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ABSTRACT Many studies have shown that thermal manipulations during the incubation (**TMI**) and naked neck gene (**Na**) positively affect heat-stressed broilers' thermotolerance, hatching process, and posthatch performance. Their combination could increase the beneficial effect on broilers reared under natural tropical climatic conditions. The aim of this study was to investigate the effects of the *Na* gene and TMI on hatching and posthatch performance of slow-growing broilers under tropical climates. The study included 1,200 hatching eggs from 2 different crosses: 1) females and males, both with a normal or fully feathered neck (*na na* group), and 2) females (with a normal neck) and males (bare neck) (*Na na* group), incubated in similar conditions until d 7. Thereafter, they were assigned to 3 subgroups for each cross: the control group (**C**) was incubated at standard incubation conditions (37.8°C, 60% RH). The TMI-1 group was subjected to TMI-1 (T = 38.5°C, RH = 65%, E10−18, 6 h/d) and TMI-2 group to TMI-2 (T = 39.5° C, RH = 65%, E7−16, 12 h/d). Between 450 and 504 h of incubation, eggs were checked for hatching events. During the posthatch phase, chicks from each incubation subgroups (*Na na*-C, *Na na*-TMI-1, *Na na*-TMI-2, *na na*-C, *na na*-TMI-1, *na na*-TMI-2) were raised for 12 wk at a tropical natural ambient temperature. Hatchability, hatching time, chick's temperature, final body weight (**FBW**), and feed conversion ratio (**FCR**) were determined. The results revealed that the *Na* gene reduced ($P < 0.05$) hatchability. The control group had the highest mortality rate compared to TMI-1 and TMI-2 groups. There was an interaction between genotype and TMI on incubation duration, hatching weight, chick quality, FBW, and FCR ($P < 0.05$). In conclusion, the *Na* gene influenced the effects of thermal manipulation. TMI-1 combined with *Na* gene improved the productive performances of broilers in a tropical climate.

Key words: thermal manipulation, *Na* gene, hatching process, posthatch growth, heat-stressed broiler

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INTRODUCTION

Heat stress is the main environmental factor that causes loss of productivity and high mortality rates on poultry farms (Terim Kapakin et al., 2013). According to Havenstein et al. (2003), broiler chickens are more susceptible to thermal stress, and their vital functions such as thermoregulation are negatively affected by it. Over the years, the genetic selection of broiler strains has mainly focused on the increased growth rate of the birds. However, little attention has been given to the cardiovascular and pulmonary systems that play essential roles in birds' thermoregulation, making them less developed and potentially less able to sustain the higher growth rate of the birds (Havenstein et al., 2003).

Different genetic, technical, or dietary strategies have been explored to improve the tolerance of birds to variations in thermal conditions (Oke, 2018; Meteyake et al., 2020). One of the genetic strategies is the use of slow-growing broiler genotypes. These genotypes may vary on their feather characteristics. One is the presence or lack of feathers on the neck. This characteristic depends on an autosomal and incompletely dominant gene (with 2 alleles, *Na* and *na*). The "Na" allele of this gene is associated with the limitation of feathering in the neck. Birds with the homozygous *na na* genotype have a normal or fully feathered neck, whereas those with the homozygous *Na Na* genotype have a complete lack of feathering in

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the neck (bare neck). The heterozygous genotype (*Na na*) leads to the presence of isolated tufts of feathers on the ventral position of the neck (tufted neck) (Crawford, 1976). According to Galal (2000), the reduction of feathers would promote thermolysis and allow good thermal stress resistance. Many studies showed that heterozygous Na na and homozygous Na Na birds can mitigate adverse effects of heat stress on body weight gain, feed intake, and feed conversion ratio (**FCR**) (Chen et al., 2009; Rajkumar et al., 2011; Adomako et al., 2014). Therefore, naked neck gene may represent a low-cost solution that is particularly attractive to developing countries with warm climates (Fernandes et al., 2023).

Another possible solution to heat stress is heat acclimatization. The embryonic and early postnatal stages are the best periods to improve the resistance of poultry to heat through thermal manipulations (Yahav, 2009). According to Yahav and McMurtry (2001), adaptation to ambient conditions depends on a mechanism that can be manipulated by exploiting the immature thermoregulation of the birds at the perinatal stage. In order to improve thermal tolerance and the well-being of birds without altering growth and the economic viability of poultry sectors, many studies on thermal manipulations in the perinatal period have been conducted in temperate and tropical climates (Piestun et al., 2008; Tona et al., 2008; Nideou et al., 2019; Meteyake et al., 2020; Oke et al., 2020). These techniques induce rapid physiological and metabolic changes that last throughout the life of the animal (Loyau et al., 2015).

Despite the advances made in the improvement of thermotolerance of commercial broiler chickens, only a few studies have focused on the thermal manipulation in slow-growing broilers strain under tropical climates, studies on the combined effect of genotype and thermal manipulation during incubation (**TMI**) are scarce. It can be hypothesized that the naked neck gene (***Na* gene**) and TMI improve the heat resistance of slow-growing broilers subjected to chronic heat stress. It can also be hypothesized that their combination would make broilers reared in tropical climatic conditions more resistant to tropical climates. Thus, the current research aimed to investigate 1) the effect of TMI, 2) the effect of genotype, and 3) the combined effects of TMI and genotype on thermotolerance, hatching, and posthatch performance of slow-growing broilers subjected to chronic heat stress.

MATERIALS AND METHODS

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.

