.203	0.102
	P-value	0.0013	0.0005	0.0040	0.0877	0.0001
19	36.5	37.43^{b}	37.46^{b}	38.50^{ab}	37.83^{b}	37.81^{b}
	37	37.67^{b}	37.80^{b}	38.35^{b}	38.16^{b}	38.00^{b}
	37.5	38.49^{a}	38.45^{a}	38.99^{a}	38.81^{a}	38.69^{a}
	SEM	0.178	0.190	0.186	0.210	0.105
	P-value	0.0007	0.0035	0.0554	0.0091	0.0001
20	36.5	37.23^{b}	37.45^{b}	38.74^{a}	37.40^{b}	37.71^{b}
	37	37.63^{b}	37.43^{b}	38.20^{b}	38.00^{ab}	37.82^{b}
	37.5	38.25^{a}	38.22^{a}	39.23^{a}	38.45^{a}	38.54^{a}
	SEM	0.150	0.226	0.180	0.284	0.127
	P-value	0.0002	0.0311	0.0016	0.0469	0.0001

$^{a\text{-}c}$Means with different superscripts within a column differ significantly ($P \leq 0.05$).

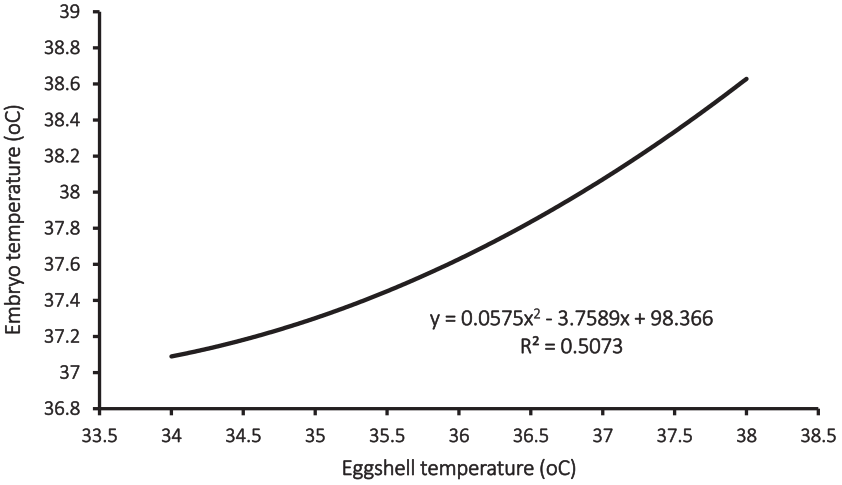

Figure 3. Relationship between eggshell temperature and embryo temperature during incubation of Cobb 500 broiler eggs from 15 to 20 d of incubation.

Table 4. Effect of different incubation temperatures on embryo rectal temperature of Cobb 500 broiler strain during the late period of incubation.

	Incubation temperature (°C)				
Days	36.5	37	37.5	SEM	*P*-value
15	34.00[b]	36.60[a]	37.22[a]	0.315	0.0001
16	36.52	36.14	36.98	0.321	0.2410
17	36.63	36.86	37.30	0.296	0.3047
18	36.96[b]	37.64[a]	38.00[a]	0.145	0.0012
19	36.98	37.48	37.42	0.207	0.2360
20	35.98[b]	37.20[a]	37.70[a]	0.288	0.0078

[a-b]Means with different superscripts within a row differ significantly ($P \leq 0.05$).

Table 6. Effect of different incubation temperatures on embryo length (mm) of Cobb 500 broiler strain.

	Incubation temperature (°C)				
Days	36.5	37	37.5	SEM	*P*-value
15	10.11	9.43	9.76	0.273	0.2653
16	11.71	11.56	11.40	0.209	0.6026
17	11.81	11.86	12.26	0.299	0.5208
18	12.53[ab]	13.07[a]	12.08[b]	0.285	0.0120
19	13.19[b]	14.43[a]	13.78[ab]	0.224	0.0120
20	14.54	13.20	14.65	0.463	0.0972

[a-b]Means with different superscripts within a row differ significantly ($P \leq 0.05$).

