JWPR
Journal of World's Poultry Research
2024, Scienceline Publication
J. World Poult. Res. 14(3): 264-272, 2024
Research Paper
DOI: https://dx.doi.org/10.36380/jwpr.2024.27
PII: S2322455X2400027-14
ISSN: 2322-455X

Effects of Thermal Manipulation During the Second Half of Incubation on Embryo Physiology, Hatching Parameters, and Quality of Broiler Chickens in Tropical Climate of Togo

Rachida A.Tankouano[1]*, Hèzouwè Meteyake [1], Oyegunle Emmanuel Oke[1], Povi Lawson-evi[2], and Kokou Tona[1]

[1]*Regional Centre of Excellence in Poultry Science, University of Lomé, Togo (CERSA/UL), 01 B.P. 1515 Lomé, Togo*
[2]*Physiopathology, Bioactive Substances and Safety Research Unit (PSBI Unit), Faculty of Science, University of Lomé, Togo, P.O. Box 1515, Togo*

*Corresponding author's E-mail: amorrach@gmail.com

Received: June 23, 2024, Revised: July 30, 2024, Accepted: August 27, 2024, Published: September 25, 2024

ABSTRACT

Chickens are sensitive to environmental challenges caused by temperature. The current study aimed to determine the effects of heat manipulation during embryonic development on the physiological responses of Goliath chickens. A total of 2000 hatching eggs from 48-week-old breeders were weighed, numbered, and randomly distributed equally into 4 incubators. Each incubator received 500 eggs (4 replicates of 125 eggs each). Eggs in two of the incubators were rotated hourly at a 45° angle and maintained at 37.8°C and 60% relative humidity (T0 groups). Between embryonic days (ED) 10 and 18 of incubation, the eggs from the other two incubators were heated to 38.5°C for 6 hours per day (T1 groups). The eggs were reweighed and candled, and viable eggs were moved to the hatching baskets at ED 18 of incubation. Hatching eggs were examined individually for hatching events every three hours during the final three days of incubation. On day 21, blood samples were collected from 12 chicks per group for hormonal and biochemical analyses. The evaluated blood parameters included Triiodothyronine (T3), T4 (thyroxine), cortisol, uric acid, lactate dehydrogenase, and total protein. At hatch, chicks were weighed and their quality (survival after hatching and performance standards) was evaluated. Data were collected on embryonic development, hatching window, hatching events, biochemical parameters, and hormonal concentrations. Results indicated that hatchability, chick's weight, Tri-iodothyronine, and corticosterone were higher in the T1 group, compared to the control group. At hatch on day 21, the pipping muscle of chicks in the treated group (T1) was significantly heavier than that of the control group, while the embryonic mortality rate was significantly higher in the T0 group. In conclusion, applying heat treatment for 6 hours at 38.5°C from ED10-ED18 of embryogenesis increased significantly the hatching rate, the pipping muscle, and the chick's weight in this study.

Keywords: Embryonic development, Physiology, Slow-growing broiler, Thermal manipulation, Tropical climate

INTRODUCTION

Poultry farming is one of the fastest-growing livestock industries in tropical nations. This expansion is caused by the prominent position that poultry products play on household menus, the absence of religious restrictions, their high nutritious value, and the ease of production (Jaovelo, 2007). Poultry meat is particularly popular since it is low in fat, an excellent source of protein, and unlike red meat, it does not raise the risk of certain diseases like metabolic or cardiovascular disorders (Pan et al., 2011; Jilo and Hasan, 2022; Connolly and Campbell, 2023).

Stress is the collection of responses to any external demand or challenge that causes the flock of hens to adjust to an unusual occurrence (Khan and Liu, 2012; Oke et al., 2022; Onagbesan et al., 2023). Providing ideal environmental conditions for chicken development, growth, and production is a prerequisite for poultry farming to operate at its peak efficiency (Muchacka et al., 2012; Oke et al., 2021). Heat stress occurs when an animal generates more internal heat than it can dissipate externally (Elizabeth et al., 2023). Chickens are more sensitive to environmental challenges posed by

temperature, particularly heat stress (Nawab et al., 2018). Heat stress is a significant factor contributing to financial losses in the poultry sector (Lin et al., 2006; Lu et al., 2007). It increases the mortality rate and reduces growth performance (Kumar et al., 2021; Belhadj et al., 2016). Compared to domestic chickens, broilers are more vulnerable to high temperatures (Gous and Morris, 2005), although the reaction to heat differs from one chicken to another according to their genetic upbringing (Altan et al., 2003; Star et al., 2008; Felver-Gant et al., 2012). In addition to the fast-growing strains, heat stress negatively affects the slow-growing strains (Tan et al., 2010; Soleimani et al., 2011; Rimoldi et al., 2015).

