MANAGEMENT AND PRODUCTION

Incubation temperature alters thermal preference and response to heat stress of broiler chickens along the rearing phase

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ABSTRACT The current study aimed to investigate whether embryonic temperature manipulation may alter thermal preference throughout the rearing phase of broiler chickens and how this manipulation may affect response to thermal challenge, metabolism, growth rate and feed intake rate. Eggs were exposed to a constant incubation temperature [machine temperatures: 36°C (Low), 37.5°C (Control), and 39°C (High); eggshell temperature of 37.4 ± 0.08°C, 37.8 ± 0.15°C, and 38.8 ± 0.33°C, respectively] from d 13 till hatching. Low treatment chickens showed lower plasma T3 and GH levels at d 1 of age and lower T3 level at d 42 of age compared to the Control treatment. Preferred ambient, rectal temperature, T4 level, growth rate, food intake rate, and response to thermal challenge were not altered in these chickens. On the other hand, High-treatment chickens exhibited high preferred ambient temperature and rectal temperature during the first 2 wk post-hatch, lower plasma T3 level at d 21 and 42 and a delayed increase in respiratory movement in response to thermal challenge compared to the Control treatment. However, chickens subjected to the Control and High treatments did not differ in T4 and GH level and performance. We conclude that exposure to high temperature during late embryonic development has long-lasting effects on the thermoregulatory system of broiler chickens by affecting the heat tolerance of these chickens. Moreover, the preferred ambient temperature of the chickens from heat-treated eggs correspond to those recommended for the strain under study, whereas for the cold-treated and control-chickens it was 1°C below, indicating that incubation temperature might have consequences on the ambient temperature chickens require during the rearing phase.

Key words: broilers, embryonic development, preferred ambient temperature, thermal challenge, thermal manipulation

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INTRODUCTION

Environmental temperature during poultry rearing is one of the physical factors that determine phenotypic expression of the chickens' genetic potential for production and growth. Indeed, deviations from the recommended temperature for a given broiler strain might affect performance by decreasing production of meat or other poultry products, causing economic losses (Geraert et al., 1996; Yahav and Hurwitz, 1996; Yahav et al., 1997).

Manipulation of environmental conditions during embryonic development has long-lasting effects on the resulting phenotypes, including physiological modifications needed to develop heat tolerance. Thus, thermal manipulation during incubation may be a potential mechanism to modulate performance, health, and well-being during later life. In fact, several studies have observed effects of thermal manipulation during incubation on various chicken characteristics post-hatch, including higher muscle growth (Halevy et al., 2001; Piestun et al., 2009; Loyau et al., 2013), higher gastrointestinal growth (Uni et al., 2001), and improved heat tolerance when chickens are thermally challenged (Yahav et al., 2004, 2005; Collin et al., 2005; Piestun et al., 2008; Yalçin et al., 2008; Collin et al., 2011; Druyan et al., 2012).

Prenatal thermal manipulation might also influence the preferred temperature of chickens in later life. Ducks and turkeys incubated in the last wk at low (34.5°C) or high (38.5°C) temperatures decreased and increased, respectively, their preferred ambient temperature until they reached 10 d of age (Nichelmann, 2004). Tzschentke (2007) verified that ducklings continuously exposed to a high temperature (38.5°C) from d 28 of incubation

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also increased their preferred temperature until they reached 10 d of age. In contrast, Walstra et al. (2010), showed that intermittent exposure of eggs of layer hens to high eggshell temperature (40°C) for short periods at the end of incubation decreased their preferred temperature in the first wk of rearing. Therefore, continuous or intermittent prenatal thermal manipulation appear to alter the thermal preference in turkeys, ducks, and layer and broiler chicks in their initial phase of rearing, a period in which these precocial birds acquire body temperature control (Nichelmann and Tzschentke, 2002). However, effects of thermal manipulation on preferred temperature in broilers is poorly studied, and furthermore, we still do not know how thermal manipulation affects the preferred temperature and the response to a thermal challenge of chickens throughout the rearing period.

Therefore, our objectives of the present study were: (1) to investigate for how long temperature manipulation during embryonic development affects thermal preference of broiler chickens during the rearing phase; (2) whether body temperature is associated with a chicken's thermal preference, and (3) how temperature manipulation during embryonic development affects the chickens' response to thermal challenge, their metabolism and in terms of growth rate and feed intake rate. The answers to these questions may contribute to further understanding of the effects of thermal manipulation during incubation on the posthatch ontogeny of thermoregulation.

MATERIALS AND METHODS

Experimental Conditions

The experimental protocol used in this study was approved by the local Ethics Committee on Animal Use (CEUA, protocol n° 021086/11), of the College of Agricultural and Veterinary Sciences, of the São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil.

A total of 900 Cobb500® eggs from one broiler breeder flock at 59 wk of age were obtained from a commercial hatchery (Globoaves, Itirapina, São Paulo, Brazil), weighed, and distributed homogeneously by average weight (67 to 72 g) over six incubators (150 eggs each). The incubator temperature was set at 37.5°C until d 12 of incubation. From d 13 until hatching, thermal programming was applied by decreasing the incubator temperature to 36°C (Low), increasing it to 39°C (High) or maintaining it at 37.5°C (Control), giving to eggshell a temperature of 37.4 ± 0.08°C, 37.8 ± 0.15°C, and 38.8 ± 0.33°C, respectively (measured every 30 min by the thermistors attached to the eggshells of 5 individual fertile eggs, per incubator, and the data stored in data loggers connected to a computer). It was used two repetitions (two incubators) per temperature profile. Relative humidity (**RH**) was maintained at 60% in all incubators until hatching. This procedure was established to eliminate effects of RH in the incubator on embryonic development and mortality. Egg rotation (45° of rotation/2 h) was maintained until d 18 of incubation. Thermal programming from d 13 of development onward was determined based on the knowledge that functional maturation of the hypothalamo-pituitary-thyroid axis is established around 13 to 14 d of incubation (Jenkins and Porter, 2004).