Experimental Design

A total of 1,200 hatching eggs each from 2 different crosses of slow-growing broilers were used (Figure 1). The first cross was between Sasso SA51 females and Ruby XL

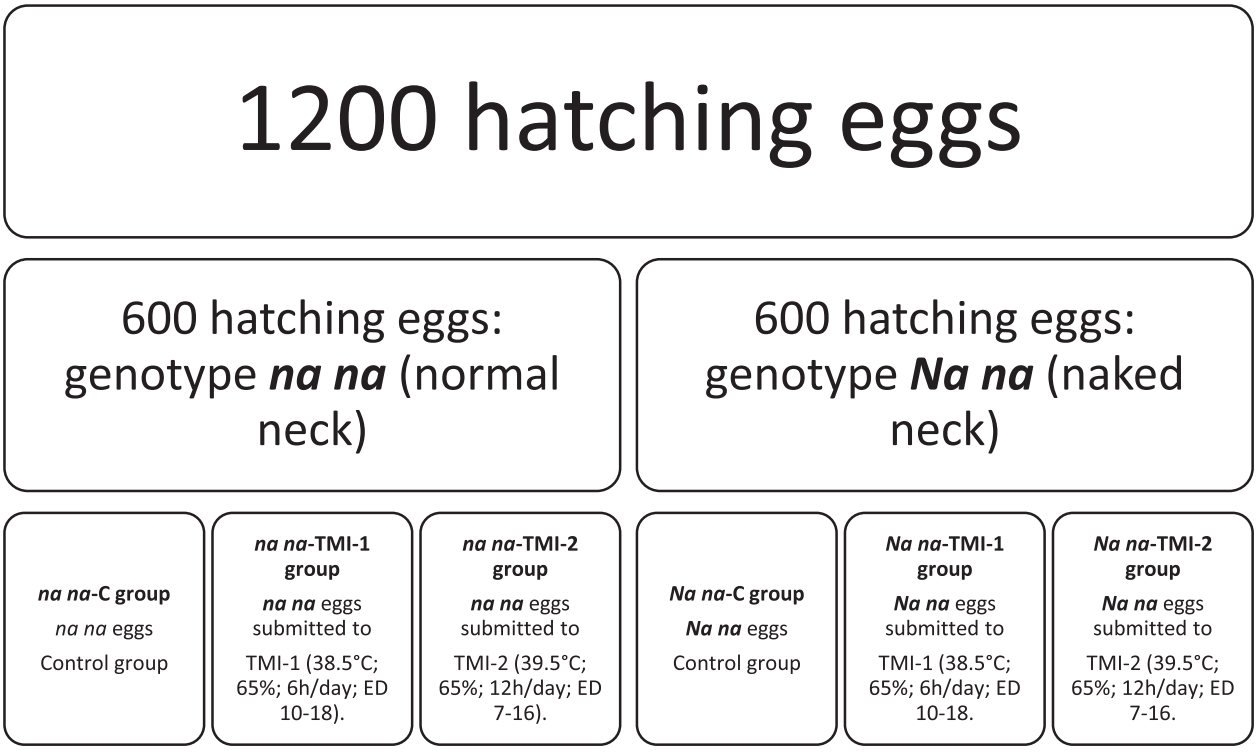

Figure 1. Experimental design. C: control group ; TMI-1: group submitted to thermal manipulation during incubation 1; TMI-2: group submitted to thermal manipulation during incubation 2; *na na*: normal neck group; *Na na*: naked neck group; TMI: thermal manipulation during incubation; *na na*-C: normal neck eggs from control group; *na na*-TMI-1: normal neck eggs subjected to TMI-1; *na na*-TMI-2: normal neck eggs subjected to TMI-2; *Na na*-C: naked neck eggs from control group; *Na na*-TMI-1: naked neck eggs subjected to TMI-1; *Na na*-TMI-2: naked neck eggs subjected to TMI-2.

males, both with a normal neck (*na na* genotype) and aged 45 wk. Eggs from this cross had *na na* genotype with normal-feathered neck chickens (*na na* group). The other cross was between SA51 females (normal neck, *na na* genotype) and Ruby N males (bare neck, *Na Na* genotype) also aged 45 wk. Their Eggs had the *Na na* genotype, with a tufted neck (*Na na* group). Prior to being set for incubation, all the eggs were numbered and weighed. They were incubated in the same incubator (Petersime Vision, Zulte, Belgium) (with a capacity of 9,600 eggs) at 37.8°C and 60% relative humidity (**RH**) until the embryonic day (**ED**) 7 when eggs from each genotype were randomly allocated to 3 subgroups (control, TMI-1, and TMI-2 groups) of 200 eggs (4 replication of 50 eggs per sub group per cross). The control group (**C**) was maintained at standard incubation conditions (37.8°C, 60% RH) whereas the TMI-1 group was subjected to thermal manipulation during incubation 1 (TMI-1: 38.5°C, RH = 65%, E10−18, 6 h/d; according to Yalçin et al., 2008) and TMI-2 group to thermal manipulation during incubation 2 (TMI-2: 39.5°C, RH = 65%, E7−16, 12 h/d; according to Piestun et al., 2008). Eggs from these 3 groups were incubated in 3 similar incubators (SmartPro-Combi, PasReform, the Netherlands; with a capacity of 600 eggs each) and turned every hour at 90° until ED 18 when all the eggs were candled. Then, those with evidence of a living embryo were weighed and transferred to hatching baskets. At hatch (E21), 125 chicks from each subgroup (*Na na*-C, *Na na*-TMI-1, *Na na*-TMI-2, *na na*-C, *na na*-TMI-1, and *na na*-TMI-2) were then transferred to the farm and raised for 12 wk in a completely randomized design. After 3 wk of brooding, the chickens were raised at a natural tropical ambient temperature in an open-sided poultry house, with 5 replicates of 25 chickens per group randomly assigned to floor pens. They were subjected to the same prophylactic and light program (23 h of light and 1 h of darkness). All the birds were fed ad libitum and received the same feed (starter: crude protein = 21%, metabolizable energy = 2,860 kcal/kg; grower: crude protein = 20%, metabolizable energy = 2,950 kcal/kg).

