MANAGEMENT AND PRODUCTION

Effects of heat stress on pullet cloacal and body temperature

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ABSTRACT One measure of the thermal status of poultry is cloacal temperature measured with a cloacal thermometer; however, this method requires handling the bird, is invasive, and can be stressful. Infrared thermography is an alternative means for assessing bird thermal status. The objective of this study was to investigate the body temperature response of pullets subjected to different environmental air temperatures during the growing phase and to evaluate the relationship between the cloacal temperature and the body parts surface temperature. A total of 648 chicks (Lohmann LSL Lite) were used in 2 different phases, phase I (day 1 through 6 wk of age) and phase II (week 7 through 17). During phase I, chicks were reared at 1 of 3 different thermal environments: thermal comfort $(35^{\circ}C-19^{\circ}C)$, mild heat stress $(38^{\circ}C-22^{\circ}C)$, or mild cold stress $(28^{\circ}C-17^{\circ}C)$. In phase II, pullets were randomly redistributed to 1 of 4 daytime temperature treatments: 20°C; 25°C; 30°C; and 35°C, all with night time temperature of 20°C. Cloacal temperature and body surface temperature for 8 parts (head, eye, comb, chest, back, wing, leg, head area, and body area) were obtained weekly from 4 to 2 birds per treatment, respectively, during phase II. There were no effects for the interactions between the 2 experimental phases for cloacal and body parts surface temperature. There was a strong correlation (P < 0.001) between cloacal temperature and each body part temperature; cloacal temperature followed a quadratic response to environmental air temperature treatments. Pullets subjected to $35^{\circ}C/20^{\circ}C$ and $30^{\circ}C/20^{\circ}C$ had the highest body parts temperatures compared with the other 2 treatments (P < 0.05). The leg surface temperature was greatest in all treatments, and the chest the lowest. Regression between cloacal and body parts temperature had a 95% predictive accuracy of better than 0.4°C, suggesting a useful alternative to direct cloacal temperature measurement.

Key words: acclimation, infrared thermography, poultry, thermal environment, thermoregulation

INTRODUCTION

The thermal comfort (**TC**) zone for homeothermic animals is characterized by a range of environmental temperatures, within which animals have minimal and nearly constant energy expenditure for maintaining body temperature (**BT**) (Curtis, 1983; Chang et al., 2018). Body temperature for poultry normally varies from 41°C to 42°C (Wilson, 1948; Welker et al., 2008), and to maintain BT in this range, the 2020 Poultry Science 99:2469–2477 https://doi.org/10.1016/j.psj.2019.11.062

thermoregulatory system adjusts physiological responses to increase or decrease body heat loss (Sturkie, 1986; Sahin et al., 2009; Taylor et al., 2014). Outside of the TC zone in situations characterized by heat stress (**HS**) or cold stress, birds further adjust their metabolism to further compensate their energy balance (Nascimento et al., 2013; Hester et al., 2015; Arcila et al., 2018; Chang et al., 2018).

Exposing birds at an early age to a nonlethal high environmental temperature can induce acclimation so that they will better cope with high ambient temperature later in life (Sykes and Fataftah, 1986; Yahav et al., 1997). Acclimation during early life was used as a tool for maintaining broiler performance in presence of HS conditions during the production period (Yahav and McMurtry, 2001).

Birds are adversely affected by HS, as it can reduce their productive performance, negatively affect their

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well-being, and in severe HS situations can lead to death (Yanagi Junior et al., 2002; Tao and Xin, 2003; Al-Ramanneh et al., 2016; Arcila et al., 2018). Especially in large-scale production systems, in which maximum efficiency of production is sought, it is important to minimize exposure to HS. For this, it is necessary to use methods for adjusting the environmental temperature based on bird's requirements (Edgar et al., 2013; Abreu et al., 2017; Chang et al., 2018).

Measuring BT is a method to assess the severity of HS (Unruh et al., 2017; Chang et al., 2018). The standard tool for BT measurement is the cloacal thermometer; however, this method is invasive, requires handling the birds, can be stressful, and can yield altered or misleading results (Eddy et al., 2001; Edgar et al., 2013; McManus et al., 2016; Vicente-Pérez et al., 2016; Andrade et al., 2017). Handling birds to measure temperature can cause a biased measurement because the induced stress during handling can change BT. Moe et al. (2017) found a drop of 2°C in the comb and eye temperature after 1 min of handling. One tool which can replace the cloacal thermometer is the thermographic camera (McManus et al., 2016). This noninvasive device measures infrared radiant emission from a surface and can be used at a distance from the bird, thus removing the need to handle birds (Giloh et al., 2012; Edgar et al., 2013; Metzner et al., 2014). However, the relation between bird body parts surface temperature and cloacal temperature for different thermal environments has not been established.

The objective of this study was to investigate the BT (cloacal and surface) response of pullets subjected to different thermal environments during the growing phase and to find the relationship between the cloacal temperature and the body parts surface temperature.