Rearing

After hatching, 115 male chicks from each incubation treatment were housed in one of five boxes (2.50 × 1.50 m, 23 chicks per box) for 6 wk within a climate chamber. Ambient temperature within the chamber was decreased each wk as recommended in the rearing manual (Cobb 500). Temperature and RH inside the chamber were registered 3 times daily during the experimental period. Per chamber two digital thermohygrometers, located at equidistant points within the chamber were used. From the first to the sixth wk of the experiment, the average weekly values of temperature and RH, were 33.3°C, 29.8°C, 27.3°C, 25.4°C, 23.0°C, and 21.0°C and 65%, 76%, 68%, 57%, 65%, and 51%, respectively. Throughout the experimental period, water and feed were available ad libitum. The diet of the chickens consisted of two feed types, based on corn and soybean meal: (1 to 21 d: ME = 2,883 Kcal/kg, PB = 21.27%) and growth (22 to 42 d: ME = 3,121 Kcal/kg, PB = 18.86%), formulated according to the requirements established for broiler chickens under tropical conditions by Rostagno et al. (2011). The chicks were vaccinated against Marek and Bouba Avian disease in the hatchery, and against Newcastle and Gumboro disease during rearing, following the vaccination program recommended for Cobb strain.

Thermal Preference Test

The thermal preference test used in this study was based on the methodology of Myhre et al. (1975) and Walstra et al. (2010). Two thermal preference test chambers with similar dimensions, thermal gradient system, and registration of temperature and chicken location were used. Briefly, each test chamber presented a thermal gradient from 19°C to 40°C along its length, ambient temperature and bird position were registered by 12 thermal sensors and 12 infrared sensors, respectively, positioned in opposite sides along of the chamber's length. Data for temperature and chicken position were obtained and stored each minute, by means of a software program developed for monitoring of the temperature and chicken location.

Thermal preference tests were simultaneously realized in the two test chambers at d 1 to 2, 7 to 8, 14 to 15, 21 to 22, 28 to 29, and 35 to 36 of age, using two chickens at the same time within each test chamber (n = 12 chickens/treatment). At start of the test, chickens were positioned in the central region of the chambers and during the first 30 min they had the opportunity to explore them. After completion of this period, the

chickens were repositioned in the central region of the chambers and a new period of 60 min followed, during which the temperature gradient and chicken movement and position in the chambers were registered. After the tests, preferred ambient temperature was established for each chicken, and corresponded to ambient temperature in which the bird remained for period longer (≥22 min, in the present study). If the chicken remained for a similar time at two or more temperatures, the mean temperature was calculated. The rectal temperature of the chickens was measured with a digital thermometer before the thermal preference tests.

Thermal Challenge Test

Thermal challenge tests were performed at d 3 to 4, 8 to 9, 15 to 16, 22 to 23, and 29 to 30 of age, based on the methodology of Walstra et al. (2010). For this test, two acrylic climate chambers (side × side × height: 80 × 80 × 80 cm) equipped with heaters, fans, and humidifiers, allowing a temperature variation of 15 to 40°C were used. During the tests the same chickens were used as in the thermal preference tests of the previous d, totaling 12 chickens/treatment/age. During the tests, the chickens were kept for 45 min under preferred ambient temperature (obtained in the previous day). Thereafter, to thermal challenge, the chamber temperature was adjusted to 5°C above preferred ambient temperature. The chamber reached a challenge temperature after 3 min. The broilers were exposed to heat challenge for 45 min. Finally, after the thermal challenge, the chamber temperature was returned to thermal preference, under which the chickens remained for another 45 min. Rectal temperature, measured with a digital thermometer, and respiratory movements, obtained through observation of movements per min, were recorded every 45 min; just before the thermal challenge, at the end of the thermal challenge, and 45 min after the end of the thermal challenge. After the tests, chickens used were marked so that they would not be used in the thermal preference and thermal challenge tests in the next weeks. This exclusion was done to prevent adaptation of chickens to the chambers and prevent thermal conditioning to high temperatures during thermal transition of the chickens, which occurs approximately up to d 10 post-hatch (Tazawa et al., 1988; Nichelmann and Tzschentke, 2002).

Plasma Hormone

Blood samples were collected by brachial vein puncture using a syringe at d 21 and 42 of age and stored in Eppendorf tubes, containing EDTA (15 μL/mL blood). Blood was centrifuged at 2,000 rpm for 10 min at 4°C to obtain plasma. Plasma was stored at –70°C until analysis. Plasma samples were analyzed for growth hormone (GH, ng/dL), 3,5,30-tri-iodo-l-thyronine (T_3, ng/mL), and 3,5,30,50-tetraiodo-l-thyronine (T_4, ng/dL). The total concentrations of T3, T4, and GH were measured by radioimmunoassays (RIA), with kits "Coat-a-count" (T3 AccuBind ELISA KIT, Monobind cod 125–300B; T4 AccuBind ELISA KIT, Monobind cod 225–300B; and chicken growth hormone ELISA kit, MyBioSource, cod MBS266317, San Diego, CA), using a gamma radiation counter (Gamma-C12, Diagnostic Products Corp., Los Angeles, CA).