Effect of Incubation Temperature on Bone Development and Bone Mineral Characteristics

The tibia lengths measured from ED15 to ED20 were not significantly different except at ED15 where it was longer at 37.0°C temperature compared to 36.5°C and 37.5°C. Similarly, the femur lengths were longer in the 37.0°C incubation temperature compared to 36.5°C and 37.5°C at ED15, DE18, and ED19. The femur lengths for the rest of the days of incubation considered were not different ($P > 0.05$) between treatments (Table 11). The incubation temperatures 37°C, 37.5°C, and 36.5°C resulted in tibia lengths of 1.65 cm, 1.42 cm, and 1.39 cm, respectively. The femur lengths were different between incubation temperatures at ED15. The femur lengths at 36.5°C, 37.0°C, and 37.5°C were 1.49 cm, 2.24 cm, and 1.90 cm. The femur lengths at ED16, ED17 and ED20 were not different. But at ED18 the femur was longer in 37°C incubation temperature compared to 36.5°C but not different from 37.5°C, which was also not different from 36.5°C. However, at ED19 the femur was longer in 37°C incubation temperature compared to 36.5°C and 37.5°C. The bone calcium and phosphorus levels in day-old chicks increased with incubation temperature with 36.5°C, 37°C, and 37.5°C recording calcium levels of 13.75, 16.59, and 22%, respectively (Table 12). The trend was the same for phosphorus levels in the bone. The increasing trend of calcium and phosphorus levels followed the same trend at wk 4 and wk 8 of broiler growth.

Table 5. Effect of different incubation temperatures on daily wet and dry embryo weight (g) of Cobb 500 broiler strain during the late period of incubation.

	Wet embryo weight (g)					Dry embryo weight (g)				
Days	36.5°C	37°C	37.5°C	SEM	*P*-value	36.5°C	37°C	37.5°C	SEM	*P*-value
15	25.89	26.94	29.20	2.165	0.5614	15.41	19.83	14.97	2.050	0.2095
16	32.88	32.94	30.76	1.571	0.5788	21.83	22.50	23.84	0.794	0.2526
17	38.71	37.89	37.75	1.437	0.8856	28.06	27.32	28.54	1.032	0.6901
18	39.07	44.76	40.03	2.625	0.2806	17.59[b]	25.79[a]	21.72[ab]	2.483	0.0948
19	45.86	47.85	48.96	1.294	0.2719	37.48	31.68	35.87	2.473	0.2959
20	49.44	48.03	50.79	1.690	0.5511	37.58	38.69	35.74	0.923	0.1499

[a-b]Means with different superscripts within a row differ significantly ($P \leq 0.05$).

Table 7. Effect of different incubation temperatures on eggshell thickness (mm) of Cobb 500 broiler strain during the late period of incubation.

	Incubation temperature (°C)				
Days	36.5	37	37.5	SEM	*P*-value
15	0.56	0.54	0.54	0.022	0.8859
16	0.51[b]	0.58[a]	0.54[ab]	0.017	0.0495
17	0.57	0.57	0.54	0.013	0.2893
18	0.56	0.54	0.56	0.019	0.7486
19	0.52	0.50	0.50	0.007	0.0831
20	0.51	0.50	0.49	0.009	0.6480

[a-b]Means with different superscripts within a row differ significantly ($P \leq 0.05$).

Table 8. Effect of different incubation temperature on external pipping and hatching times of Cobb 500 broiler chicks.

Incubation temperature (°C)	External pipping	Hatching time
36.5	486.25[a]	504.58[a]
37	484.84[a]	502.94[ab]
37.5	481.75[b]	501.13[b]
SEM	0.980	0.876
P-value	0.0043	0.0212

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

Effect of Incubation Temperature on Broiler Growth Characteristics

The weekly broiler growth characteristics largely showed no significant differences in weekly feed intake, weight gain, and FCR between incubation temperatures. The results showed that at wk 5 the feed intake decreased with increasing incubation temperature (Table 13). The trend was similar in the weight gain at wk 4 and 5 and the FCR at wk 4, which all decreased with increasing incubation temperature. However, the total feed intake, the average weight gain, and average FCR were all not different between the treatments (Table 13).