Eggs Weight Loss, Hatching Events, and Hatchability

Eggs weights (**EW**) were recorded at E0 and E18 and were used to determine the relative egg weight loss (**EWL**) at E18 of incubation using the Formula (1):

$$\text{EWL } (\%) = \left(\frac{\text{EW0 (g)} - \text{EW18 (g)}}{\text{EW0 (g)}}\right) \times 100 \qquad (1)$$

where EW0 is the eggs weight at E0 and EW18 is the eggs weight at E18.

From the 450th hour to the end of incubation (504th hour of incubation), the time of the occurrence of external pipping (**EP**) and chick hatching for individual eggs was recorded every 3 h. The hatched chicks were recorded. These data were used to determine the EP time (time between setting and EP), EP duration (duration between EP and hatching), the total incubation duration (time between setting and hatching) and the spread of hatch according to treatments. Hatchability of fertile eggs was also determined using the Formula (2):

$$\text{Hatchability } (\%) = \frac{\text{Number of hatched chicks at the end of incubation}}{\text{Number of fertiles eggs transferred to hatching basket}} \times 100 \qquad (2)$$

Chick Quality and Day-Old Chick Body Weights at Hatching

At the end of incubation, chick quality was determined on fifty chicks per treatment group using Tona scoring method (Tona et al., 2003). According to this method, the quality criteria such as the reflex, down and appearance, eyes, the conformation of legs, navel area, yolk sac, remaining membranes, and yolk were scored. The chick quality score was defined as the sum of the scores assigned to each quality criterion. The day-old chicks' body weight was also measured after hatching. At the end of hatching, clear and unhatched eggs were broken to determine macroscopically true fertility and the stage of embryonic mortality.

Meteorological Data During the Rearing Phase

Temperatures and relative humidity (Table 1) in the poultry house were recorded every day at 7:00 am, 10:00 am, 1:00 pm and 5:00 pm using thermo-hygrometers. These data were used to calculate the temperature-humidity index (**THI**) using Eq. (4) used by Bueno et al. (2020):

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

Table 1. Temperature-humidity index (THI) and mean temperature and relative humidity (RH) values in the open poultry house during the rearing phase.

	Schedules of the day			
Parameters	07:00	10:00	13:00	17:00
Temperature (°C)	26.08 ± 0.04	29.17 ± 0.06	31.38 ± 0.11	26.58 ± 0.08
HR (%)	77.92	71.75	60.58	71.9
THI	76.34	80.30	81.75	75.46

°C, degree Celsius; THI, temperature-humidity index.

Posthatch Growth Performance

At the end of the hatching, 125 chicks per treatment were transferred to the research farm and were weighed to determine their initial body weight. During the experimental period, all the birds were weekly weighed per group. The daily weight gain (**DWG**) was computed as the difference in body weight between 2 consecutive measurements divided by the number of days between weights. The amount of feed given and the remaining feed were measured daily. These data were used to calculate the daily feed intake (**DFI**) using this equation:

$$\text{DFI (g)} = \frac{\text{Feed given (g)} - \text{remaining feed (g)}}{\text{Number of birds} \times \text{number of days}} \quad (5)$$

The FCR was computed by dividing DFI by DWG. The mortalities were recorded daily. These data allow the calculation of the mortality rate (**MR**) using this formula:

$$\text{MR (\%)} = \frac{\text{Number of dead birds during a period}}{\text{Number of birds at the beginning of the period}} \times 100 \quad (6)$$

Blood Sample Collection and Hormonal Analysis (T3, T4)

At the end of the rearing period, blood samples ($n = 15$ per treatment) were collected from brachial veins in nonheparinized tubes with a 2 mL syringe and centrifuged at 3,000 rpm for 15 min. The serum obtained was stored in a freezer at −20°C until analyzed. A volume of 100 μL of this serum was used for triiodothyronine (**T3**), thyroxin (**T4**), and corticosterone concentrations determination by using the automated VIDAS systems and each of VIDAS T3 kit (reference: 30403-01), VIDAS T4 kit (reference: 30404-01), and VIDAS cortisol kit (reference: 30451) all distributed by BioMerieux S.A. (Lyon, France) and which use an enzyme-linked fluorescent assay (**ELFA**) technique.