Growth and Feed Intake Rates, and Survival

Growth rate was measured by weighing chicks on d 1, 21, and 42 of age. Feed intake rate was measured for d 1 to 21 and d 22 to 42. Chicken survival was determined for the rearing period until 42 d of age. All data were determined for each replicate.

Statistical Analyses

Data were assessed with one-way analysis of variance (ANOVA), using the General Linear Models procedure of SAS 9.2 software package (SAS Institute, 2009). Model assumptions were approved for both means and residuals before analyses. Preferred ambient and rectal temperature, hormone concentration, and performance results (BW, FI) were tested with respect to the effects of incubation temperature (IT; cold: 36°C; control: 37.5°C; and heat: 39°C), and within each age, using the following model: $Y = \mu + \text{Temperature} + e$, in which Y = dependent variable, μ = overall mean, and e = residual error. Rectal temperatures and respiratory movements, registered just before, at the end, and 45 min after thermal challenge, were analyzed with the same model. Differences between the rectal temperatures and respiratory movements registered just before and at the end of the thermal challenge ($\Delta 1$) or registered at the end and 45 min after the thermal challenge ($\Delta 2$) were also analyzed with the same statistical model. When necessary, in case of significant differences (at 5%), the means were compared by Tukey's test.

RESULTS

Thermal Preference and Body Temperature

The thermal preference test was performed at d 1 to 2, 14 to 15, 21 to 22, 28 to 29, and 35 to 36 posthatch. However, at d 35 to 36 chickens did not show enough mobility to determine their preferred temperature. Chickens from the High incubation temperature treatment preferred a significantly higher ambient temperature and had higher rectal temperatures before the preference test until d 14 to 15, compared to Low and Control incubation temperature treatments (Figure 1A, B). There was no difference in these variables between the Low and Control incubation temperature treatments. After this age, no significant difference in thermal preference and rectal temperatures were

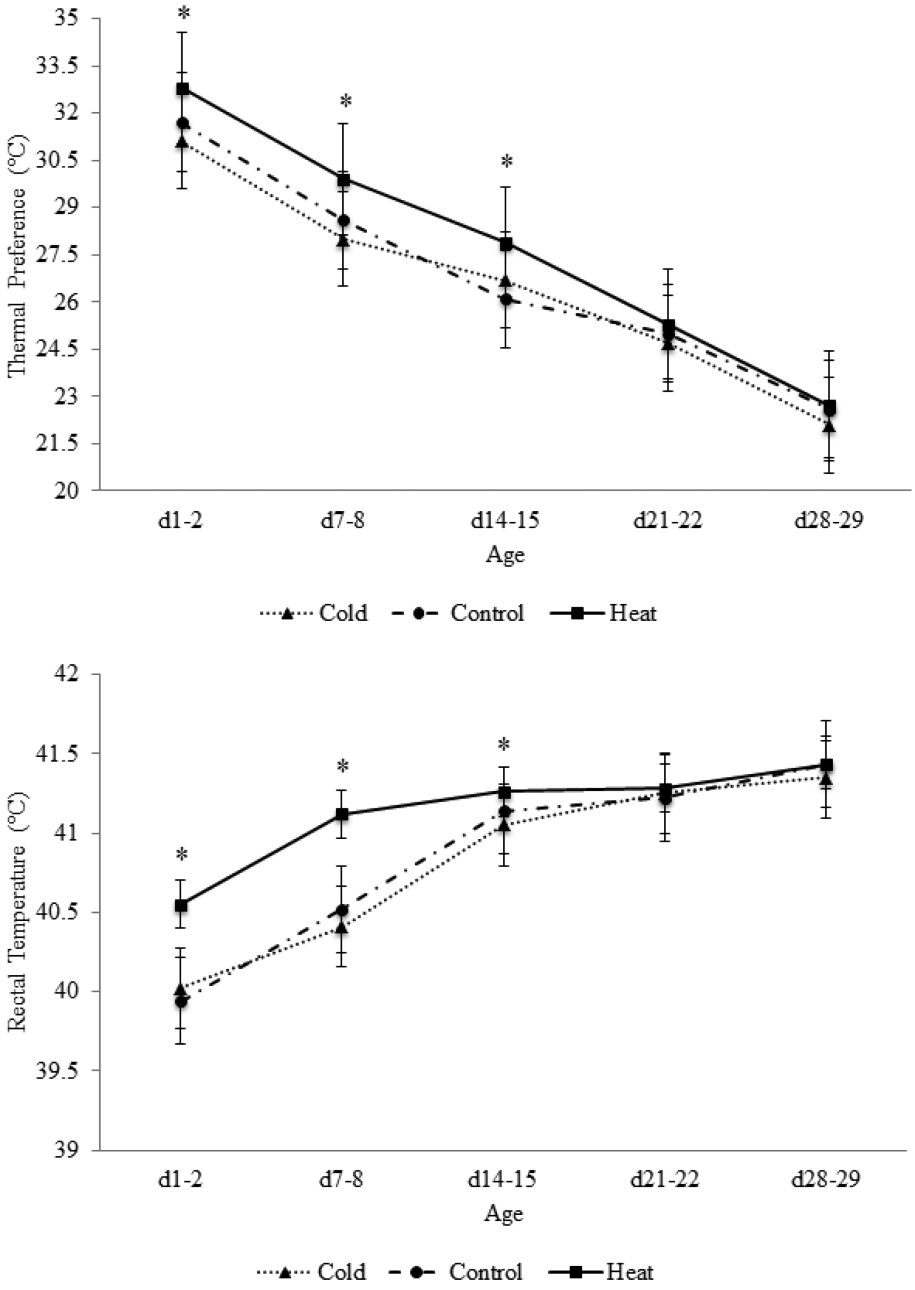

Figure 1. Thermal preference (A) and rectal temperature (B) of broiler chickens obtained from eggs subjected to Low (36.0°C), Control (37.5°C), or High (39.0°C) incubation temperature from d 13 of incubation till hatching. *: means obtained from high incubated chickens differ from the other groups at the same age ($P < 0.05$). Data are expressed as mean ± SD, n = 12 per incubation temperature per age.

found among chickens obtained from different incubation temperatures.

