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Thermal Manipulation During Incubation: Effects on Embryo Development, Production Performance, Meat Quality, and Thermal Tolerance of Broiler Chickens

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ABSTRACT

Thermal manipulations during the embryonic period have positive effects on thermotolerance and the productive performance of broilers subjected to acute heat stress. This study aimed to investigate the potential effects of Thermal manipulation during incubation (TMI) on productive performances and thermotolerance of broiler chickens growing in tropical climates. A total of 900 Cobb 500 broiler chicken eggs from a 35-week-old breeder flock were incubated in standard incubation conditions (37.8°C, 60% relative humidity) until day 7, when they were divided into 3 groups (300 eggs per group). The control group (C) was incubated at standard incubation conditions while T6 and T12 groups were subjected to, respectively, 6 hours/day and 12 hours/day of TMI (T° = 39.5°C, relative humidity = 65%, Embryonic day = 7-16). The relative embryo and albumen weight were determined from 10 to 18 days of incubation. The hatching event was checked between 450 and 504 hours of incubation, and egg hatchability, chick quality, and cloacal temperature were also determined. One hundred and twenty-five chicks from each incubation group were transferred to the farm and raised for 6 weeks. During this period, their post-hatch performances were determined. At week 6, blood samples were collected to measure T3, T4, and corticosterone hormone levels. Then, the 6-week-old broilers were slaughtered to determine meat yield and quality. Results showed that the chick's rectal temperature was significantly reduced in T6 and T12 groups compared to the C group, while hatchability and one-day-old chick weight were not affected. Final body weight and feed conversion ratio were significantly improved in the T12 group, compared to other groups. Thermal manipulation during incubation for 6 and 12 hours significantly reduced mortality rate and pectoralis major muscle drip loss while it increased muscle pH at 24 hours post-mortem (pH24). Corticosterone, T3, and T4 plasma hormone levels at week 6 were also significantly reduced by TMI. Therefore, exposing hatching eggs to 39.5°C and 65% of relative humidity from days 7 to 16 of incubation for 12 hours/day is recommended for the poultry industry in tropical climates.

Keywords: Chronic heat stress, Fast-growing broilers, Hatching and post-hatch performances, Thermal manipulation, Thermotolerance, Meat quality

INTRODUCTION

The poultry industry has been considered in recent years as a promising and emerging sector to satisfy the growing demand for animal protein in developing countries (Ayssiwede et al., 2009). However, this sector is confronted by many constraints, including heat stress. According to Lin et al. (2006), heat stress is the main limiting factor for the poultry industry in tropical and subtropical regions. Temperatures ranging from 35 to 45°C in some parts of the year are common in some tropical areas, causing morbidity and mortality in poultry (Akbarian et al., 2013). The situation is exacerbated by the globally averaged surface temperature projected to increase by 1.4 to 5.8°C over the period 1990 to 2100 (IPCC, 2001). It is known that the optimal temperature

range for broiler and layer production is 18-22°C (Charles, 2002; Aengwanich and Simaraks, 2004), with slight variations on both sides due to differences in age, sex, and line/strain. However, in the tropics and subtropics, chickens are generally kept in open houses and are therefore exposed daily to high environmental temperatures. Therefore they are susceptible to heat stress.

The ambient heat is one of the major constraints in poultry farming because of the enormous economic losses it causes in terms of mortality and reduction of productivity (Tesseraud and Temim, 1999). Heat stress causes a disturbance in the physiological status of chickens, including a reduction in certain hormones (Mujahid et al., 2007). Low T3 and high T4 hormone levels were observed in 42-day-old broilers under cyclic heat stress (Bueno et al. 2017). Sohail et al. (2010) also found an increase in plasma corticosterone levels in broilers reared under heat stress for 8 hours per day from 22 to 42 days.

The efficiency of certain vital functions, such as thermoregulation, is often impaired in broilers because the genetic selection of broiler strains is mainly aimed at increasing the growth rate of the animals (Havenstein et al., 2003). The cardiovascular and pulmonary systems, which play an important role in poultry thermoregulation, are less developed, therefore, cannot cope with the broiler chickens' growth rate (Havenstein et al., 2003). This makes them more sensitive to ambient heat and vulnerable to metabolic problems such as ascites, sudden death syndrome, and leg problems (De Smit et al., 2005). This is compounded by the fact that these commercial broilers (Cobb and Ross), usually raised in tropical and subtropical climates, are not mainly selected for these climates.