DISCUSSION

EST is an important determinant of embryonic metabolism, and therefore, a predictor of overheating in incubating embryos (Hamidu et al., 2018). Eggshell temperature during incubation greatly affects embryo development, chick quality at hatch, and subsequently various broiler physiological systems (Wijnen et al.,

Table 9. Impact of different incubation temperatures on chick quality assessment of Cobb 500 broiler strain.

	Parameters measured						
Incubation temperature (°C)	Chick weight (g)	Chick length (mm)	Shank length (mm)	Wet YFBW (g)	Dry YFBM (g)	Wet RYS (g)	Dry RYS (g)
36.5	46.18	18.22	1.99	38.12	10.94	6.39	5.14
37	43.89	17.48	2.07	36.69	10.32	5.28	3.94
37.5	44.54	17.58	2.08	36.84	10.39	7.47	5.43
SEM	1.115	0.403	0.071	0.935	1.608	1.555	0.806
P-value	0.3572	0.3984	0.6810	0.5153	0.9579	0.6209	0.4076

Abbreviations: DYFBM, dry yolk free body mass; Dry RYS; dry residual yolk sac; wet YRS, wet residual yolk Sac; YFBW, yolk free body weight.

Table 10. Effect of different incubation temperatures on the blood parameters of day-old chicks of Cobb 500 broilers.

	Incubation temperature (°C)				
Blood parameters	36.5°C	37°C	37.5°C	SEM	*P*-value
WBC/UL	168,750.00	140,650.00	171,900.00	16241.767	0.4327
RBC/UL	1,233,500.00	1,765,000.00	2,070,000.00	659,198.31	0.6947
HGBg/DL	8.05	6.75	9.00	1.167	0.4829
%HCT	29.25	24.65	29.85	4.038	0.6514
MCV/fl	135.15[c]	139.80[b]	144.20[a]	0.671	0.0057
MCH/pg	37.20[b]	38.15[b]	43.45[a]	0.329	0.0017
MCHCg	27.50[b]	27.30[b]	30.15[a]	0.290	0.0104
PLT/Ul	7,500.00	5,500.00	11,000.00	4,915.960	0.7478
% LYM	97.15	98.20	98.30	0.375	0.2003
% NEUT	2.85	1.80	1.70	0.375	0.2003
LYM /UL	163,950.00	138,150.00	169,000.00	15,880.544	0.4418
NEUT/UL	4,800.00	2,500.00	2,900.00	752.773	0.2162
RDWSD/fl	39.70	52.95	52.75	8.234	0.5098
% RDWCV	19.35	20.20	18.00	0.724	0.2434
PDW/ fl	3.35	4.25	3.50	3.242	0.9783
MPV / fl	7.85	7.85	7.35	0.337	0.6103
% PLCR	18.00	13.35	14.70	3.643	0.6845
% PCT	0.01	0.01	0.01	0.005	1.0000

Abbreviations: fl, femtolitre; g/DL, gram per decilitre; HGB, hemoglobin; HCT, hematocrits; LYM, lymphocite; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; NEUT, neutrophils, pg, pictogram; PLT, platelet test; PDW, platelets distribution width; PLCR, platelet large cell ratio; PCT, procalcitonin; RBC, red blood cells; RDWSD, red cell distribution width standard deviation; RDWCV, red cell distribution width coefficient of variation; WBC, white blood cells; UL, microlitre.

[a-c]Means with different superscripts within a row differ significantly ($P \leq 0.05$).

Table 11. Effect of different incubation temperatures on chick bone tibia and femur lengths of Cobb 500 broiler after incubation.