During the hottest months, the appropriate microclimatic parameters are often exceeded, disrupting the homeostasis of the chickens' internal environment. Consequently, the management of poultry and the equipment used in hot weather must be reevaluated to reduce heat stress (Akşit et al., 2006; Kpomasse et al., 2023).

Perinatal or postnatal acclimatization through thermal manipulation is one way to help chickens adjust to climate change and enhance their growth performance (Collin et al., 2007; Yalçin et al., 2008; Meteyake et al., 2020). Growth performances, metabolic rate physiological response, and hatching of poikilothermic embryos can be affected by variations of temperature from the standard incubation temperatures range of 37 to 37.5°C, (Tazawa et al., 2004; Black and Burggren, 2004). Lowering the incubation temperature increases incubation time and inhibits embryo growth (Black and Burggren, 2004), while elevated temperatures accelerate embryo growth and development (Willemsen et al., 2010; Narinç et al., 2016). Embryo weights were lower on embryonic day (ED) 18 when the eggs were exposed to a temperature of 39.6°C for 6 hours daily from ED10 to ED18 of incubation, even though the weights were similar to the control (Yalçin et al., 2005) or a bit lower than the control group (Yalçin et al., 2005). Because epigenetic adaptation to elevated or low post-hatch environmental temperatures is induced during the pre-hatch period, lower or higher incubation temperatures affect post-hatch thermoregulation systems (Nichelmann and Tzschentke, 2002; Al Amaz et al., 2024; Iraqi et al., 2024). Several studies have been conducted on the acclimatization of fast-growing broilers, but fewer studies have been carried out on slow-growing broilers, especially on Goliath chicken embryos which are also known to be slow-growing strains (Madougou, 2023). Hence, this study aimed to assess the physiological reactions of Goliath chicken embryos subjected to embryonic thermal manipulations from day 10 of embryogenesis to day 18 under tropical climate conditions.

MATERIALS AND METHODS

Ethical approval

The current study was performed with strict adherence to the University of Lome/Togo's Guide for the Care and Use of Experimental Animals (008/2021/BC-BPA/FDS-UL).

Experimental design

This experiment was carried out at the Regional Centre of Excellence for Poultry Science (CERSA) experimental unit at the University of Lomé.

A total of 2000 Goliath hatching eggs from 48-week-old breeders stored for 7 days were used. The eggs were purchased from a production farm in the Republic of Benin. These eggs were weighed, numbered, and incubated until day 10 of incubation in the same incubator (© Petersime Incubator, Belgium) at the appropriate temperatures and humidity conditions (37.8°C, 60%). On day 10 of incubation, the eggs were divided randomly into four groups (500 eggs each) and incubated in four different incubators of the same model (PasReform, Zeddam, SmartProCombi model, Netherlands). Each incubator had 4 replicates of 125 eggs. From ED10 to ED18, the eggs from two incubators (T1 groups) were subjected to 38.5°C and relative humidity (RH) of 60% for six hours daily, whereas the eggs from the other two incubators (T0 groups) were maintained at standard conditions. Eggs from all treated groups were incubated in complete darkness. On day 18 of incubation, the eggs were candled, and the fertile ones were weighed and conveyed in the hatcher for three days of hatching (until day 21 of incubation; Yalçin et al., 2008)

Egg and embryo weights

Before the setting of eggs and at ED18, egg weight (EWT) was recorded. These weights were used to determine the egg weight loss (EWTL) at ED18 of incubation using Formula 1.

$$\text{Egg weight loss (\%)} = \frac{EWT(ED0) - EWT(ED18)}{ED0} \text{ x } 100$$

(Formula 1)

Where ED 0 indicates the day the eggs were placed in the incubator.

At ED10, ED14, and ED18 12 eggs/treatment were broken at each embryonic day to measure embryo weights.