Various approaches, including genetic, technical, or feeding strategies, can be implemented to improve the tolerance of chickens to variations in thermal conditions (Oke et al., 2017; Oke, 2018, Meteyake et al., 2020). Heat acclimation is a strategy that could increase the thermotolerance of fast-growing broilers (Meteyake et al., 2020). The embryonic and early postnatal periods are the best times to improve the heat tolerance of chickens through thermal manipulation (Yahav 2009). When broilers are exposed to thermal stress during these periods, they acquire thermotolerance more easily in continued life (Al-Zghoul et al., 2013; Loyau et al., 2016). It was reported that adaptation to environmental conditions could be manipulated by exploiting the immaturity of the thermoregulatory mechanism system of chickens at the perinatal stage (Yahav and Mcmurtry 2001). In order to improve the thermal tolerance and welfare of poultry without altering growth and, thus, the economic viability of poultry industries, numerous studies on thermal manipulations in the perinatal and neonatal period have been conducted (Piestun et al., 2008; Nideou et al., 2019; Meteyake et al., 2020). These techniques induce rapid physiological and metabolic changes that persist for the life of the animal (Collin et al., 2011). A study by Meteyake et al. (2020) indicated positive effects of embryonic and neonatal acclimation techniques when applied individually, and in combination on Ross 308 broilers raised in a tropical climate; however, some deleterious effects, such as low hatchability, were observed. It became necessary to conduct further studies to establish optimal techniques. The adverse effect observed could be due to the duration of thermal manipulation applied to hatching eggs (Meteyake et al., 2020). Additionally, despite the plethora of reports on the thermotolerance of chickens, there is a scarcity of information on the effect of thermal manipulation duration during incubation on the productive performances of broilers reared in a tropical climate and in real uncontrolled environmental conditions (temperature and humidity). Thus, the present study aimed to investigate the effects of thermal manipulation during incubation (TMI) on, first, embryo development and production performances, second, thermo-tolerance, and third, meat quality of broiler chickens reared under a chronic hot environment.

MATERIAL AND METHODS

Ethical approval

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Experimental Animals (008/2021/BC-BPA/FDS-UL) of the University of Lome, Togo.

Study design

A total of 900 Cobb 500 broiler chicken hatching eggs from 35-week-old broiler breeders, provided by Incubel Hoogstraten, Belgium, were used for this study. Before incubation, these eggs were weighed, identified, and incubated in the same incubator (Petersime® Vision, Zulte, Belgium) at standard conditions (37.8°C and 60% of relative humidity [RH]) until day 7. Eggs were then divided randomly into three incubation treatment groups of 300 eggs each, including the control group (C group) and two thermal manipulation groups (T6 and T12 groups, Figure 1). The control group (C) was maintained at standard conditions while T6 and T12 groups were

subjected to a temperature of 39.5°C and 65% of RH for 6 and 12 hours daily, respectively, during the embryonic day (ED) 7-16. On day 18 of incubation, all the eggs were candled, and those with evidence of living embryos were weighed and transferred from the turning trays to hatching baskets, where they were subjected to standard conditions until ED 21.

At ED 21, 125 chicks per incubation group, selected randomly, were transferred to the farm and raised for 6 weeks. After 10 days of brooding, the chickens were raised at a natural ambient temperature, humidity, and ventilation in an open-sided poultry house (25 broilers with 5 replicates per group) and were randomly assigned to floor pens). They were reared on a floor litter with a stocking density of 10 broilers per m^2 (2 x 1.25 x 1.8 m for each pen) from 10 days of age onward and were subjected to the same prophylactic and light program (23 hours of light and 1 hour of darkness). All the chicks were vaccinated against the infectious bursal disease (BUR-706, France) vaccine via drinking water at the age of 5 and 19 days. Newcastle disease (Avinew® NeO, Boehringer Ingelheim, Lyon, France) and Avian infectious bronchitis disease (Bioral, Boehringer Ingelheim, Lyon, France) vaccines were also orally administrated at the age of 7 and 21 days. All the broilers were fed *ad libitum* and received the same non-pelleted feed (Table 1). At 6 weeks of age, 15 chickens per group were slaughtered for meat yield, and quality measurement, and blood samples were collected to determine levels of T3, T4, and corticosterone hormones.

Table 1. Calculated composition of experimental feed during the starter (0-10 days of age) and grower (11-42 days of age)

Ingredient (%)	Starter (1-10 days)	Grower (10-42 days)
White maize	53.5	62
Wheat bran	4	3
Fish meal[1]	8	8
Roasted soybeans	21.5	19.5
Brewery by product	5	2
Oyster shell	2	2.5
Broiler concentrate	5	2
Dl-methionine	0.5	0.5
L-lysine	0.5	0.5
Total	100	100
Chemical nutritional characteristics		
Metabolizable energy (kcal/kg)	3000	3065
Crude protein (%)	21.30	19.59
Crude fiber (%)	4.9	4.68
Lysine	1.45	1.33
Methionine ((%)	0.98	0.88
Methionine + cysteine (%)	1.20	1.08
Calcium	1.55	1.34
Total phosphorus	0.56	0.61

[1]A commercial fish meal containing 40% crude protein produced in Senegal and used by West African breeders.