Plasma Hormone Levels

Plasma T_3 and GH levels were significantly lower ($P < 0.05$) at d 1 in chickens from the Low incubation temperature in comparison to High and Control treatments, which did not differ from each other (Table 1). T3 level at d 21 was lower for chickens obtained from the High incubation temperature than for both other temperatures, whereas at d 42 High and Low incubation temperature resulted in a lower T3 level than the Control incubation temperature. Plasma T_4 did not differ among incubation temperatures at any age, whereas GH at d 21 and d 42 did not differ among incubation temperatures either

Body Weight, Feed Intake and Survival During Rearing

Incubation temperature did not affect BW at d 1, 21, and 42. Feed intake was evaluated on two periods: 1 to 21 and 22 to 42 d. Chickens from the High incubation temperature showed lower feed intake than the Low incubation temperature, but only for the period of

Table 1. Effect of incubation temperature from d 13 of incubation till hatching (36°C (Low), 37.5°C (Control) or 39°C (High)) on plasma triiodothyronine (T3, ng/mL), thyroxine (T4, ng/dL), and growth hormone (GH) concentration at d 1, 21, and 42 of age and on body weight and feed intake during rearing of broiler chickens.

		Incubation temperatures				
Variables	Ages	Low	Control	High	*P*-value	CV (%)
T3 (ng/dL)	d 1	3.01[b]	4.07[a]	3.93[a]	0.0119	21.04
	d 21	1.78[a]	1.69[a]	1.45[b]	0.0002	25.17
	d 42	1.22[b]	1.34[a]	1.18[b]	0.0314	32.18
T4 (ng/dLl)	d 1	6.03	5.89	6.37	0.1215	26.22
	d 21	7.13	6.73	7.79	0.1541	8.01
	d 42	9.11	9.30	9.45	0.2004	11.16
GH (ng/dL)	d 1	1.54[b]	1.75[a]	1.70[a]	0.0024	32.33
	d 21	3.80	4.56	3.67	0.2822	41.17
	d 42	1.96	1.40	1.49	0.2516	44.69
Body weight (g)	d 1	48.50	48.94	48.98	0.8173	6.58
	d 21	810.30	787.60	790.83	0.2356	6.40
	d 42	2,325.44	2,325.28	2,245.76	0.1540	5.24
Feed intake (g)	d 1–21	941.27[a]	922.22[a,b]	836.40[b]	<0.0001	4.1
	d 21–42	2,492	2,471	2,416	0.8786	12.87

Plasma concentrations of T3, T4, and GH as well as body weight (BW) were measured at d 1 and d 21 and d42 d of age. Feed Intake was measured for the periods of 1–21 and 22–42 d. Broiler chickens tested belonged to the Low (36°C), the Control (37.5°C) or High (39°C) treatments referring to the temperatures of incubation during fetal development. [a,b]: Means lacking a common letter within a row differ ($P < 0.05$).

1 to 21 d, with the Control incubation temperature in between and not different from both other groups. Between d 21 and 42 feed intake was not affected by incubation temperature. Chicken survival until d 42 of age was not influenced by incubation temperatures (Low: 96.8%; Control: 95.7%; High: 93.6%; $P = 0.2312$).

Thermal Challenge

Low- and control incubated chickens showed, in all analyzed ages, an increase in rectal temperature during the thermal challenge. Rectal temperatures decreased again when the chickens were exposed back to their preferred temperature after the thermal challenge. However, only chickens at d 8 to 9 decreased their rectal temperatures to similar values as before the challenge (Figure 2A and B). High incubated chickens showed comparable results for the ages d 2 to 3, d 22 to 23, and d 29 to 30, but at these ages rectal temperatures did not return back to the values presented before the thermal challenge. High incubated chickens at d 8 to 9 and d 15 to 16 did not exhibit a change in rectal temperature when challenged (Figure 2C).

The differences between the rectal temperatures registered before and at the end of the thermal challenge ($\Delta 1$) or at the end and 45 min after the end of the thermal challenge ($\Delta 2$) were compared among Low, Control and High incubated chickens and are shown in Figure 3A. High incubated chickens showed lower $\Delta 1$ and $\Delta 2$ at d 8 to 9 and d 15 to 16 compared to Low and Control incubated chickens, between which there was no difference.

Respiratory movements of Low and Control incubated chickens increased during the thermal challenge at d 22 to 23 and d 29 to 30 and remained high after it (Figure 4A and B), whereas the same holds for the High incubated chickens at d 29 to 30 only (Figure 4C). Regardless of the treatments, no changes in the respiratory movements frequency in response to thermal challenge was shown by chickens in the other analyzed ages.

The differences between the respiratory movements registered at the end of the thermal challenge and that before it ($\Delta 1$) or between the end of the thermal challenge and 45 min after the end of it ($\Delta 2$) were compared among Low, Control and High incubated within each age (Figure 5). High incubated chickens showed lower $\Delta 2$ at d 22 to 23 and d 28 to 29 compared to chickens from the other two incubation temperatures, which were similar. Independent of the treatment, no difference in A1 and A2 due to thermal challenge was recorded in the other analyzed ages.