Hatching event, embryonic mortality, hatchability, and chick quality

Every three hours starting on day 19 of incubation, the time of internal pipping (IP), external pipping (EP), and hatching for each egg was recorded. The number of chicks hatched was counted. To determine the early and late embryonic mortalities, the unhatched eggs were broken and examined macroscopically at the end of incubation. Deaths before the 18th day of incubation were classified as early death. Deaths that happened at IP, during IP and EP, or when the embryo was positioned incorrectly were considered late embryonic mortality. The data collected were used to determine the spread of hatch according to various treatments, the entire incubation period (between setting and hatching), the hatchability (Formula 2), and the embryonic mortalities (Formula 3). The quality of the chicks at the hatch was evaluated using the Tona scoring system (Tona et al., 2004). The major objective of this method was to score physical attributes, such as response, appearance, down and eyes, legs conformation, navel area, yolk sac, and remaining membranes and yolk.

The total of the ratings given to each quality parameter was used to create the chicks' quality score :

$$\text{Hatchability (\%)} = \frac{\text{Total number of Hatched eggs}}{\text{Total number of Fertile Eggs}} \text{ x } 100$$

(Formula 2)

Organs, day-old chick body weights, and cloaca temperature at hatch

On day 21 after hatching, the weights of the liver, heart, and pipping muscles were calculated by cervical dislocation on a sample of 12 chicks per treatment. These data were used to determine body weights and the absolute weights of the heart and liver. An electronic thermometer inserted about 3 cm into the colon was used to record the cloaca temperatures of the same chicks at hatch.

$$\text{Mortality (\%)} = \frac{\text{Total number of dead embryos}}{\text{Total number of Fertile Eggs}} \text{ x } 100$$

(Formula 3)

Blood biochemical traits, hematology, and hormonal analysis

At hatch (day 21), blood samples were collected from 12 chicks via the wing vein using insulin syringes (1CC), to collect blood samples (1ml) into anti-coagulant-free tubes. These samples were used to evaluate uric acid, Lactate Dehydrogenase (LDH), total proteins, triiodothyronine (T3), thyroxine (T4), and corticosterone. In preparation for analysis, serum samples (obtained from centrifuged blood (15000g for 15 min) were frozen and kept at -20°C. Using the Biolabo kit (France), a spectrophotometer was used to quantify proteins, uric acid, and LDH. ELFA equipment and the Vidas kit were used to measure the serum T3 and T4 concentrations. Utilizing Cobas equipment and the Eclia technique, corticosterone concentration was determined (Repetto et al., 2017). The same chickens' blood was also drawn into heparinized tubes, where blood cells (Lymphocytes and Heterophils) were identified.

Statistical analysis

Data were analyzed using R software (R Core Team Development, 2023; Version 4.3.1). Descriptive statistics, including the Shapiro-Wilk normality test, means and standard errors, were calculated for the main quantitative variables. For variables with a normal distribution, the Student's parametric test was applied to compare the means between the treatment groups. On the other hand, the non-parametric Wilcoxon test was employed for variables that did not have a normal distribution. To compare the proportions between the various groups, the Chi-square test was also performed. The results were presented as the mean ± the Standard Deviation (SD). The significance rate was 5%.

RESULTS AND DISCUSSION

Embryonic development

Figure 1 shows the impact of thermal manipulation on embryonic development from day 10 to day 18 of incubation. The heat treatment did not affect the development of embryos ($p > 0.05$). These results confirm those reported by Al-Zghoul et al. (2019) but contradict those reported by Horowitz (1986), indicating that heat treatments had an instantaneous impact on the development of embryos, resulting in slowed growth by day 14. The heat treatment, which in their case reached 39.6°C, may have contributed to this outcome.

Hatching window

The spread of the hatch in relation to various heat treatments is depicted in Figure 2. Chicks in the T1 group began hatching three hours earlier than those in the T0 group. The first chicks in the T1 group were observed at 451 hours (day 19 of incubation), with the peak hatch occurring at 472 hours (day 20 of incubation). In contrast, chicks in the T0 group started hatching at 454 hours (day 19 of incubation), reaching their peak at 478 hours (day 20 of incubation). The T0 group exhibited a shorter hatching window compared to the T1 group.