Statistical Analysis

The data were analyzed using the R statistical software package. Shapiro-Wilk's test was used to check the normal distribution of data, and Levene's test to prove the homogeneity of variance. Data on hatching percentages were analyzed using a logistic regression model. The generalized linear model Procedure was used to analyze the EWL, hatching event, day-old chicks' body weights, chick quality, and posthatch performance (feed intake, body weight, FCR). Scores of quality were transformed into log (Y+1) values and then retransformed into the original values after the analysis. Data were analyzed as a completely randomized design with a 2 × 3 factorial arrangement of treatments. When the treatment effects of the general model were statistically significant, the means were further compared using Tukey's post-test. For all analyses, P value of 0.05 was used as the level of significance. The model was:

$$\text{Yijk} = \mu + \text{Ti} + \text{Pj} + (\text{T} * \text{P})\,\text{ij} + \text{eijk}$$

where Yijk is the egg weight loss, EP time, EP duration, incubation duration, day-old chicks body weights, chicks quality, and feed intake, body weight, DWG, FCR; μ is the overall mean; Ti is the effect of genotype; Pj is the effect of TMI; (T*P)ij is the interaction between genotype and TMI; and eijk is the random error.

RESULTS

Genotype and TMI had various effects on hatching performance (EWL, hatching window, incubation duration, EP time and duration, hatchability, chick quality and chicks weight), posthatch performance (daily feed intake, DWG, FCR, and mortality rates) and thermotolerance (T3, T4, and corticosterone concentration) of chickens.

Eggs Weight Loss at E18 and Hatching Process

A significant interaction ($P = 0.0281$) effect of genotype and TMI was observed on EWL at E18 (Table 2). In the *na na* genotype, TMI-2 eggs lost less weight than

Table 2. Effect of TMI and genotype on egg weight loss (EWL), external pipping duration (EP dur), external pipping time (EP time), incubation duration (Inc dur), and hatchability.

Genotype	*na na*			*Na na*			*P* value		
Treatments	C	TMI-1	TMI-2	C	TMI-1	TMI-2	GNT	TMI	GNT × TMI
EWL (%)	13.62 ± 0.37^{ab}	12.78 ± 0.27^{ab}	11.69 ± 0.28^{c}	13.66 ± 0.33^{a}	12.17 ± 0.22^{bc}	$12.71 \pm 0{,}31^{ab}$	0.554	<0.0001	0.028
EP dur (h)	15.13 ± 0.12^{a}	11.89 ± 0.20^{dc}	14.23 ± 0.20^{b}	12.55 ± 0.19^{c}	10.51 ± 0.15^{e}	$11.70 \pm 0{,}26^{d}$	<0.0001	<0.0001	0.002
EP time (h)	$465.31 \pm 0{,}68^{bc}$	466.67 ± 0.69^{ab}	463.57 ± 0.77^{c}	469.25 ± 0.84^{a}	465.81 ± 0.61^{bc}	464.12 ± 0.96^{bc}	0.060	<0.0001	0.005
Inc dur (h)	480.45 ± 0.40^{ab}	478.56 ± 0.45^{bc}	477.80 ± 0.66^{cd}	481.80 ± 0.72^{a}	476.32 ± 0.55^{cd}	475.83 ± 0.88^{d}	0.060	<0.0001	0.007
Hatchability (%)	92.54 ± 0.91^{a}	92.63 ± 0.81^{a}	92.33 ± 0.90^{a}	90.66 ± 0.93^{b}	89.97 ± 0.92^{b}	89.70 ± 0.90^{b}	0.0045	0.7990	0.8751

[a–d]For each column and each factor, data sharing no common letter were different ($P < 0.05$).

C: control group; TMI-1: group submitted to TMI-1; TMI-2: group submitted to TMI-2; *na na*: normal neck group; *Na na*: tufted neck group; TMI: thermal manipulation during incubation; *na na*-C: normal neck eggs from control group; *na na*-TMI-1: normal neck eggs subjected to TMI-1; *na na*-TMI-2: normal neck eggs subjected to TMI-2; *Na na*-C: tufted neck eggs from control group; *Na na*-TMI-1: tufted neck eggs subjected to TMI-1; *Na na*-TMI-2: tufted neck eggs subjected to TMI-2; GNT: genotype.

control and TMI-1 eggs, whereas in the *Na na* genotype, the control eggs lost more weight than the TMI-1 eggs only. But control and TMI-2 eggs weight loss being not statistically different.

The duration of EP, the average time of EP and hatching (incubation duration) were significantly affected ($P = 0.002$; $P = 0.0059$ and $P = 0.007$, respectively) by the interaction of TMI and genotype (Table 2). In both genotypes, TMI-1 and TMI-2 treatments induced a lower duration of EP than for control eggs, the effects being more pronounced for TMI-1 than for TMI-2, especially in the *na na* genotype. In the *na na* genotype, EP time was reduced in the TMI-2 group compared to the TMI-1 group, while in the *Na na* genotype, TMI-1 and TMI-2 induced a lower EP time than control incubation. Accordingly, in the *Na na* genotype, the incubation duration was shorter ($P < 0.0001$) in both TMI-1 and TMI-2 groups compared to the control group while it was reduced only in TMI-2 group compare to control group in *na na* genotype, incubation time in TMI-1 group being statistically not different from that of the 2 other groups.

Thermal manipulations during embryogenesis also affected the spread of hatch depending on the genotype considered (Figure 2). Indeed, hatching of chicks commenced 6 h and 12 h earlier in *na na*-TMI-2 than *na na*-TMI-1 and *na na*-C, respectively. However, *Na na*-TMI-2 chicks hatched 3 h and 9 h earlier than *Na na*-TMI-1 and *Na na*-C, respectively. In both genotypes, the spread of hatch was shorter in the control group than in TMI-1 and TMI-2 groups. It was 18 h, 21 h, and 33 h in *na na*-C, *na na*-TMI-1 and *na na*-TMI-2 groups, respectively, while it was 21 h, 27 h, and 27 h in *Na na*-C, *Na na*-TMI-1, and *Na na*-TMI-2 groups, respectively. The peak of the hatching curve was reached at the 480^{th}, 480^{th}, and 474^{th} hours of incubation in *na na*-C, *na na*-TMI-1, and *na na*-TMI-2 groups, respectively. It was reached at the 474^{th}, 477^{th}, and 474^{th} hours of incubation in *Na na*-C, *Na na*-TMI-1, and *Na na*-TMI-2 groups, respectively.