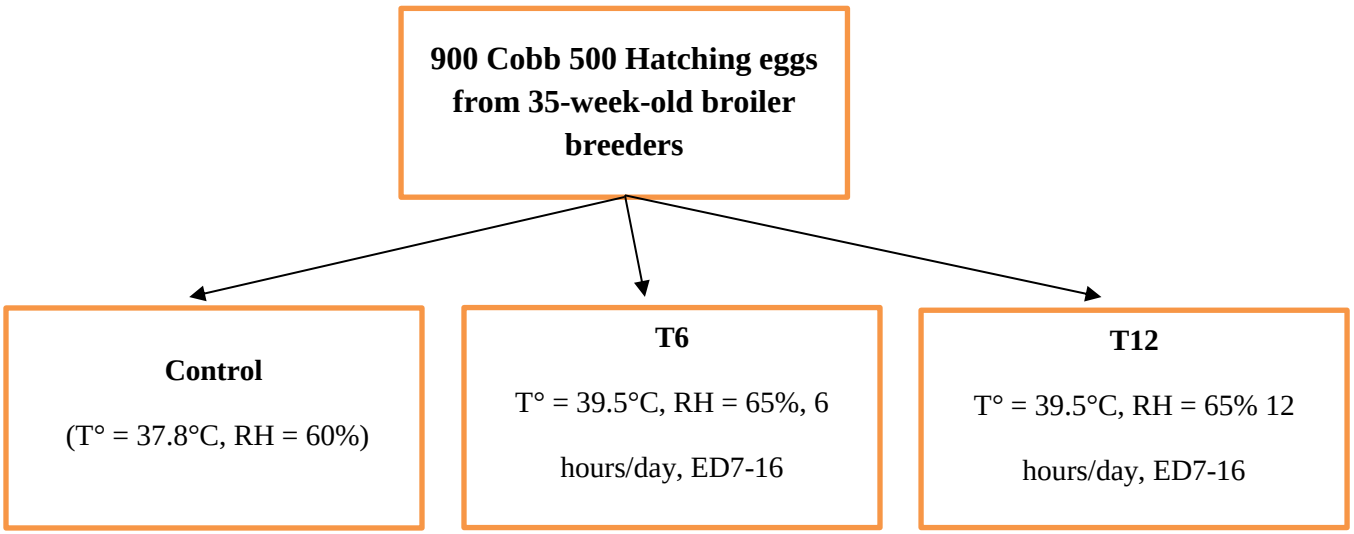

Figure 1. Study design. RH: Relative humidity; T°: Temperature; ED: Embryonic day

Data collection

Embryo and albumen weight, hatching event, hatchability, and chick quality

At embryo days 10, 12, 14, 16, and 18, a total sample of 10 eggs per group was broken, and the embryo and albumen weight were measured. These data were used to determine the Relative embryo weight (REW) and Relative albumen weight (RAW) using Formula 1:

$$\text{REW or RAW (\%)} = \frac{\text{Absolute Embryo or Albumen weight}}{\text{Egg weight}} \text{ X } 100 \qquad \text{(Formula 1)}$$

From 450 to 504 hours of incubation, the time of external pipping (EP) and hatching for individual eggs were recorded every three hours. The hatched chicks were recorded. These data were used to determine the external pipping time (time between setting and external pipping), external pipping duration (duration between external pipping and hatching), and the total incubation duration (time between setting and hatching). Fertile hatchability and total embryo mortality (TEM) were also determined using formulas 2 and 3.

$$\text{Fertile hatchability (\%)} = \frac{\text{Number of hatched chicks at the end of incubation}}{\text{Number of fertile eggs transferred to the hatching basket}} \text{ X } 100 \quad \text{(Formula 2)}$$

$$\text{Total embryo mortality (\%)} = \frac{\text{Number of unhatched but fertile eggs}}{\text{Number of fertile eggs transferred to the hatching basket}} \text{ X } 100 \quad \text{(Formula 3)}$$

One-day-old chick body weights and quality

At 21 days of incubation, the hatched chicks were weighed, and the chick quality was determined according to the Tona scoring method (Tona et al., 2003). According to this method, physical parameters were scored, including reflex, down and appearance, eyes, the conformation of legs, navel area, yolk sac, remaining membranes, and yolk. The chick quality score was defined as the sum of the scores assigned to each quality parameter.

Meteorological data during the rearing phase

Temperatures and RH in the poultry house were recorded daily at 07:00 a.m., 10:00 a.m., 01:00 p.m., and 04:00 p.m. using thermo-hygrometers (HTC-1, China). These data were used to calculate the temperature-humidity index (THI) using Formula 4 used by Bueno et al. (2020):

$$\text{THI} = 0.8\text{T} + \left(\frac{\text{RH}(\text{T}-14.3)}{100}\right) + 46.3 \qquad \text{(Formula 4)}$$

THI: Temperature-humidity index, T: Ambient temperature, RH: Relative humidity

Post-hatch growth performance and mortality rate

At the end of the hatch, chicks were transferred to the farm and weighed to determine their initial body weight. During the experimental period, amount of feed consumption, body weight, and mortalities were recorded weekly. Feed intake was determined as the difference between the amount of feed given and the remaining feed. The body weight gain was calculated as the difference between initial and final body weight. These data were used to determine the feed conversion ratio by dividing feed intake by body weight gain.

Blood sample collection and hormonal analysis

At an internal pipping stage, at hatch, and 6 weeks post-hatch, the blood samples (n = 15 per group) were collected (from the jugular vein at IP stage and brachial veins at hatch and at 6 weeks post-hatch) in heparinized tubes and centrifuged at 3,000 rpm for 15 minutes. The samples of plasma obtained were stored in a freezer at -20°C. A volume of 100 µL of plasma was used for tri-iodothyronine (T3), thyroxin (T4), and corticosterone concentrations determination by using the automated VIDAS systems, which is an enzyme-linked fluorescent assay (ELFA) technique. Antibody anti-T3 of mutton, marked by phosphatase alkaline and sodium azide, antibody anti-T4, marked by phosphatase alkaline and methylisothiazolone, and a derivative of cortisol, marked by phosphatase alkaline and sodium azide, provided by VIDAS were used, for the determination of the concentrations of T3, T4, and corticosterone, respectively. All the samples were run in a certain assay for each hormone to avoid inter-assay variability. Corticosterone concentration was determined just at 6 weeks of age.