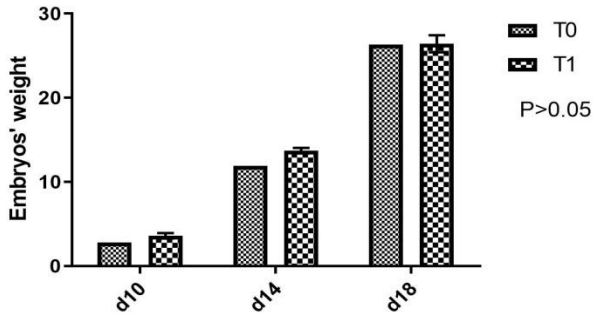

Figure 1. Effects of thermal manipulation on embryonic development (gr) of Goliath chickens from day 10 to day 18 of embryogenesis for 6 hours at 38.5°C. T0: Control group, T1: Thermal manipulated group

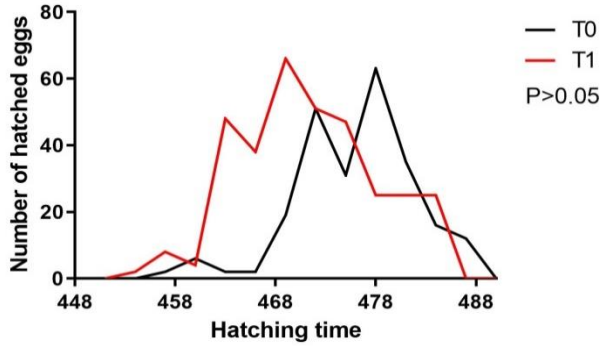

Figure 2. Effect of thermal manipulation on the hatching window of Goliath chickens from day 10 to day 18 of embryogenesis for 6 hours at 38.5°C. T0: Control group, T1: Thermal manipulated group

Internal pipping, external pipping, hatching durations, and cloacal temperature

Table 1 shows the effect of heat treatment on IP, EP, and hatching durations. Raising the temperature to 38.5 for 6 hours from ED10 to ED18 affected the duration of IP ($p < 0.05$), EP ($p < 0.05$), and cloaca temperature ($p < 0.001$). The difference was not significant between the two groups for the duration of hatching ($p > 0.05$). Embryos from the treated batch started the IP, EP, and hatching earlier than those in the control group (T0). The quality of chicks (surviving hatching, and performance standards) at hatch was similar in T0 and T1 groups ($p > 0.05$). The significant difference in the duration of IP and EP between the two treatment groups might be due to the fact that embryos use more oxygen when the temperature is higher. Because of that increased demand, the embryos must switch to pulmonary respiration in order to meet their oxygen requirements. This rise in oxygen demand may encourage the embryos to pip and hatch earlier (Molenaar et al., 2010). This result confirms those reported by Piestun et al. (2013) but contradicts those reported by Willemsen et al. (2010) who found that high heat treatment delayed the hatching process (IP, EP, and hatch) in the treated group. Willemsen et al. (2010) applied a heat treatment of 40.6°C from day 16 of incubation to day 18.5 of incubation. This incubation period is very critical for the development of embryos (Kpodo and Proszkowiec-Weglarz, 2023) and could explain why the results are contradictory. In his study, the thermal manipulation was applied during the late embryonic development. The higher cloaca temperature in the T1 group ($p < 0.05$) may be due to increased thermal manipulation induced by the metabolic rate, resulting in higher heat production by the chickens. In the event of future chronic heat stress, the heat therapy may cause a metabolic and stress response, suggesting a potential increase in thermotolerance. These results are in line with those found by several authors (Narinç et al., 2016; Al-Rukibat et al., 2017; Al-Zghoul, 2018; Saleh et al., 2020). These authors applied respectively 39.6 °C for 6 hours daily from day 10 to day 18 of incubation, 38.5°C and 40°C for 6 hours at day 16, 9 hours at day 17, and 12 hours at day 18 of incubation; 38.5°C, 39°C, 39.5°C and 40°C for 6 hours from day 12 to day 18 of incubation; 39°C for 18 hours daily from day 10 to day 18 of incubation. They all concluded that thermal manipulation improved the thermotolerance of chicks. Al-Zghoul et al. (2019) added that the dynamics of heat shock proteins (HSPs) and heat shock factors (HSF) mRNA expression were changed by heat treatment, and this was linked to an increased development of thermotolerance.