MATERIALS AND METHODS

Experimental Design

All animal care procedures were approved by the Ethics Commission on the research use of Farm Animals of Federal University of Viçosa (CEUAP-UFV Protocol No. 37/2016).

This research was conducted using 648 commercial (Lohmann LSL Lite) egg type chicks, randomly allocated in 4 identical environmental controlled chambers. Each chamber measured 3.20 m length, 2.44 m width, and 2.38 m high. The control and real-time monitoring of chamber temperature was done with an individual control system for each chamber, consisting of an electronic microcontroller (Model MT-531R Plus; Full Gauge Controls, Canoas/RS, Brazil), connected to a heater (Model AB Split 1; Britania Eletrodomesticos S.A. Pirabeiraba, SC, Brazil) and an air conditioner (Model ABS 12FC 2LX; Komeco, Manaus, AM, Brazil). Chamber air temperature and relative humidity were recorded every 5 min by dataloggers (Model HOBO U14-001, Onset, USA; specifications: temperature accuracy of $\pm 0.21^{\circ}$ C from 0°Cto 50°C and resolution of 0.02° C and relative humidity accuracy of $\pm 2.5\%$ from 10 to 90% and resolution of 0.05%). Chamber relative humidity was controlled in the range of 40 to 60% with an ultrasonic air humidifier (Model HUL535 W; Kaz USA, Inc., Marlborough, MA). Two continuously running axial exhaust fans (Model FD08025 AMB; Ambition Technology Company, Guangdong, China) were used to provide fresh air to each chamber during the whole experimental period.

In each environmental chamber, birds were randomly allocated to cages with dimensions of 0.50 \times 0.50×0.50 m (length × width × height). Chick placement density was $140 \text{ cm}^2 \text{ chick}^{-1}$ for the first 4 wk (17 chicks cage -1), and from the beginning of the fifth week until the end of the sixth week, the placement density was $285 \text{ cm}^2 \text{ chick}^{-1}$ (9 chicks cage⁻¹); from the sixth week through the 17th wk, stocking density was $357 \text{ cm}^2 \text{ pullet}^{-1}$ (7 pullets cage⁻¹) per industry guidelines (Lohmann, 2016). Density adjustments were accomplished by random culling. Each cage was equipped with 0.5 m of linear feeder at the cage front, and 1 nipple drinker placed on a side midway between the front and back. Jug waterers and a layer of newspapers on the cage bottom (to facilitate feed access) were provided during the first week to assist chick starting, and an additional nipple drinker was placed until the sixth week per industry guidelines (Lohmann, 2016). All birds received feed and water *ad libitum*. The birds were fed a starter diet until the sixth week and thereafter a grower ration according to Rostagno et al. (2011).

The light program adopted was that recommended by the lineage manual (Lohmann, 2016). The Light:Dark (L:D) hourly schedule was 24L:0D, 16L:8D, for days 1 to 2, and 3 to 6 respectively. From the second week, lighting was reduced by 1 h per week, until 10L:14D on the sixth week, which was maintained through the 17th wk. Lighting was provided by 2 incandescent bulbs (60 W each), located in line in the center of the chamber and 1m apart from each.

The research was conducted in 2 phases, phase I (from day 1 until the end of the sixth week) and phase II (from the seventh week until the end of the 17th wk). Each phase was organized as follows:

Phase I

Phase I treatments were selected to provide pullets that were acclimated to different rearing environments for subsequent HS challenge. Chicks were randomly distributed into 3 of the environmental chambers, each with a different thermal environment, as delineated in Table 1.

Phase II

Birds from each thermal environment in phase I were uniformly and randomly redistributed into the 4 different environmental chambers, each with a different thermal environment, designated as follows: TC (20° C/ 20° C); presumed mild heat stress (**MiHs**, 25° C/ 20° C);

Table 1. Values of the air temperature for each chamber during phase I.

Thermal environment	1st w k $(^{\circ}\mathrm{C})$	2nd wk (°C)	3rd week (°C)	4th week (°C)	5th–6th week $^{\circ}\mathrm{C})$
Mild cold stress Literature thermal comfort Mild heat stress	$28 \\ 35-31 \\ 38$	$25 \\ 28 \\ 31$	23 26 29	20 23 26	17 19 22

(Literature thermal comfort: Rostagno et al., 2011; Lohmann, 2016).

presumed moderate heat stress (**MoHs**, $30^{\circ}C/20^{\circ}C$); presumed severe heat stress (**SeHs**, $35^{\circ}C/20^{\circ}C$). During the night (from 7:00 pm–7:00 am), the air temperature was reduced to $20^{\circ}C$ for all chambers. Each thermal environment received 5 cages (replicates) of birds from each of the 3 phase I treatments, with 7 pullets per cage, as depicted in Figure 1.