Body and surface temperature

Neck and breast temperature were measured on 30-day-old chicks per group using an infrared thermometer. The rectal temperature was also measured using an electronic thermometer.

Meat yield and quality

At week 6, 15 broilers per group were slaughtered. Their carcass, breast, and tigh were weighed to calculate their yields in relation to body weight. The ultimate pH (pHu or pH24) of the *pectoralis major* muscle was measured at 24 hours postmortem using a pH meter (HI9125 portable waterproof pH/ORP meter, HANNA Instrument, Italy) by inserting the electrode in the left pectoralis major muscle (Zhang et al., 2017). The drip loss of *pectoralis major* muscle was also determined, as described by Zhang et al. (2017). Meat samples with a size of 3 cm (length) × 2 cm (width) × 1 cm (thickness), were weighed (W1) and suspended parallel to the longitudinal axis of the myofibers in netting and vacuumed bags and stored at 4°C. Samples were weighed after 48 hours (W2) hanging. The drip loss was calculated using the following formula:

$$\text{Drip loss at 48 hours (\%)} = \frac{W1 - W2}{W1} \text{ X } 100 \quad \text{(Formula 5)}$$

Where, W1 is weight at slaughter time and W2 denotes weight 48 hours after slaughter.

Statistical analysis

Graph Pad Prism 8.0 statistical analysis software was used for data analysis. ANOVA one-way test was used for statistical analysis. Shapiro-Wilk's test was used to check the normal distribution of data, and Levene's test to prove the homogeneity of variance. Percentage data were transformed into Arcsin values and then re-transformed into the original values after the analysis. The comparison between the different groups after ANOVA was made using the Tukey test. The significant level was set at $p < 0.05$.

RESULTS

Embryo weight

The evolution of the absolute and relative weights of embryos according to the treatments has been shown in Figure 2 (A and B). The figures show the increasing trajectory of absolute and relative embryo weights with age. The embryos of the three groups had similar absolute weights from day 10 to 16 ($p > 0.05$). However, on day 18 of incubation, embryos from C and T12 groups had a higher absolute weight than those of the T6 groups ($p < 0.05$). Regarding relative weight, the significant effect of embryo acclimation was only observed on days 14 (C = T12 > T6; $p < 0.05$) and 16 (C = T6 > T12; $p < 0.05$) of incubation.

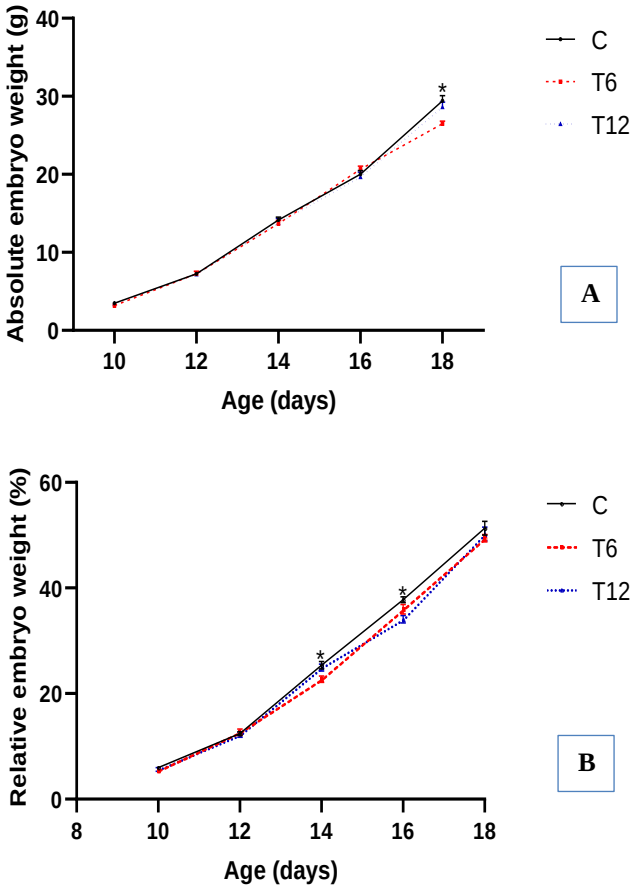

Figure 2. Absolute (A) and relative embryo weight (B) according to thermal manipulation and chicken embryo age. *: significant difference; $p < 0.05$; C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

Albumen weight

Absolute and relative albumen weights decreased with embryo age (figures 3A and 3B). Absolute and relative albumen weights were similar in all three groups except on day 16 of incubation, where the C group had a lower absolute weight ($p < 0.05$) and a lower relative weight ($p < 0.05$) than the other groups (T6 and T12).