Egg Hatchability, Embryo Mortality, Day-Old Chick Body Weight, Quality Score, and Body Temperature

Hatchability was reduced ($P = 0.0045$; Table 2) in the *Na na* genotype as compared to the *na na* one, whatever the thermal treatment considered, with in average 2.39% less hatchability with this genotype. There were interactions of TMI and genotype on chick weight and quality ($P = 0.046$ and $P = 0.039$, respectively; Table 3). *Na na*-TMI-2 group body weight was lower than that of *Na na*-TMI-1, with the *na na* genotype chick weight not being affected by thermal treatments. Chick quality was lower in the TMI-2 group than in control and TMI-1 group in the *na na* genotype, whereas it was not different among groups of *Na na* genotype, with a significant interaction between both factors ($P = 0.0391$).

Body temperature of hatchlings, in average of 39.7°C, was not affected by the genotype, the embryo thermal treatment or the interaction of both factors (data not shown).

Posthatch Growth Performances, Daily Feed Intake, Feed Conversion Ratio, and Mortality Rate

Broiler body weight at 12 wk of age was significantly influenced by the interaction of genotype and TMI ($P < 0.0001$; Table 4); TMI-1 chickens had the highest body weights in both groups (+1.51% than controls in *na na* and +5.11% than controls in *Na na* genotype), with TMI-2 chickens being also heavier than controls in the *na na* genotype. Control *Na na* chickens were also 0.81% heavier than their *na na* counterparts. DWG was significantly affected by the interaction of genotype and TMI ($P = 0.0457$; Table 4). The DWG was statistically not different in all the groups except in the *Na na-TMI-1* group where the broilers grew more than in the other groups. Additionally, there was a significant interaction

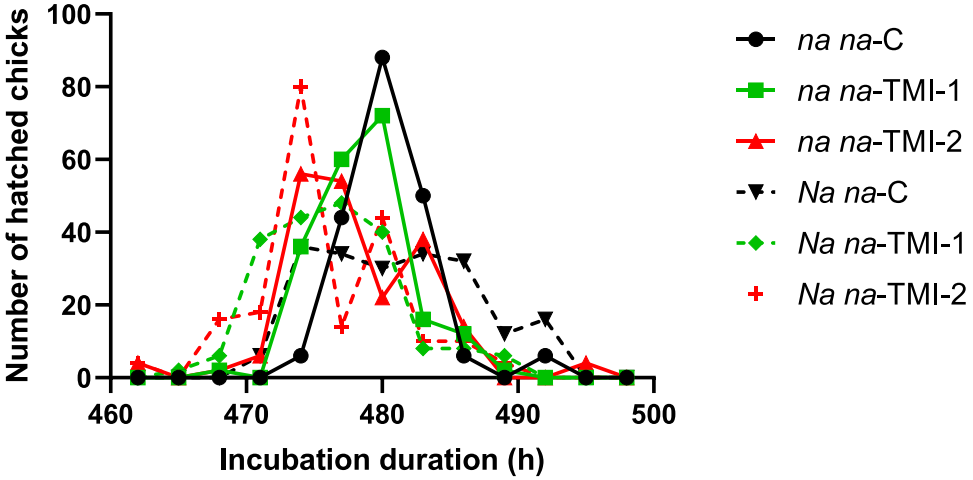

Figure 2. Effect of TMI and genotype on the spread of hatch. TMI: thermal manipulation during incubation; *na na*-C: normal neck eggs from control group; *na na*-TMI-1: normal neck eggs subjected to TMI-1; *na na*-TMI-2: normal neck eggs subjected to TMI-2; *Na na*-C: naked neck eggs from control group; *Na na*-TMI-1: naked neck eggs subjected to TMI-1; *Na na*-TMI-2: naked neck eggs subjected to TMI-2.

Table 3. Effect of TMI and genotype on day-old chick's weight and quality.

Genotype	*na na*			*Na na*			*P* value		
Treatments	C	TMI-1	TMI-2	C	TMI-1	TMI-2	GNT	TMI	GNT × TMI
Chick weight (g)	39.5 ± 0.50^{a}	38.0 ± 0.41^{ab}	37.9 ± 0.46^{ab}	39.0 ± 0.47^{ab}	39.43 ± 0.36^{a}	37.43 ± 0.43^{b}	0.580	0.0008	0.040
Chick quality (out of 100)	93.60 ± 0.36^{a}	93.33 ± 0.46^{a}	91.67 ± 0.42^{b}	93.33 ± 0.49^{ab}	93.40 ± 0.44^{ab}	93.47 ± 0.42^{a}	0.140	0.080	0.040

[a,b]For each column and each factors, data sharing no common letter are different ($P < 0.05$).

C: control group; TMI-1: group submitted to TMI-1; TMI-2: group submitted to TMI-2; *na na*: normal neck group; *Na na*: tufted neck group; TMI: thermal manipulation during incubation; *na na*-C: normal neck eggs from control group; *na na*-TMI-1: normal neck eggs subjected to TMI-1; *na na*-TMI-2: normal neck eggs subjected to TMI-2; *Na na*-C: tufted neck eggs from control group; *Na na*-TMI-1: tufted neck eggs subjected to TMI-1; *Na na*-TMI-2: tufted neck eggs subjected to TMI-2; GNT: genotype.