DISCUSSION

We investigated how thermal manipulation affects the preferred temperature, metabolism, growth rate, feed intake rate, and the response to thermal challenge of broiler chickens during the rearing period.

Up to 14 to 15 d of age, in comparison with the recommended ambient temperature in the rearing profile for the studied strain, our data have shown that this temperature coincides with the preferred temperature by chickens from the High incubation treatment, which is 1°C above the preferred temperature of Cold and Control treatments. Indeed, the chickens from the High incubation temperature preferred a significantly higher ambient temperature and had higher rectal temperatures until d 14 to 15 than those from both other incubation temperatures. These data suggest that incubation of eggs at high temperature, increasing eggshell temperature, affects the rectal temperature of the chickens and consequently determines the thermal preference of these chickens in the first part of the rearing phase. Rectal

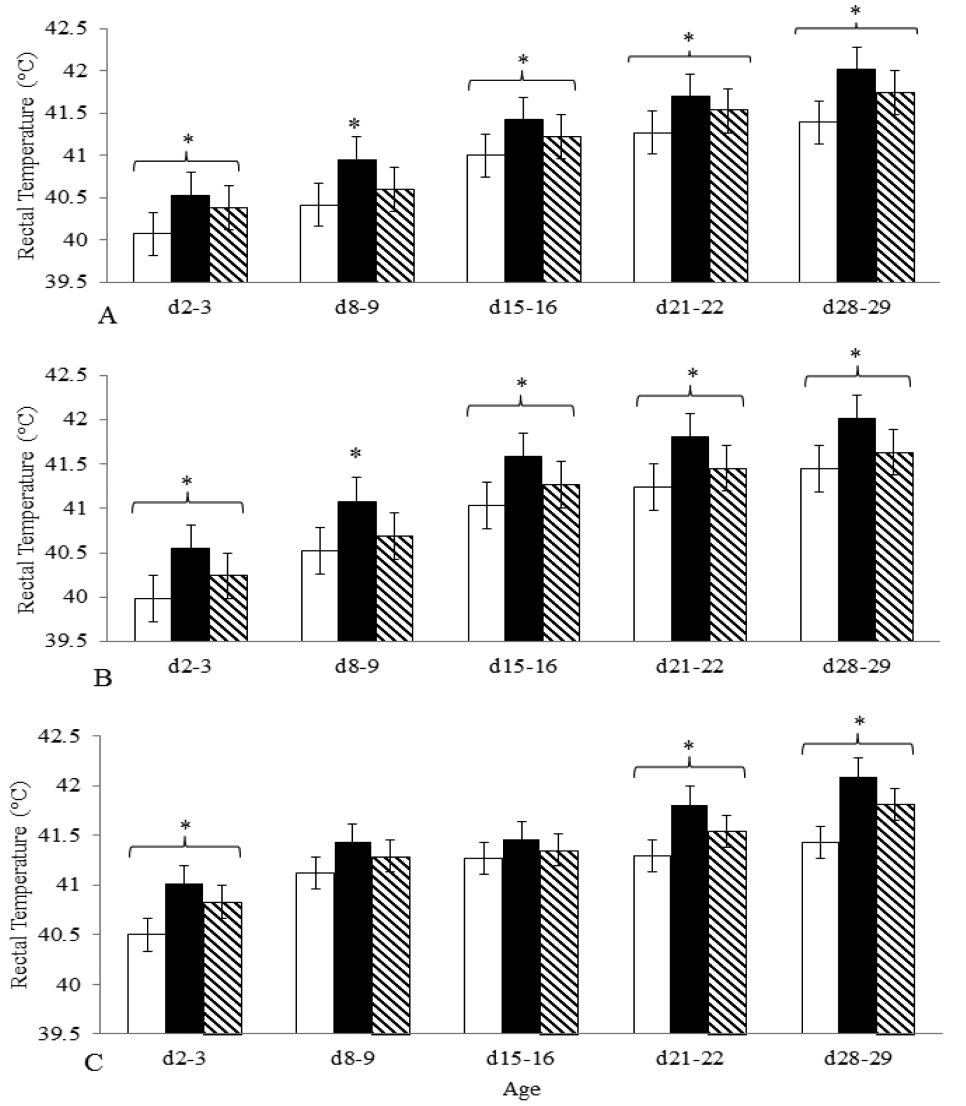

Figure 2. Rectal temperature of broiler chickens incubated at Low (36°C, A), Control (37.5°C, B), or High (39°C, C) temperatures from d 13 of incubation till hatching when exposed to ambient temperatures at thermal preference (before thermal challenge), at 5°C above thermal preference for 45 min (thermal challenge), and upon return to thermal preference (45 min after the thermal challenge) at different ages. *: asterisk above the bars indicates that means were different from other treatments at the same age; * above the bracket indicates difference among the means at the same age ($P < 0.05$). Data are expressed as mean ± SD, n = 12 per treatment per age.

temperature has been shown to reflect heat exchanges between the animal and the environment, because the difference between the surface and ambient temperature is the driving force for sensible heat exchange. Therefore, it can be expected that with a higher rectal temperature, the skin temperature will be higher as well, which would result in a lower preferred ambient temperature (Yahav and Plavnik, 1999; Yahav et al., 2005; Piestun et al., 2008). However, in the current study opposite results were found with a higher preferred temperature. This might be due to the fact that we have demonstrated that High-incubated chickens had a thinner and more vascularized skin than the Control- and Low-incubated chickens (Morita, unpublished results), which may allow a greater heat exchange, resulting in a preference for higher ambient temperature.