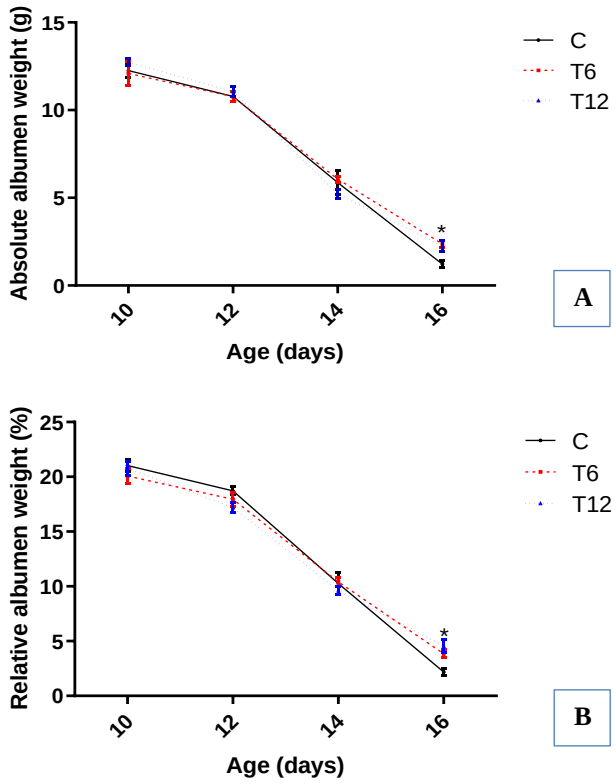

Figure 3. Absolute (A) and Relative albumen weight (B) according to thermal manipulation and chicken embryo age. *: significant difference; $p < 0.05$; C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

Egg weight loss at 18 days of incubation and hatching performance

The effect of thermal manipulation during incubation (TMI) on egg weight loss and hatching performance has been shown in Table 2. The egg weights before setting were 62.08 ± 0.17 g, 62.12 ± 0.17 g, and 62.25 ± 0.17 g, respectively in C, T6, and T12 groups. Egg weight loss at 18 days of incubation was higher in the control group compared to the T12 group ($p < 0.05$), however, there was no significant difference between T6 and other groups. Both duration of thermal manipulation significantly increased external pipping time ($p < 0.05$). The T6 group had external pipping about 4 hours and 2 hours after C and T12 groups, respectively. The T6 group had a significantly lower external pipping duration and a higher total incubation duration, compared to the other groups ($p < 0.05$). The T12 group had no significant external pipping duration and total incubation duration compared to the control. Hatchability and body weight of chicks of all groups at hatch were not significantly ($p > 0.05$) different among all the groups, while TMI during 12 hours decreased the chick's quality ($p < 0.05$).

Rectal, neck, and breast temperature

The effect of heat treatments on rectal, neck, and breast temperatures is shown in Table 3. The rectal temperature of chicks in the T12 group was significantly the lowest ($p < 0.05$). It was followed by the temperature of T6 group and C group, which had the highest rectal temperature. Regarding neck and breast temperature, it was significantly higher in the T6 group compared to the other groups ($p < 0.05$).

Meteorological data in the opened poultry house

The evolution of ambient temperature, RH, and THI in the poultry house is shown in Table 4. The ambient temperature increased to reach a peak at 01:00 pm and then decreased. The RH followed an opposite trend to that of ambient temperature. The highest THI was reached at 01.00 pm.

Table 2. Effect of thermal manipulation on hatching process, fertile hatchability, body weight, and quality of broiler chickens

Parameters	C	T6	T12	p value
Egg weight loss at ED 18 (%)	14.38 ± 0.15^{a}	13.7 ± 0.2^{ab}	13.41 ± 0.19^{b}	0.0007
EP time (hours)	466.1 ± 0.4394^{c}	470.8 ± 0.5321^{a}	468.6 ± 0.5287^{b}	<0.0001
EP duration (hours)	15.71 ± 0.508^{a}	13.57 ± 0.429^{b}	14.03 ± 0.556^{ab}	0.0059
Incubation time (hours)	481.8 ± 0.498^{b}	484.4 ± 0.465^{a}	482.6 ± 0.526^{b}	0.0005
Fertile hatchability (%)	84.68 ± 2.829	84.44 ± 2.750	83.59 ± 2.922	0.9602
Day old chick body weight (g)	44.89 ± 0.386	44.69 ± 0.451	44.27 ± 0.468	0.6121
Tona score (%)	85 ± 0.8460^{a}	82 ± 1.046^{a}	71 ± 1.369^{b}	<0,0001

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, EP: External pipping, ED: Embryonic day

Table 3. Effect of thermal manipulation on rectal, neck, and side temperature of broiler chickens at hatch (at day 21 of incubation)

Parameters	C	T6	T12	p value
Rectal temperature (°C)	38.89 ± 0.07^{a}	38.59 ± 0.08^{b}	38.20 ± 0.0795^{c}	<0.0001
Neck temperature (°C)	36.34 ± 0.1635^{b}	37.12 ± 0.1680^{a}	36.51 ± 0.2294^{ab}	0.0121
Breast temperature (°C)	37.18 ± 0.1908^{b}	38.16 ± 0.1989^{a}	37.28 ± 0.2549^{b}	0.0041

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, °C: Degree Celsius

Post-hatch performance

The post-hatch performance of the broiler chickens according to treatment is shown in Table 5. Daily feed intake (DFI) was reduced significantly by the thermal treatment ($p < 0.05$). The body weight at 6 weeks of age and the average daily weight gain were increased significantly by a TMI of 12 hours ($p < 0.05$). The feed conversion ratio was reduced significantly in the T12 group compared to the C group ($p < 0.05$). The FCR of the T6 group was not significantly different from the C group and T12 group. The mortality rate (MR) was significantly reduced by TMI ($p < 0.05$). The lowest MR was recorded in the T12 group.