	Tibia (mm)					Femur (mm)				
Days	36.5°C	37.0°C	37.5°C	SEM	*P*-value	36.5°C	37.0°C	37.5°C	SEM	*P*-value
15	1.39[b]	1.65[a]	1.42[b]	0.066	0.0325	1.49[c]	2.24[a]	1.90[b]	0.105	0.0015
16	1.56	1.60	1.64	0.038	0.3986	2.15	2.27	2.30	0.099	0.4694
17	1.68	1.70	1.77	0.074	0.6489	2.55	2.68	2.52	0.087	0.3944
18	1.74	1.74	1.73	0.048	0.9702	2.64[b]	3.04[a]	2.90[ab]	0.120	0.0881
19	1.94	2.13	2.04	0.074	0.2709	3.18[b]	3.40[a]	3.19[b]	0.063	0.0576
20	2.08	2.03	2.07	0.063	0.8288	3.25	3.48	3.43	0.160	0.5702

[a-b]Means of different superscripts within a row differ significantly ($P \leq 0.05$).

Table 12. Effect of different incubation temperatures on calcium and phosphorous levels of Cobb 500 broiler chick leg bone (tibia) at hatch and during grow-out.

Duration	Incubation temperature (°C)	Calcium (%)	Phosphorus (%)
Day-old	36.5	13.75[b]	3.87[b]
	37	16.59[ab]	6.43[a]
	37.5	22.01[a]	8.55[a]
	SEM	1.819	0.497
	P-value	0.1031	0.0158
Wk 4	36.5	13.76[c]	3.88[c]
	37	16.60[b]	6.44[b]
	37.5	22.02[a]	8.56[a]
	SEM	1.820	0.018
	P-value	0.0085	0.0004
Wk 8	36.5	13.77[c]	3.89[c]
	37	16.61[b]	6.45[b]
	37.5	22.03[a]	8.57[a]
	SEM	1.821	0.085
	P-value	0.0029	0.0001

[a-c]Means of different superscripts within a column differ significantly ($P \leq 0.05$).

2020). Until recently, the recommended EST of incubation has been 37.8°C throughout incubation, which was regarded optimal (Lourens et al., 2005). However, this seems to be changing because of differences in physiological development of eggs from different broiler genetic strains and maternal flock ages and differences in their embryonic metabolism especially in the late period of incubation (Hamidu et al., 2018, Wijnen et al., 2020). There is evidence to show that as the days of incubation increase at constant incubation temperature (usually 37.5°C until 18 d) the EST is usually lower as a result of lowered embryonic metabolism. However, with increasing days of incubation embryonic metabolism begins to increase and by the third and last period of incubation embryonic metabolism and heat production is so high that the EST temperature is higher than the air and incubation temperature (French, 2009). This can create stressful condition for the developing embryo in the plateau stage of oxygen consumption (ED16 to ED19) (Astorino et al., 2005) resulting in overheating and increased embryonic mortality (Hamidu et al., 2018). Therefore, incubation temperature control is required to compensate for excess heat production from the embryo to optimize embryo development and chick quality.

In the current study, the EST increased chronologically as days of incubation increased, which could be due to increased metabolic activities as embryonic development advanced (Uddin and Hamidu, 2014). Consistently, the EST in the 37.5°C incubation temperature was higher throughout the days of incubation monitored. However, the EST may not be up to stressful levels and appeared to be within the acceptable range suggested by previous studies (Lourens et al., 2005; Molenaar et al., 2011b). The EST registered in these Cobb 500 eggs were lower than at the higher incubation temperature (37.5°C), which resulted in almost 40°C EST at the same incubation temperature (37.5°C) in Ross 708 eggs but similar to Ross 308 eggs in a previous study (Hamidu et al., 2018). Historically, the Cobb 500 broiler genetic strain has shown lower metabolism and EST compared to Ross 308 or Ross 708 and could trigger hatching time challenging (Hamidu et al., 2007, 2018).

Table 13. Effect of different incubation temperatures during the late period of incubation on the feed intake, body weight gain and feed conversion ratio of Cobb 500 broilers over 8 wk period.