Table 4. Effect of TMI and genotype on daily weight gain (DWG), final body weight (BW), daily feed intake (DFI), feed conversion ratio (FCR), and mortality rate (MR).

Genotype	*na na*			*Na na*			*P* value		
Treatments	C	TMI-1	TMI-2	C	TMI-1	TMI-2	GNT	TMI	GNT × TMI
DWG (g)	23.63 ± 0.2^{b}	23.84 ± 0.1^{b}	24.02 ± 0.07^{b}	23.82 ± 0.08^{b}	25.08 ± 0.24^{a}	$23.88 \pm 0{,}26^{b}$	0.004	0.001	0.040
FBW (g)	2025.2 ± 1.7^{d}	2055.8 ± 1.2^{b}	2038.8 ± 1.9^{c}	2041.8 ± 1.75^{c}	2146.3 ± 3.80^{a}	2043.5 ± 0.90^{c}	<0.0001	<0.0001	<0.0001
DFI (g)	74.28 ± 0.7^{b}	72.91 ± 0.61^{bc}	71.17 ± 0.9^{c}	77.02 ± 0.61^{a}	73.20 ± 0.46^{bc}	68.42 ± 0.57^{d}	0.850	<0.0001	0.001
FCR (g:g)	3.14 ± 0.03^{a}	3.008 ± 0.02^{b}	2.98 ± 0.02^{bc}	3.23 ± 0.02^{a}	2.91 ± 0.02^{cd}	2.86 ± 0.02^{d}	0.030	<0.0001	0.0001
MR (%)	9.60 ± 0.98^{a}	4.00 ± 1.26^{b}	3.20 ± 0.80^{b}	7.20 ± 1.49^{a}	2.40 ± 0.98^{b}	4.80 ± 0.80^{b}	0.374	0.0001	0.1701

[a–d]For each column and each factors, data sharing no common letter are different ($P < 0.05$).

C: control group; TMI-1: group submitted to TMI-1; TMI-2: group submitted to TMI-2; *na na*: normal neck group; *Na na*: tufted neck group; TMI: thermal manipulation during incubation; *na na*-C: normal neck broilers from control group; *na na*-TMI-1: normal neck broilers subjected to TMI-1; *na na*-TMI-2: normal neck broilers subjected to TMI-2; *Na na*-C: tufted neck broilers from control group; *Na na*-TMI-1: tufted neck broilers subjected to TMI-1; *Na na*-TMI-2: tufted neck broilers subjected to TMI-2; DWG: daily weight gain; FBW: final body weight at 84 d of age; DFI: daily feed intake; FCR: feed conversion ratio; MR: mortality rate; GNT: genotype.

of genotype and TMI recorded ($P = 0.0001$) for feed intake and FCR ($P = 0.0001$; Table 4). The DFI in Na na-C was higher than that of *na na*-C. TMI-2 treatment reduced the feed intake in both genotype while TMI-1 treatment reduced the DFI only in *Na na* group. It could also be noted that the reduction in DFI by the TMI-2 treatment was greater in *Na na* chickens. Regarding FCR, it was improved by both TMI treatment in each genotype. FCR was the highest in *Na na*-C and *na na*-C group, followed by *na na*-TMI-1 and *na na*-TMI-2 which were not different. The FCR was the lowest in *Na na*-TMI-1.

The mortality rate was reduced (with in average 5.2 and 4.4% less mortality rate respectively in TMI-1 and TMI-2, $P = 0.0001$; Table 4) in the TMI groups as compared to the control group. Moreover, it was not statistically different between TMI-1 and TMI-2 group.

T3, T4 Levels, and Corticosterone at D 84 of Age

Serum T3 and T4 concentrations at 12 wk of age were significantly influenced by the interaction of genotype and TMI ($P < 0.0001$; Table 5). The lowest T3 concentrations were observed in the *Na na*-C group and both TMI-1 groups while the highest value was obtained in the *Na na-TMI-2* group. Regarding T4 concentration, it was the highest in the *Na na*-C group, followed by *Na na*-TMI-2 which was not different from the *na na*-C group but higher than the *na na*-TMI-1 group. The lowest T4 concentration was in *na na*-TMI-2 group. The serum corticosterone concentration at 12 wk of age was significantly influenced by the interaction of genotype and TMI ($P = 0.0384$; Table 5). Tufted-neck TMI-1 broilers had lower concentrations of corticosterone than both control groups, *na na*-TMI-1 and *Na na*-TMI-2 groups.

DISCUSSION

This study aimed to investigate the effects of thermal manipulation during incubation on hatching and post-hatch performance in 2 genotypes of slow-growing broilers reared in a tropical environment. Overall, the genotype and thermal manipulations during thermogenesis

Table 5. Effect of TMI and genotype on T3, T4, and corticosterone level at d 84.