T3, T4 and corticosterone hormone

The effect of TMI on thyroid hormone levels at the internal pipping stage, hatch, and at 6 weeks post-hatch is shown in Table 6. Regardless of the stage (at the internal pipping stage, at hatch, and at 6 weeks post-hatch), the level of T3 was significantly lower in TMI groups, compared to the control ($p < 0.05$). The level of T4 at the internal pipping and 6 weeks post-hatch was significantly lower in the T6 and T12 groups compared to the control group ($p < 0.05$). Plasma corticosterone concentration at 6 weeks of age was significantly lower in T6 and T12 groups compared to the control group ($p < 0.05$, Figure 4).

Meats yields, ultimate pH, and drip loss

The effect of thermal treatment on meat yields is shown in Table 7. The carcass yield of the control group was significantly lower than the other groups, which had similar carcass yields ($p < 0.05$). The thigh yield was not affected by thermal manipulation, while breast yield was increased significantly with 12 hours of TMI compared to the control group ($p < 0.05$). The pHu of meat was significantly higher in T6 group compared to the control group ($p < 0.05$, Figure 5), while drip loss was reduced significantly by thermal manipulation irrespective of TMI duration ($p < 0.05$, Figure 6).

Table 4. Temperature-humidity index, mean temperature, and relative humidity values in the open poultry house during the rearing phase

Schedules of the day / Parameters	7.00 am	10.00 am	01.00 pm	04.00 pm
Temperature (°C)	25.91 ± 0.25	30.5 ± 0.29	33.5 ± 0.2	29.77 ± 0.4
Relative Humidity (%)	46.665	44.835	35.815	39.335
THI	72.44	77.96	79.97	76.20

°C: Degree Celsius, THI: Temperature- humidity index

Table 5. Effect of thermal manipulation on post-hatch performance of broiler chickens

Parameters	C	T6	T12	p value
DFI (g)	76.72 ± 0.46^{a}	70.28 ± 0.411^{b}	70.03 ± 0.551^{b}	< 0.0001
DWG (g)	37.78 ± 0.34^{b}	37.72 ± 0.22^{b}	39.22 ± 0.28^{a}	0.0073
FCR	2.03 ± 0.05^{a}	1.89 ± 0.06^{ab}	1.79 ± 0.09^{b}	0.0335
MR (%)	9.09 ± 0.52^{a}	6.58 ± 0.60^{b}	3.927 ± 0.672^{c}	0.0007
FBW (g)	1631.6 ± 15.5^{b}	1629 ± 12.2^{b}	1691 ± 16^{a}	0.0250

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, DFI: Daily feed intake, DWG: Daily weight gain, FCR: Feed conversion ratio, MR: Mortality rate, FBW: Final body weight.

Table 6. Effect of thermal manipulation on thyroid hormones of broiler chickens

Stage	Parameters	C	T6	T12	p value
IP	T3 (nmol/l)	27.02 ± 0.646[a]	16.29 ± 0.962[b]	12.68 ± 0.114[c]	<0.0001
	T4 (nmol/l)	33.32 ± 0.356[a]	25.21 ± 0.778[b]	19.31 ± 0.009[c]	<0.0001
Hatch	T3 (nmol/l)	3.748 ± 0.386[a]	2.049 ± 0.170[b]	2.542 ± 0.153[b]	0.0012
	T4 (nmol/l)	24.63 ± 0.934[b]	36.09 ± 0.329[a]	37.91 ± 0.832[a]	<0.0001
Six weeks post-hatch	T3 (nmol/l)	2.763 ± 0.018[a]	2.11 ± 0.1091[c]	2.438 ± 0.0414[b]	<0.0001
	T4 (nmol/l)	16.29 ± 0.464[a]	6.025 ± 0.221[c]	9.728 ± 0.2425[b]	<0.0001

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$). C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment, nmol/l: nanomol per liter, IP: Internal pipping

Table 7. Effect of thermal manipulation on meat yields of broiler chickens

Parameters	C	T6	T12	p value
Carcass yield (%)	70.21 ± 0.2222[b]	72.43 ± 0.4498[a]	72.74 ± 0.3032[a]	<0.0001
Breast yield (%)	22.82 ± 0.3277[b]	23.50 ± 0.3673[ab]	24.46 ± 0.2934[a]	0.0044
Thigh yield (%)	22.14 ± 0.4907	21.95 ± 0.4907	20.89 ± 0.5618	0.1668

[a,b] Means within rows with different superscripts differ significantly ($p < 0.05$) C: control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

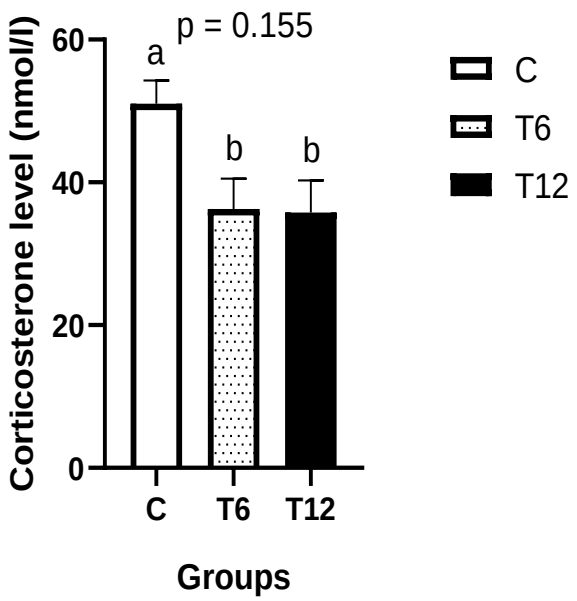

Figure 4. Effect of thermal manipulation during incubation on plasma corticosterone concentration of 6 weeks old broiler chickens. [a,b] Different letters indicate significant ($p < 0.05$) differences among treatments C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