Table 1. Effects of thermal manipulation on hatching parameters of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters \ Treatments	T0	T1	p-value
IP time (h)	450.7 ± 3.14^{a}	446.5 ± 2.87^{a}	0.33
EP time (h)	461.5 ± 3.14^{a}	457.0 ± 3^{a}	0.33
Total incubation duration (h)	473.2 ± 3.03^{a}	467.5 ± 3.4^{a}	0.20
Duration between IP and EP (h)	10.77 ± 0^{a}	10.5 ± 0^{b}	0.03
Duration between EP and Hatching(h)	11.7 ± 0^{a}	10.5 ± 0^{b}	0.03
Duration between IP and Hatching (h)	22.47 ± 0^{a}	21 ± 0^{a}	0.34
Cloacal temperature of chicks (°C)	37.98 ± 0.22^{b}	39.99 ± 0.06^{a}	< 0.001
Tona score	96.55 ± 0.47^{a}	96.02 ± 0.58^{a}	0.74

IP: Internal pipping, EP: External pipping, h: Hour, P-value: Probability. All results are presented as mean ± SD; ^{a,b} Means with different superscripts are significantly different in a row, T0: Control group, T1: Thermal manipulated group

Weight loss, hatching rate, and mortality rate

Table 2 shows the results of thermal manipulation on weight loss from incubated eggs, hatching rate, and mortality. No significant difference was recorded in terms of weight loss ($p > 0.05$) but raising the temperature to 38.5°C affected the early mortality rate ($p < 0.05$) and the late mortality rate ($p < 0.05$). Lower, early, and late mortality rates were recorded in treatment T1. The hatching rate of batch T1 was higher than that of the T0 group ($p < 0.05$).

Table 2. Effect of thermal manipulation on weight loss, hatching, and mortalities rate of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters (%)	Treatments		p-value
	T0	T1	
Weight loss	13.02[a]	13.98[a]	1
Hatchability	85.43[b]	89.22[a]	0.03
EM	5.6[a]	4.25[b]	0.04
LM	8.87 [a]	6.35[b]	0.01

EM: Early mortality, LM: Late mortality. All results are presented as mean ± SD; [a,b] Means with different superscripts are significantly different in a row, T0: Control group, T1: Thermal manipulated group.

The weight of the pipping muscle and the high level of T3 (triiodothyronine) in T1 group chicks can be used to explain the hatching rates obtained. Chicks' pipping muscles are crucial in the process of hatching. The mechanical strength needed for the chick to break the eggshell and come out is supplied by the pipping muscles (Pulikanti et al., 2010). Heat stress resulted in an increased thyroid hormone T3 and corticosterone concentration in the T1 group. These hormones play an important role in the hatching process, providing the chicks the energy they need to hatch. The higher the T3 and T4 concentrations, the higher the chicks' energy level. For the control of metabolic processes, T3 and T4 are crucial. They affect the turnover of lipids and carbohydrates, protein synthesis, and basal metabolic rate. They promote the mobilization of energy reserves, such as lipids and proteins, needed to sustain energy during the hatching phase. This mobilization is crucial if the embryo is to complete the hatching process with sufficient energy (Al-Zghoul, 2018).

Compared to the chicks in the T0 group, which had a lower concentration of T3, the highly active chicks in the T1 group hatched earlier. Delayed hatching can cause chick mortality within the egg, leading to a lower hatching rate. These findings contradict those reported by Al-Rukibat et al. (2017), who found that thermal manipulation did not affect the hatching rate. The discrepancies between studies could be due to genetic differences. The higher embryonic mortality in the chicks of the control batch (T0) could be explained by the low weight of the pipping muscle, allowing the chicks to spin inside their shells, rip the membrane, and break the shell.