This hypothesis is supported by data showing that the differences between the rectal temperature and environment temperature was the same among the three treatments for whole period studied. Furthermore, according to De Basilio et al. (2003), rectal temperature of young chickens is considered to be an effective indicator of later heat tolerance, so this difference observed among chickens from the heat incubation treatment and the other groups may indicate a carry-over effect of thermal programming during incubation on postnatal life.

Our findings partially agree with those obtained by Nichelmann (2004) and Tzschentke (2007). Nichelmann

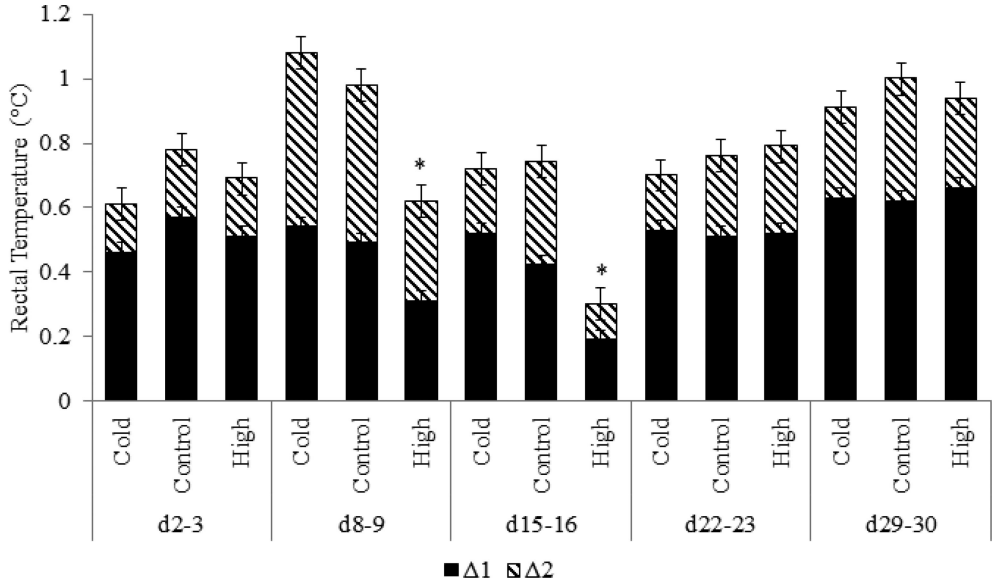

Figure 3. Comparisons of the differences in rectal temperature (Δ1 and Δ2) among Low (36°C), Control (37.5°C) and High (39°C) incubated chickens, at different ages. Δ1: difference in rectal temperature between the end of the thermal challenge (5°C above thermal preference for 45 min) and just before thermal challenge (under thermal preference). Δ2: differences in rectal temperature between the end of the thermal challenge (5°C above thermal preference for 45 min) and upon return to thermal preference (45 min after the end of the thermal challenge). *: asterisk above the bars indicates that means were different from others in the same age ($P < 0.05$). Data are expressed as mean ± SD, n = 12 per treatment per age.

(2004) exposed eggs from ducks and turkeys to high temperature (38.5°C) in the last wk of incubation. These birds also demonstrated higher rectal temperature at 1 d of age and preferred higher ambient temperatures until 10 d of age. Tzschentke (2007) found that continuous exposure to high temperature (38.5°C) from d 28 of incubation also increased the ducklings preferred temperature until 10 d of age. However, our findings contrast to the results obtained by Nichelmann (2004) who incubated eggs at a low temperature (34.5°C). In that case, the birds preferred lower ambient temperatures. Our data also differs from those presented by Walstra et al. (2010) who exposed eggs of layer hens intermittently to high eggshell temperature (40°C) and found that the chickens did not change their rectal temperature and preferred lower ambient temperature. They differ also from results of Piestun et al. (2008) that verified lower body weight and temperature at hatching when eggs were continuously exposed to thermal manipulation at 39.5°C and 65% RH from embryonic d 7 to 16. These discrepancies may be due to the different bird species used and most probably the thermal manipulation protocol used (temperature, duration, period, and constant vs intermittent). These ambiguous results also suggest that thermal manipulation during the embryonic phase is a very sensitive tool to induce thermotolerance in later life, but only when applied in the right way.

We also investigated whether incubation temperature had metabolic effects by assessing plasma hormonal levels in the chickens. Thyroid hormones and GH are indicators of metabolic status and animal growth rate, respectively (Todini, 2007). On one hand, chickens obtained from the Low incubation treatment had lower levels of T3 and GH at d 1, but at 42 d of age, only the T3 levels were lower. The low levels of T3 and GH presented by the chickens from the Low-incubated eggs indicates lower metabolism and growth compared to chicks of the Control and High incubation treatments and may be because the exposure of eggs to low temperatures slows fetal development and increases the duration of incubation (Morita, unpublished results). On the other hand, chickens from the High incubation treatment only showed lower T3 concentrations and at 21 d compared to Low and Control incubation treatments, and at 42 d compared to Control incubation treatment, indicative of lower metabolism. In this study we found that, for the period of 1 to 21 d of age, chickens from the High incubation treatment consumed less feed than the Low incubated ones. This difference in the feed intake might be related to heavier residual yolk-sac presented by chicks from High-incubated eggs compared to chicks from Low-incubated eggs, as verified by Almeida et al. (2015) for the same chickens utilized in this study. The initial growth period of chickens is characterized by the maturation of the thermoregulatory system (Whittow and Tazawa, 1991; Nichelmann and Tzschentke, 2002). Therefore, the difference in the feed intake rate between these groups might be due to different feeding requirements to sustain their metabolic heat production and maintain their body temperature also. From this point of view, the low T3 levels presented by the chickens from the High incubation treatment seem to be a thermoregulatory mechanism used by these chickens to reduce feed intake and consequently metabolic heat production, already that their body