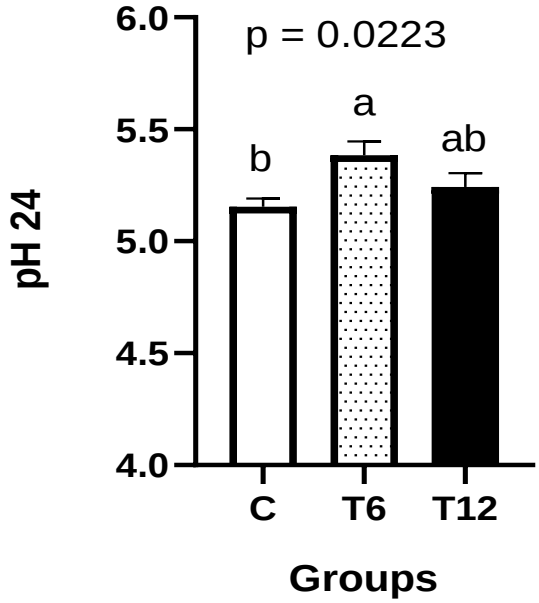

Figure 5. Effect of thermal manipulation on meat ultimate pH of broiler chickens. [a,b] Different letters indicate significant ($p < 0.05$) differences among treatments C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

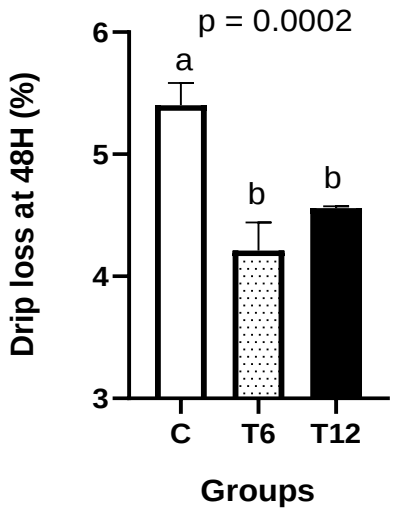

Figure 6. Effect of thermal manipulation on meat drip loss of broiler chickens at 48 hours post-slaughter. [a,b] Different letters indicate significant ($p < 0.05$) differences among treatments C: Control group, T6: Group subjected to 6 hours of thermal treatment, T12: Group subjected to 12 hours of thermal treatment.

DISCUSSION

This study clearly showed that the duration of thermal manipulation during incubation affected embryo development, hatching and post-hatching performance, meat quality, and thermotolerance of Cobb broilers. Regarding the effects of TMI on embryo development at certain embryonic ages, the present study indicated a significant effect of TMI on absolute and relative yolk sac, albumen, and embryo weights. These results are similar to those of Molenaar et al. (2011) and Maatjens et al. (2014), who observed that eggshell temperature influenced yolk absorption and embryo development during incubation.

Regarding hatching performance, similar to the findings of a study by Meteyake et al. (2020), indicating the 12-hour TMI reduced egg weight loss at 18 days of age. The increase in incubation temperature was combined with 65% RH to reduce water loss caused by the high temperature. Relative humidity of 65% appears too high for a 12 h TMI but not for 6 hours since egg weight loss was similar to controls in the group subjected to a 6 hours TMI.

Incubation time was longer in T6 group compared to T12 group, which had a similar incubation time to the control group in the present study. Thermal manipulation during incubation would have reduced the metabolism of the embryos. This could explain why the relative weight of embryos in the T6 group was lower compared to the other groups at certain ages. Moraes et al. (2004) observed a delay in the hatching processes of eggs incubated at 39.0°C for 2 hours per day from day 13 to day 17 of incubation.

Higher incubation temperature depresses hatchability depending upon the embryogenesis stage and temperature's duration and intensity (Halle and Tzschentke, 2011). Thermal manipulation during incubation did not significantly affect hatchability and chick weight in the current study. The present results are in contrast with those of Meteyake et al. (2020), who observed a reduction in hatchability and body weight of Ross 308 day-old chicks subjected to TMI (39.5°C and 65% for 12 hours). This discrepancy could be attributed to the genetic strain of the broiler (Cobb 500) used in the present study. Therefore, a TMI applied for 12 hours would not be enough to induce an augmentation of embryonic mortality in Cobb 500 strain. However, a decrease in the quality of chicks from the eggs subjected to TMI of 12 hours was observed. This result is similar to the studies by Piestun et al. (2015) and Meteyake et al. (2020). A duration of 12 hours of thermal manipulation was sufficient to elicit negative effects on the navel closure and then the chick's quality in this study.