Absolute weight of chicks, heart, liver, and pipping muscle

Table 3 shows the effects of heat treatment on the absolute weight of day-old chicks, heart weight, liver weight, and pipping muscle weight. The weight of chicks in T1 was significantly higher than that of the chicks in the T0 group ($p < 0.05$). The same tendency was observed for the pipping muscles ($p < 0.05$). However, there was no difference in the weight of the heart ($p > 0.05$) and liver ($p > 0.05$). These outcomes (high chicks' weight and pipping muscle in the T1 group) could be explained by the fact that high temperatures are known to speed up not only the metabolic rate but also the growth and development of muscle tissues (Meltzer, 1983). This result confirms the findings reported by Piestun et al. (2015). Piestun et al. (2015) applied a heat treatment of 39.5°C from day 7 to day 16 of incubation for 12 hours. It was concluded that the thermal manipulation had a positive effect on embryo growth with an improved chick's weight at the hatch. This result can also be explained by the effective use (due to accelerated metabolism) of the energy reserves in the egg which resulted in body tissue enlargement (Piestun et al., 2015). In addition, the heat treatment influenced hormone regulation by increasing T3 levels in the T1 batch. These hormones are like growth hormones. Higher levels of T3 can promote the growth of body tissue in chicks, leading to larger size at hatch. The results confirm those reported by Abuoghaba et al. (2018) and Al-Rukibat et al. (2017) but contradict those reported by Yahav et al. (2004) and Tona et al. (2004), who found that a thermal manipulation of 38.5°C applied between ED16 and 18 for 3 hours did not affect the hatching weight of Cobb chicks. This could be explained by the period of application and the type of boiler used.

Table 3. Effect of thermal manipulation on the absolute weight of chick, heart, liver, and pipping muscle of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
Chick (g)	36.03 ± 0.59[b]	38.26 ± 0.56[a]	< 0.001
Heart (g)	0.086 ± 0.01[a]	0.092 ± 0.00[a]	0.74
Liver (g)	0.76 ± 0.06[a]	0.86 ± 0.07[a]	0.18
Pipping muscle (g)	0.14 ± 0.02[b]	0.20 ± 0.03[a]	0.02

[a,b] Means with different superscripts are significantly different in a row; All results are presented as mean ± SD, T0: Control group, T1: Thermal manipulated group.

Relative weight of chicks, heart, liver, and pipping muscle

Table 4 shows the result of heat treatment on the relative weight of day-old chicks, heart weight, liver weight, and pipping muscle weight. At the setting, the weight of the eggs was similar across the treatments ($p > 0.05$). The weight of chicks in batch T1 was higher than that of the chicks in the T0 group ($p < 0.05$). The same tendency was observed for the weights of the liver ($p < 0.05$) and pipping muscles ($p < 0.05$). However, there was no difference in the weight of the heart ($p > 0.05$). These results (high chicks' weight and pipping muscle in the T1 group) could be explained by the fact that heat is known to accelerate the growth and development of muscle tissues as well as the metabolic rate (Meltzer, 1983). This result confirms the findings reported by Piestun et al. (2015). In addition, there is a positive correlation between the liver's weight and body weight (Hassan, 2009). These results contradict those reported by Yalcin et al. (2008) who found a lower absolute liver and heart weight under the same heat treatment conditions (38.5°C for 6 hours, from incubation day 10 to day 18). The difference here could probably be due to genetic factors. Cobb500 which is a fast-growing broiler was used in their study while in this study a slow-growing breed was used.

Table 4. Effect of thermal manipulation on the relative weight of chick, heart, liver, and pipping muscle of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
Egg's weight (g)	49.34 ± 0,59[a]	48.42 ± 0,48[a]	0.24
Relative chick weight (%)	73.02 ± 0.01[b]	79.01 ± 0.00[a]	< 0.001
Relative heart weight (%)	0.25 ± 0.01[a]	0.24 ± 0.01[a]	0.57
Relative liver weight(%)	1.99 ± 0.01[b]	2.39 ± 0.01[a]	< 0.001
Relative pipping muscle'weight (%)	0.39 ± 0.02[b]	0.52 ± 0.01[a]	< 0.001

[a,b] Means with different superscripts are significantly different in a row, All results are presented as mean ± SD, T0: Control group, T1: Thermal manipulated group.