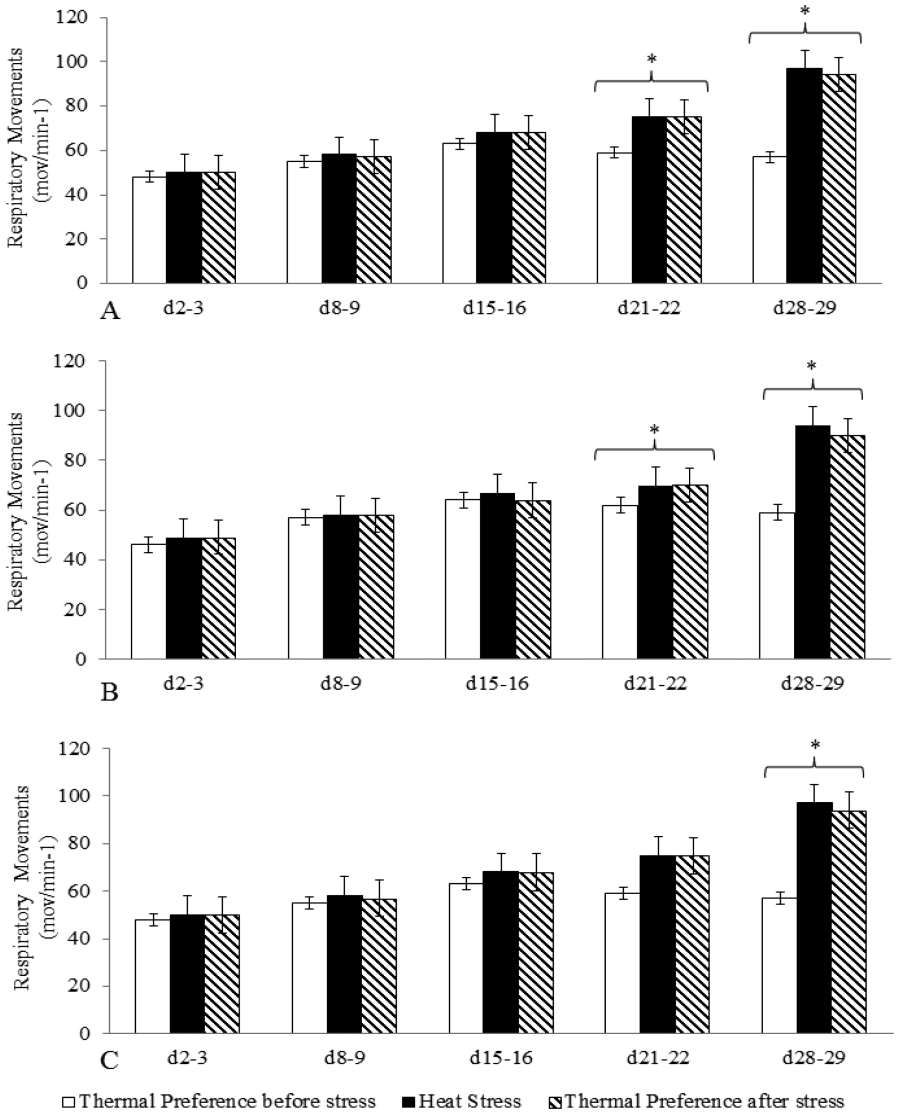

Figure 4. Respiratory movements frequency of broiler chickens incubated at Low (36°C, A), Control (37.5°C, B), or High (39°C, C) temperatures from d 13 of incubation till hatching when exposed to ambient temperatures at thermal preference (before thermal challenge), at 5°C above thermal preference for 45 min (thermal challenge), and upon return to thermal preference (45 min after the thermal challenge) at different ages. *: above the bar indicates that means were different from other treatments at the same age; * above the bracket indicates difference among the means at the same age ($P < 0.05$). Data are expressed as mean ± SD, n = 12 per treatment per age.

temperature is higher till d 15 to 16. Our results agree with those obtained by Willemsen et al. (2011) who found that continuous thermal programming using temperature 2 and 3°C higher than the standard incubation temperature of broiler chicken eggs for the period d 16 to 18.5, also reduced the chickens' thyroid hormone plasma levels.

In rats, reduction of thyroid hormone levels causes reduction of feed intake, body weight, and body temperature (Alva-Sánchez et al., 2012). Although it is expected that chicks with lower metabolism to have lower food intake rates and slower growth rates, the lower levels of T3 found here for chickens did not affect all these variables. Chickens from the High incubation treatment had lower feed intake until 42 d of age, but their body weight at this age did not differ of the body weight presented by chickens from the other incubation treatments. One issue raised by this study was whether the broiler maturation and growth would be affected by differences in hatch times. However, incubation duration was longer for Low incubation treatment than for Control and High incubation treatments, which did not differ (Almeida et al., 2015), indicating no relationship between incubation length and maturation and growth rates.