	Weekly feed intake (g)					Weekly weight gain					Weekly FCR				
Incubation temperature (°C)	36.5°C	37.0°C	37.5°C	SEM	*P*-value	36.5°C	37.0°C	37.5°C	SEM	*P*-value	36.5°C	37.0°C	37.5°C	SEM	*P*-value
Wk															
1	0.1	0.1	0.11	0.007	0.3876	0.15	0.14	0.15	0.006	0.2647	0.68	0.74	0.74	0.034	0.4359
2	0.33	0.33	0.31	0.01	0.3933	0.3	0.3	0.3	0.009	0.7636	1.11	1.11	1.02	0.051	0.4355
3	0.17	0.17	0.16	0.053	0.9965	0.58	0.57	0.55	0.016	0.4253	5.77	6.03	5.99	0.297	0.8074
4	1.69	1.61	1.51	0.486	0.9965	0.71[a]	0.65[ab]	0.62[b]	0.022	0.0492	2.41[b]	2.71[ab]	2.96[a]	0.126	0.0418
5	0.52[a]	0.47[b]	0.45[ab]	0.014	0.0173	0.91[a]	0.84[ab]	0.79[b]	0.022	0.0159	0.58	0.56	0.57	0.015	0.7272
6	0.66	0.66	0.61	0.023	0.2950	1.38[a]	1.26[b]	1.24[b]	0.032	0.0190	0.48	0.52	0.5	0.015	0.2239
7	0.98	0.99	0.95	0.015	0.1139	1.73	1.69	1.65	0.049	0.4398	0.57	0.59	0.58	0.015	0.6215
8	1.12	1.19	1.1	0.029	0.1632	2.19	2.23	2.13	0.062	0.5005	0.51	0.53	0.52	0.011	0.5482
Total and averages	5.58	5.51	5.22	0.56	0.8916	2.04	2.1	1.97	0.061	0.4085	2.73	2.63	2.66	0.259	0.9627

[a-b]Means of different superscripts within a row differ significantly ($P \leq 0.05$).

The early pipping and hatching time of chicks at 37.5°C is also reported in earlier research (Hamidu et al., 2018). An optimum incubation temperature (recommended to be 37.0°C) allows the embryos to have normal metabolism between 15 and 21 d of incubation, reducing overheating and resulting in hatching within an appropriate hatch window, which increases chick quality (Hamidu et al., 2018). Heat stress could cause changes in chick quality indicators including hematocrit values (blood erythrocyte levels) which increases in heat stress conditions (Borges et al., 2003).

In a related study, broiler breeder eggs exposed to low (36°C), control (37.5°C), or high (39°C) incubation temperatures from 13 d of incubation onward showed higher EST in the high-temperature incubated eggs (EST = 38.8 ± 0.33°C) compared to low-temperature incubated eggs (EST = 37.4 ± 0.08°C) and control temperature eggs (EST = 37.8 ± 0.15°C) (Morita et al., 2020). Generally, irrespective of maternal flock age within a strain it has been observed that the EST increases with increasing incubation temperature (Gualhanone et al., 2012). The embryo temperature was partly dependent on the eggshell temperature and therefore control of EST could be important in reducing overheating in the embryo, which could result in increased embryo mortality during the last stage of incubation (Lourens et al., 2005). It has been previously established that EST is greatly dependent on incubation temperature. However, any impact of the two factors on embryo temperature is not well known. The impact of these on embryonic metabolism has been widely investigated because metabolism is assumed to be greatly influenced by embryo temperature (Hamidu et al., 2018).

Chick quality is subjective in assessment but the indicators include chick weight, chick length, shank length, yoke-free body mass, residual yolk sac and navel score, bone length, and level of mineralization, all resulting in low mortality within 5 d post hatch. Several factors affect chick quality at the breeder farm and hatchery (Tona et al., 2001; Yeboah et al., 2019). Breeder farm factors such as breed or genetic strains, flock age, and egg handling practices such as eggs storage affect the egg quality and chick quality. The hatchery factors include incubation temperature, relative humidity, ventilation, gas exchange, egg turning, and technical skills of staff. These factors should be managed well for good quality chick, which should result in vibrant, alert and active chicks, high number of uniform chicks, have well-healed navel and have good weight and length correlating well with final broiler body weight. It is demonstrated that high EST during the third week of incubation results in reduced embryo length on ED18 and chick length at hatch (Lourens et al., 2005). This appears to show a favorable relationship between embryo length and chick length. The best indicator of chick quality has been argued to be chick length (Molenaar et al., 2010), however, this is disputable because there are other indicators such as residual yolk sac, which is an important factor for chick quality assessment (Yeboah et al., 2019). Since chick length is influenced by embryo length a longer embryo will result in a longer chick and subsequently may give quality chicks. In a related study chick length was longer at 37.0°C incubation temperature compared to 36.0°C, 36.5°C, and 37.5°C (Hamidu et al., 2018). The quality of the 1-day-old chick is important for a good start by the chick and for the final performance of the bird (Meijerhof, 2009). The length of the chicken is indicative of its development and there is a positive correlation between chick length at day of hatch and broiler performance (Meijerhof, 2009).