T3, T4 concentration, corticosterone, and heterophils/lymphocytes ratio

Table 5 shows the effect of high heat treatment on stress hormones T3 and T4 and the heterophils/lymphocytes H/L ratio. Blood serum T3 was higher in group T1 ($p < 0.05$) and corticosterone concentration was also higher in group T1 ($p < 0.05$), compared to the T0 group. The heat treatment did not affect the H/L ratio and T4 concentration. Compared to T0, the higher blood serum T3 concentration in T1 chicks at hatch suggested that less T3 was required for oxidative metabolism, which reduced the amount of T3 absorbed by the cells and increased the blood serum T3 concentration over time. In addition, the increasing metabolic rate is known to increase T3 levels in the blood. When there was an increase in metabolic rate, the T3 rate also increased in the blood. There was no major difference in T4 concentration since the conversion of T4 to T3 occurred more quickly in T1 than in T0 chicks throughout embryonic development (Tona et al., 2004). The decrease in hepatic Deiodinase (D3) expression may be a contributing factor to the rise in blood serum T3 levels. The breakdown of T3 by D3 is a significant cause of determining serum T3 level, even if the hepatic D3 level has not been assessed (Decuypere and Kuhn, 1985; Darras et al., 2000). Under the action of D3, the conversion of T4 to T3 is reduced, which decreases the quantity of T3 in the blood. In addition, the conversion of T3 to T2 by D3 directly reduces the concentration of active T3 (Maia et al., 2005). High levels of hepatic D3 show increased conversion of T3 to T2 and T4 to rT3. This suggests that blood T3 levels may be reduced as the enzyme reduces the amount of active T3. Low hepatic D3 levels show decreased inactivation of T3 and conversion of T4 to rT3. This suggests that blood T3 levels may be relatively higher

Table 5. Effect of thermal manipulation on stress hormones concentration and H/L ratio of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
T3 (Pmol/l)	5.54 ± 1.33[b]	9.98 ± 2.12[a]	0.02
T4 (Pmol/l)	7.11 ± 0.87[a]	5.1 ± 0.13[a]	0.2
Corticosterone (ng/ml)	0.40 ± 0.00[b]	0.54 ± 0.01[a]	< 0.001
Ratio H/L	6.63[a]	4.33[a]	0.42

[a,b] Means with different superscripts are significantly different in a row, All results are presented as mean ± SD; T3: Triiodothyronine; T4: Thyroxine, T0: Control group, T1: Thermal manipulated group. Ratio H/L: Heterophils/lymphocytes H/L ratio

Biochemical parameters

Table 6 shows the effect of heat treatment on biochemical parameters. The heat treatment decreased the concentration of uric acid ($p < 0.05$) and increased LDH ($p < 0.05$) in group T1. In addition, there was no difference in the protein content. Heat can increase the metabolism of

embryos, accelerating the processes of purine degradation and the conversion of uric acid into other metabolic compounds (Al-Kharusi et al., 2012; Loyau et al., 2016). This could explain the lower uric acid levels observed. These outcomes confirm those reported by Moraes et al. (2003), who also got a reduction in uric acid in heat-treated batches. Heat has the potential to interfere with metabolic processes. In order to generate energy, cells might shift to a more anaerobic metabolism, which raises the synthesis of lactate, an LDH substrate.

Table 6. Effect of thermal manipulation on biochemical parameters of Goliath chickens from day 10 to day 18 of embryogenesis

Parameters	Treatments		p value
	T0	T1	
Uric acid (mg/l)	75.81±1.87[a]	65.98±1.45[b]	0.02
Lactate Dehydrogenase (UI/L)	982±1.73[b]	1260±1.16[a]	< 0.001
Total protein (g/l)	39.47±7.27[a]	37.58±6.32[a]	0.85

[a,b] Means with different superscripts are significantly different in a row; All results are presented as mean ± SD, T0: Control group, T1: Thermal manipulated group.

CONCLUSION

Applying heat treatment for 6 hours at 38.5°C from ED10 to ED18 of embryogenesis increases the hatching rate, the pipping muscle, and the chick's weight at hatch. Moreover, it did not affect the embryonic development from ED 10 to ED18. Additional investigation is important to clarify the underlying mechanisms and to assess the impact of these thermal manipulations on poultry production on a larger scale.

DECLARATIONS

Acknowledgments

The authors express their profound gratitude to the World Bank Group for funding this project through the Regional Center of Excellence in Poultry Science (CERSA).

Funding

Support for this effort was provided by the World Bank Group's Regional Center of Excellence in Poultry Science (CERSA) [IDA 5424].

Authors' contributions

Rachida Tankouano, Povi Evi Lawson, and Kokou Tona did the design of this study. Meteyake Hezouwe contributed to the conceptualization, and data analysis of the present study and drafted the manuscript. Oyegunle Emmanuel Oke helped to improve the English of the manuscript. All authors approved the final version of the manuscript.

Availability of data and materials

All the data and materials are available on request from the corresponding author.

Ethical considerations

No sentence in this manuscript has been copied. The manuscript has not been submitted for editorial review, accepted for publishing, or published anywhere else. There is no fabrication or falsification of the data.

Competing of interests

The authors of this work declare no competing interests

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