We showed that chickens from the Low and Control incubation treatments responded to the thermal challenge by first increasing rectal temperature, but never reducing it to the pre-challenge temperatures in all analyzed ages. Chickens from both treatments showed increased respiratory rate in response to thermal challenge and did not restore the respiratory rate presented pre-challenge. In contrast, chickens from the High incubation treatment also increased their rectal temperature when challenged, but only at d 2 to 3, 21 to

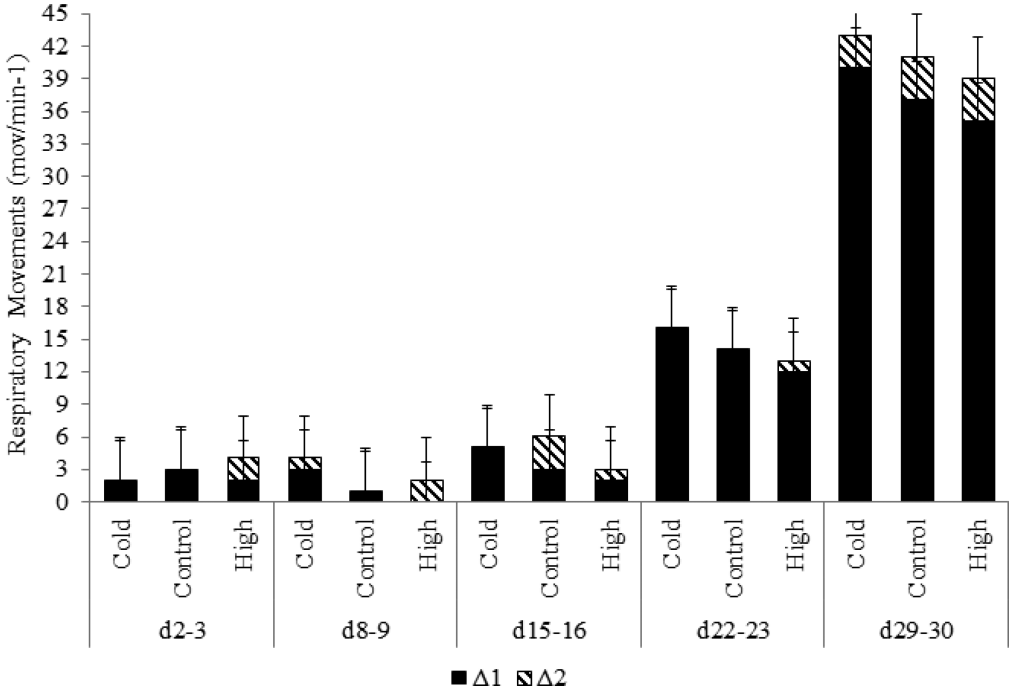

Figure 5. Comparisons of the differences in respiratory movements frequency (Δ1 and Δ2) among Low (36°C), Control (37.5°C) and High (39°C) incubated chickens, at different ages. Δ1: Differences in respiratory movements frequency between the end of the thermal challenge (5°C above thermal preference for 45 min) and just before thermal challenge (under thermal preference). A2: Differences in respiratory movements frequency between the end of the thermal challenge (5°C above thermal preference for 45 min) and upon return to thermal preference (45 min after the end of the thermal challenge). *: asterisk above the bar indicates that means were different from others in the same age ($P < 0.05$). Data are expressed as mean ± SD, n = 12 per treatment per age.

22, and 28 to 29. They also varied their respiratory rate in response to the thermal challenge, but only on d 28 to 29 and with less intensity than the other treatments. Nevertheless, just like the chickens from the Low and Control incubation treatments, these chickens also did not restore their respiratory rate 45 min post thermal challenge to the rate pre-challenge. The later response and lower intensity of the High incubation treatment indicates that continuous exposure to high temperature during incubation promotes long-lasting changes in the thermoregulatory system of chickens, including the improvement of their coping ability to high temperature exposure in the rearing phase. Willemsen et al. (2010) observed similar effects regarding the rectal temperature in response to thermal challenge for chickens from eggs exposed continuously 2°C above the standard incubation temperature. Piestun et al. (2008), on the other hand, did not find thermotolerance when they exposed chicks derived from eggs treated continuously (d 7 to 16 of incubation) with high incubation temperatures (39.5°C) to a thermal challenge (35°C, 4 h) at d 35.

Various studies involving intermittent thermal manipulation of broiler chicken eggs have shown that these chickens do not improve their thermotolerance in response to thermal challenge (Yahav et al., 2004; Collin et al., 2005; Collin et al., 2007). However, Piestun et al. (2008) did report a successful thermotolerance of broiler chickens when the eggs were exposed to intermittently thermal manipulation (12 h/ED, 39.5°C) from d 7 to 16. Indeed, utilizing similar incubation protocol, Loyau et al. (2013) also did not find effect of incubation thermal manipulation on increase of the thermotolerance when the chickens were challenged. These ambiguous results once again demonstrates that small differences in the thermal manipulation protocol (duration, temperature, period and constant or intermittent) could be of large importance for the results obtained, including the effects on thermotolerance in later life.

Although our results show incubation temperature changes influence the post-hatching thermotolerance of the broilers, from a practical view, there is no point in encouraging industry to under take incubation temperature changes if they result in decreased hatchability or post-hatch survival. The present study is part of a major research project that analyses the effects of continuous high and low incubation temperature from d 13 on chick hatchability, quality, growth and survival. According to the results already obtained, High incubation temperature (39°C) improves hatchability of about 8 to 7.6% compared to Low (36°C) and Control (37.5°C) temperatures, respectively (Almeida et al., 2015; Morita, unpublished data). Regarding posthatch survival, our study shows no influence of the incubation temperature manipulation on bird survival until 42d of age. Together, these results obtained by our research group reinforce the potential use of incubation temperature manipulation for changes in the broiler thermotolerance.

CONCLUSIONS

From the current experiment, it can be concluded that a constant thermal programming temperature during the incubation period has long term effects on thermotolerance of broiler chickens. This might have consequences on the ambient temperature chickens require during the rearing phase.

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