At an optimum incubation temperature, both femur and tibia development is very important as these can affect frame size and broiler bone quality. High EST nevertheless results in lower metatarsus weight than high EST. High temperatures in the first week of incubation stimulate later bone development, and high temperatures during the last week decrease the rate of bone development (Hammond et al., 2007). Incubating eggs at 39°C impedes the body and heart development of layer chicks and decreases the accessibility of blood ionized Ca for bone mineralization during embryo development (Sgavioli et al., 2016). This confirms that incubation temperature has an effect on bone development and could be negative or positive depending on the level of incubation temperature. Incubation temperature at 37.0°C increased shell thickness and may influence hatchability similar to eggs of young flocks which have thicker eggshells and hatch well (Gualhanone et al., 2012). It is not clear if Ca mobilization from the eggshell is greatest at a low incubation temperature compared to the higher temperatures as observed in the thickness of the eggshells. Embryos utilize up to 47% of the eggshell mass from ED10 to mobilize the Ca needed for skeletal formation during embryonic development (Gualhanone et al., 2012).

The current study showed that the levels of Ca and P measured immediately after incubation had a carry-over effect on broiler production. The levels of Ca and P increased with increasing incubation temperature and this was carried over to rearing. In another study, the concentration of ionized Ca in the blood of chicks incubated at 39°C incubation temperature was however, lower than those incubated at 37.5°C incubation temperature (Sgavioli et al., 2016). Hence, a higher incubation temperature than optimum could be deleterious to a chick's bone development and leg health. Overall, incubation temperature is expected to result in the proper development of embryos and consequently, growth during grow out. There is evidence that a lower EST, which could correspond to lower incubation temperature in wk 3 of incubation resulted in better organ development but resulted in lower grow-out performance (Wijnen et al., 2020). However, this is not reflected in the current study where no differences in feed intake, weight gain, and FCR were recorded at the end of 8 wk. Nevertheless, these growth parameters decreased with increasing incubation temperature during ED15 to 20 on the chicks during the 4th and 5th wk of rearing. Exposure to high temperature during late embryonic

development has been found to have long-lasting effects on the thermoregulatory system of broiler chickens by affecting the heat tolerance of these chickens and therefore, these chicks require higher ambient temperature for brooding (Morita et al., 2016).

Higher temperatures at 37.5°C compared to 36.5°C and 37.0°C had a more negative effect on the development of embryos, the quality of chicks, and broiler bone length although bone mineralization increased with increasing temperature of incubation. The study shows that the optimum temperature for chick embryonic development including chicks' general growth and reduced mortality during the last period of incubation is 37.0°C as also recommended in previous study (Hamidu et al., 2018). However, the temperature with the highest Ca and P levels was 37.5°C. Although incubation temperature at 37.5°C resulted in early pipping, hatching and higher Ca and P levels at day-old and rearing of broilers the optimum temperature for bone development and chicks quality appeared to be 37.0°C as measured by the tibia and femur lengths, embryo weights, and embryo length. The study shows that bone mineral levels may not be the same as bone development. Therefore, appropriate incubation temperature that gives an optimum EST according to genetic strains and flock ages (Hamidu et al., 2018) and nutritional strategies need to be put in place to increase bone development during incubation as well as chick quality. The nutritional modification of breeders should be targeted before incubation and in broilers after incubation in high temperature incubated eggs to improve growth to reduce leg problems in broilers.

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DISCLOSURES

The authors declare that there is no conflict of interest either financial or non-financial regarding the submission of this article.

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