Genotype	*na na*			*Na na*			*P* value		
Treatments	C	TMI-1	TMI-2	C	TMI-1	TMI-2	GNT	TMI	GNT × TMI
T3 (nmol/L)	10.16 ± 0.07^{b}	9.17 ± 0.11^{c}	9.61 ± 0.05^{bc}	9.46 ± 0.05^{c}	9.01 ± 0.24^{c}	11.02 ± 0.24^{a}	0.090	<0.0001	<0.0001
T4 (nmol/L)	31.46 ± 1.23^{bc}	30.24 ± 1.23^{cd}	27.46 ± 0.38^{d}	42.08 ± 0.8^{a}	27.93 ± 0.26^{d}	33.71 ± 0.26^{b}	<0.0001	<0.0001	<0.0001
Corticosterone (μg/dL)	0.52 ± 0.04^{a}	0.5 ± 0.07^{a}	0.495 ± 0.006^{ab}	0.5 ± 0.011^{a}	0.47 ± 0.004^{b}	0.498 ± 0.003^{a}	0.005	0.002	0.030

[a–d]For each column and each factors, data sharing no common letter are different ($P < 0.05$).

C: control group; TMI-1: group submitted to TMI-1; TMI-2: group submitted to TMI-2; *na na*: normal neck group; *Na na*: tufted neck group; TMI: thermal manipulation during incubation; *na na*-C: normal neck broilers from control group; *na na*-TMI-1: normal neck broilers subjected to TMI-1; *na na*-TMI-2: normal neck broilers subjected to TMI-2; *Na na*-C: tufted neck broilers from control group; *Na na*-TMI-1: tufted neck broilers subjected to TMI-1; *Na na*-TMI-2: tufted neck broilers subjected to TMI-2; GNT: genotype.

interestingly interacted on the short- and long-term performance and physiology of the birds.

First, the use of a high incubation temperature (39.5°C) in the group subjected to TMI was associated with the use of high relative humidity (65%) to avoid excessive water loss. The TMI significantly reduced EWL at E18. Comparing EWL of broiler eggs incubated at different relative humidity, Tullett and Burton (1982) reported that the higher the relative humidity, the lower the EWL. Given the lowest water loss in the heated groups compared to the control group, it could be hypothesized that the relative humidity was higher than for just compensating the higher incubation temperatures chosen for these 2 TMI (TMI-1: 38.5°C, RH = 65%, ED 10−18, 6 h/d; and TMI-2: TMI-2: 39.5°C, RH = 65%, ED 7−16, 12 h/d). Regarding the TMI-1 groups, as observed in egg water loss, the fact that incubation duration was similar to the control in the *na na* groups and lower than the control group in the *Na na* group suggests that the *na na* embryos are more sensitive to the 38.5°C temperature applied in TMI-1. Furthermore, the fact that both the Na gene and TMI accelerated metabolism and hence embryo development until hatching could explain why *Na na* eggs subjected to TMI had a shorter incubation duration than all other groups.

In the current study, hatchability was not affected by incubation conditions, which is consistent with the results of Piestun et al. (2008), who observed that exposure of Cobb eggs to 39.5°C for 12 h/d did not significantly affect embryo mortality. Previous reports have indicated that depending on the time period and primarily the daily duration of temperature manipulation, hatching rate can be decreased Piestun et al. (2008), increased (Collin et al., 2007), or not influenced (Yalçin et al., 2008). But at the same eggshell temperature, metabolism, embryo development and thus hatching performance could also be affected by eggs' genetic background (Nangsuay et al., 2015). This is reflected in this study where a significant effect of genotype on hatchability is observed. The decrease in hatchability in the *Na na* group in this study is in agreement with the results of Merat (1990) and Desta (2021) who reported that the naked neck gene was associated with increased embryonic mortality. This occurred during the last stages before hatching. The authors indicated that the reduced hatchability appeared to be due to embryo malpositions.

Body weight at hatch was reduced by TMI-2 in the *Na na* group as compared to *Na na*-TMI-1 and *na na* C groups. Our results are in agreement with the earlier findings of Meteyake et al. (2020), who explained this negative effect of TMI on chicks' body weight by the fact that the embryo subjected to TMI-2 had a rapid development because of the acceleration of their metabolism. The fact that TMI-2 did not affect the hatching body weight of *na na* chicks reflects an interaction between TMI and genotype. This is confirmed by the fact that TMI-1 did not impact hatching body weight in both genotypes. This interaction of genotype and TMI was also observed on chick quality which was reduced by TMI-2 in *na na* genotype only. These results are similar to the report of Meteyake et al. (2020) who obtained a negative effect of TMI on chick quality. This could suggest that the high temperature accelerated embryogenesis and some chick quality criteria such as the navel closure, the remaining membrane and the remaining yolk of the day-old chicks were adversely affected.

Na gene and TMI, as well as their interaction, did not affect the rectal temperature of the birds at hatch (data not shown). This result contradicts the one of Loyau et al. (2013), who obtained lower body temperatures in the chicks exposed to 39.5°C for 12 h/d at hatch than that of the control group and which is in accordance with several studies on fast-growing broilers reported by Loyau et al. (2015). The discrepancy in the findings may be due to the difference in the strains of the birds used, the present study focusing on slow-growing broilers. Even if the rectal temperature is a good criterion for thermotolerance (De basilio et al., 2003), Morita et al. (2016) found that heat treatment during incubation, could, without reducing rectal temperature, increase thermolysis by the increasing of skin temperature and then increase birds thermotolerance.

During the posthatch growth, broilers were reared in an open-sided poultry house where they were exposed to natural ambient conditions. The birds were exposed to cyclic conditions and the temperature and relative humidity varied during the day and were inversely proportional, typical of environmental conditions obtained in tropical climates. THI is an indicator that considers both temperature and relative humidity when the effects of heat stress are evaluated (Zulovich and Deshazer, 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 am and even more at 1:00 pm. This shows that the birds might be thermally stressed every day throughout the experiment, as it has been suggested that THI values above 79.92 indicate that birds are exposed to heat stress (Yakubu et al., 2018).