Thermal manipulation during incubation reduced the rectal temperature of hatched chicks in the present study. Collin et al. (2007), Al-Zghoul et al. (2015), Al-Rukibat et al. (2017) observed a low rectal temperature in chicks hatched from the eggs subjected to TMI treatments. This reduction in the body temperature suggested a reduction in metabolism, and thus, better thermotolerance, as evidenced by T3 levels in day-old chicks subjected to TMI. Thermal manipulation during incubation of 6 hours increased the body temperature of the neck and breast in day-old chicks. These results are similar to those reported by Morita et al. (2016), who observed an increase in head, back, and neck temperatures in day-old chicks from eggs subjected to continuous heat treatment of 39°C from day 13 of incubation to hatching. This thermal treatment would have reduced the thickness of the skin and increased the density of blood vessels in the skin, thereby increasing the thermolysis capacity of the chicks. These results were not observed in chicks from the eggs subjected to 12 hours of TMI. This suggests that the duration of TMI would affect broilers' thermolysis and, thus, their thermotolerance.

During the post-hatch growth phase, the chickens were reared in an open-sided poultry house in a tropical temperature where the daily ambient temperature and RH were highest between 10:00 am and 01:00 pm. The ambient temperature during this period exceeded the

optimal rearing temperature (between 22 and 24°C) for adult chickens. It was observed that temperature and RH were inversely proportional. The cyclic variation of ambient temperature and RH in the current study is consistent with environmental conditions in tropical climates. Temperature-humidity index (THI) is an indicator which is used when the effects of heat stress are evaluated (Zulovich et al., 1990). According to Purswell et al. (2012), thermal comfort indices, such as the THI integrate the effects of temperature and humidity and may offer a means to predict the effects of thermal conditions on performance. The THI values were as high as 80 around 10:00 a.m. and even more at 1:00 p.m. This shows that the broilers were thermally stressed every day throughout the experiment, as it has been shown that THI values above 79.92 indicate that hens are exposed to heat stress (Yakubu et al., 2018).

In this study, the higher daily weight gain and final body weight of chickens in the T12 group are consistent with those of Al-Zghoul et al. (2019). It was reported that TMI improved broiler weights by stimulating muscle development and growth via myoblast proliferation (Piestun et al., 2015). This effect was not observed in the T6 group, suggesting that a TMI during 6 hours in the current study was insufficient to stimulate myoblast development and growth.

The feed intake of the chickens decreased as a function of TMI duration in this study. This result confirms that Meteyake et al. (2020) observed a decreased feed intake in acclimated chickens. The decrease in feed intake would be due to a decrease in metabolism and, thus, heat production, corresponding to the low plasma T3 and T4 levels in 6-week-old acclimated chickens. The levels of thyroid hormones are important indicators of metabolic activity (Todini, 2007). Jenkins et al. (2004) stated that thyroid hormones significantly influence the metabolism, development, and thermoregulatory mechanisms of the chicks in the post-hatch period and consequently, on metabolic heat production. The decrease in heat production in chickens subjected to TMI led to better thermotolerance, as shown by the decrease of corticosterone levels in the T6 and T12 groups. Blood corticosterone levels are commonly analyzed as a stress index in chickens (Mahmoud et al., 2004). The results of the current study are similar to those of Piestun et al. (2008), who observed a decrease in blood corticosterone levels in acclimatized chickens subjected to a thermal change at the end of rearing.

The decrease in feed conversion ratio in this study could be explained by the fact that despite a decrease in the feed intake of chickens in the T12 group, they had a higher weight gain than the other group. In agreement with this study result, Meteyake et al. (2020) observed an improvement in the feed conversion ratio in chickens from eggs subjected to TMI.

The increased carcass yield of the TMI chickens in the current study is probably due to the myoblast proliferation, particularly in the breast muscle, which had a higher yield. These results are similar to those of Piestun et al. (2015), indicating that TMI stimulates muscle development and growth via myoblast proliferation. The reduced meat drip loss in the present study is consistent with the observation of Yalçin et al. (2005), who found lower drip loss in breast meat of broilers exposed to 39.6°C from embryonic day 10 to 18. According to AL-Sagan et al. (2020), pH24 is one of the most important physical traits for the qualitative profile of meat and is commonly utilized to assess the sensory qualities of the technological properties of meat. Meat pH is related to the water-holding capacity (Jung et al., 2010), which is correlated positively with the texture, juiciness, and flavor of the meat. In the present study, TMI reduced drip loss, increased the ultimate pH of the meat, and then improved the processing ability of the meat, particularly in the T6 group. These results are similar to those of Yalçin et al. (2022), who reported that an increase in the incubation temperature induced an increase of pH24.

CONCLUSION

In conclusion, thermal manipulation during incubation influenced embryo development and hatching performance. Thermal manipulation during incubation for 12 hours reduced day-old chicks' quality without affecting hatchability and body weight. Thermal manipulation during incubation affected the body and rectal temperature. Thermal manipulation during incubation decreases chicks' rectal temperature. A TMI during 6 hours increases chicks' ability to dissipate heat. Moreover, post-hatch performance and meat quality were improved by TMI. Generally, exposing hatching eggs to 39.5°C and 65% of relative humidity from day 7 to day 16 of incubation during 12 hours/day is recommended for the poultry industry in the tropical climate.

DECLARATION

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Authors' contributions

Meteyake Hèzouwè Tchilabalo contributed to the experimental design, data collection, data analysis, and manuscript drafting. Bilalissi Abidi, Kouame Yaah Aimee Emmanuelle and N'nanle Ombortime contributed to data collection and revising the manuscript. Tona Kokou contributed to the design and supervision of the experiment and to the revising of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Ethical consideration

The authors have made sure that the work complies with the journal's ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) for submission and publication.

Availability of data and materials

The necessary data will be provided by authors according to reasonable requests.

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