In both genotypes, lower feed intakes were recorded in TMI-2 birds in than controls. This lower feed intake could represent an advantage under tropical climate, inducing a lower diet-induced thermogenesis in these groups. A positive interaction between the Na gene and the TMI-2 was also observed since their association made it possible to have the lowest DFI in this group, probably allowing animals to cope better with heat stress by reducing thermogenesis. This effect was also observed to a lesser extent in the TMI-1 group only with the *Na na* genotype, confirming the interest of both this genotype and heat stimulations during embryogenesis to reduce heat increment in slow-growing chickens.

The improvement of DWG in *Na na*-TMI-1 group is consistent with the report of Piestun et al. (2015) who found that TMI applied on hatching eggs improved weight gain by stimulating myoblast proliferation and

thus muscle development and growth. The similarity of DWG between *na na* groups and the increase of DWG in *Na na*-TMI-1 compare to other *Na na* groups illustrate the interaction of genotype and TMI on DWG. It was also the case with final body weight that was improved by TMI-1 in both genotypes but more in *Na na* broilers than in *na na* group. The positive impact of naked neck gene on birds growth, observed in the current study were already found by Yalçin et al. (1997) and Deeb et al. (1999) who showed that the growth of normally feathered birds can be compromised at temperature above 32°C whereas naked neck animals show better growth rates. Regarding FCR, it was reduced by TMI in both genotypes. This result is similar to the report of Piestun et al. (2013) who found higher FCR in a control group compared to thermally manipulated broilers reared until 70 d of age. According to Piestun et al. (2009), thermal manipulation during incubation altered the energy balance of the fast-growing broilers by reducing their maintenance requirements and thus providing more energy for production. In the current study, TMI reduced FCR more in the *Na na* group than in the *na na* group. This illustrates the positive interaction between genotype and TMI on FCR. Singh et al. (1998) found that the weight of feather at slaughter is about 5% of the animal weight, most of which is protein. So the reduction of feathers by Na gene means a reduction of protein needed for feathers development and then an extra amount of protein channeled into growth of the animals. Which could explain the improvement of birds FCR by Na gene in a previous study of Chen et al. (2004). Therefore, the naked neck gene would have emphasized the improvement of FCR by TMI.

Similar to the earlier report of Meteyake et al. (2020), TMI probably allowing broilers to better thermo-tolerance, significantly reduced the mortality rate in both genotype.

Thyroid hormones are known to regulate heat production and development in mammals and in avian species (McNabb, 1988; Collin et al., 2009). According to Decuypere et al. (1981), the selective peripheral conversion of thyroxine (T4) into triiodothyronine (T3), or reverse triiodothyronine (r-T3) plays an important role in the thermoregulation of domestic fowl. There was an interaction between genotype and TMI on the serum T3 and T4 concentrations. In the fully-feathered *na na* birds, TMI-1, but not TMI-2, reduced serum T3 concentration. Concerning TMI-2 treatment, this finding is in agreement with the results of Loyau et al. (2013) who observed unchanged plasma T3 concentration compared to control incubation during an acute heat challenge in fast-growing chickens (5 h at 32°C) at 34 d of age. Nevertheless, Piestun et al. (2008) had previously observed decreased plasma T3 concentration in TMI-2 (vs. control incubation) during a heat challenge at 35°C for 5 h. The discrepancies between studies may have been due to genetics and/or the type of thermal challenge (i.e., acute vs. chronic in the present study). However, with the TMI-1 incubation treatment, the lower circulating T3 concentration seemed to occur in fully feathered slow-growing birds, suggesting a reduction in the stimulation of heat production in these animals. Furthermore, this was not the case in Na na birds, showing higher T3 concentrations in TMI-2 than in both control and TMI-1 birds, despite nondifferent serum T4 concentrations from that of the controls. In these birds, the naked neck gene may have provided an adaptive advantage to lose heat that could be overcompensated in TMI-2 birds at the level of the mechanisms regulating heat production. These regulations could potentially involve deiodinase activities or T3 uptake by the tissues, especially by the liver, for ensuring metabolic regulations (Reyns et al., 2002; Yalçin et al., 2008).

Corticosterone levels have been used as a measurement of environmental stress in birds (Beuving et al., 1989). In the current study, there was also an interaction between genotype and TMI on corticosterone levels at 84 d of age: serum corticosterone concentration was reduced by TMI-1 only in *Na na* chickens. It is well known that the naked neck gene improves heat tolerance by reducing feather cover (Galal et al., 2019). It increases also the size of head appendages (wattle and comb), instigating more body surface to thermoregulation and loss of heat (Desta, 2021). TMI also is known to decrease corticosterone levels and thus thermotolerance in heat-stressed chickens (Piestun et al., 2008). The fact that the lowest corticosterone level was obtained in the *Na na*-TMI-1 group suggests that the effect of TMI on corticosterone reduction was emphasized by the naked neck gene.

In conclusion, genotype and TMI had significant effects on hatching and posthatch performances. Despite the minor effects of the *Na* gene on hatchability and chick quality, the combination of Na gene with TMI-1 or TMI-2 treatments improved the broilers thermotolerance, body weight and FCR. The use of both naked neck gene and TMI (TMI-1: 38.5°C, RH = 65%, ED 10−18, 6 h/d) would allow better productive performances and should be recommended in tropical countries.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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