Measurements

Surface and cloacal temperature measurements were made once a week during phase II on sequential days, respectively, and in both days, the readings started at 12:30. Measurements were made over 2 days to reduce the stress of handling and started at the same time to ensure similar diurnal temperature patterns were captured. The cloacal temperature was measured with a calibrated clinical thermometer (Model Incoterm, Porto Alegre/RS, Brazil), with a temperature range of 32°C to 43.9°C and resolution of 0.1°C. To acquire each cloacal temperature, a bird was removed from the cage and placed on a table in the chamber; then, the pullet was held, and a thermometer was inserted approximately 1 cm into the cloaca and was read after temperature stabilization, typically 45 to 60 s. The cloacal temperature was collected from 4 birds randomly selected from each cage, corresponding to 57% of the birds in each replicate, that is 240 birds sampled weekly.

The infrared thermal camera (Model Therma-CAMb60; FLIR Systems, Wilsonville, OR) had a temperature range of -20° C to 120° C, an absolute accuracy of $\pm 2^{\circ}$ C, and resolution of 2048 \times 1536 pixels. The coefficient of emissivity (ε) was set at 0.95 and kept constant based on recommendations of the manufacturer and Nääs et al. (2010). The points of interest for BT were analyzed with FLIR Tools software (FLIR Systems, Inc., North Billerica, MA). Thermal images of 1 side of 2 randomly select birds from each cage were taken with the camera positioned 1.3 m from the pullets. A total of 120 birds was sampled weekly with this technique. To acquire each image, a bird was removed from the cage and placed on a table in the chamber. Birds were not held, so the time required to obtain the image depended on the bird's behavior, averaging about 3 min per bird; if a bird would not stand still after several minutes, it was returned to the cage, and another bird was randomly selected. It took about 4 h to acquire the thermal images from all 4 chambers. From each image, the temperature of 7 points (head, eye, comb, breast, back, wing, and leg) and 2 area averages (head, and body without head, neck, and legs) were collected as shown in Figure 2, where stars represent point measurements and rectangles represent

area measurements which were the average temperature of all pixels in the area.

Statistical Analyses

The phase II experimental design was completely randomized in a split-plot arrangement with 4 treatments (phase II temperatures—TC; MiHs; MoHs; SeHs) as the plots and 3 subplots consisting of the birds reared under phase I temperatures, with 5 replicate cages per each phase II treatment \times phase I assignment combination. Replicates were assumed random effects (subject) under a mixed model framework, thus the variance component associated with replicates explains possible variations between cages with the same treatment. The effects of phase II treatments, phase I treatment, and their interactions on cloacal and the body parts temperature were performed using analysis of variance (SAEG, 2007) for each week of bird temperatures collected. Differences between group means were compared by Tukey's test, with a 5% confidence level (P < 0.05) for the significance of treatment effects, interactions, and differences between means. Correlations between cloacal temperature and body parts surface temperature (head, eye, comb, chest, back, wing, leg, head area, and body area) were evaluated with the Pearson correlation coefficient (r) with significance at the 5% level. Linear and quadratic regressions were developed for the effect of environmental air temperature on cloacal temperature at 17 wk of age. Linear regressions between cloacal temperature and each body part temperature were made for the 17th wk data.

RESULTS

The chamber air temperature maintained from the seventh through the 17th wk for each chamber during phase II ranged from 0.2° C to 0.6° C warmer than the nominal treatment values (standard deviation 0.7° C– 0.9° C), and relative humidity varied between 63 and 69% (standard deviation 6–8%).

Weekly mean cloacal temperatures of pullets subjected to the 4 different environmental air temperatures are presented in Table 2. The effect of phase II air temperature was significant (P < 0.001), but not phase I temperature treatment or its interaction with phase II temperature (P > 0.05) for cloacal temperature from week 7 through week 17 of age. Pullets subjected to $35^{\circ}C/20^{\circ}C$ air temperature during the seventh through 17th wk had a consistently higher cloacal temperature (P < 0.05), regardless of the previous temperature exposure, Table 2. Pullets subjected to $20^{\circ}C/20^{\circ}C$ and



Figure 1. Experimental design of pullet distribution in the environmentally controlled chambers during phase II, from the seventh through the 17th wk of age.

 $25^{\circ}C/20^{\circ}C$ treatments had lower cloacal temperatures than the other 2 treatments (P < 0.05) and were similar for 8 of the 11 wk evaluated (P < 0.05), Table 2.

Regressions of cloacal temperature vs. environmental air temperatures of pullets at the 17th wk of age are presented in Figure 3. The coefficients in all equations were significant (P < 0.001), and the R² values were 0.70 and 0.85 for linear and quadratic, respectively. The quadratic regression fit the data better than the linear regression based on the standard error of regression and R² coefficient.

Mean body parts surface temperatures (head, eye, comb, back, chest, wing, leg, head area, and body area) of pullets subjected to the 4 different environmental air temperatures are presented in Table 3 for week 17. The effects of interaction between the 2 phases, phase I and phase II, were not significant for any of the body parts evaluated for the 17th wk of age.



Figure 2. Side view of a pullet with the body parts of interest identified with the green stars for points (head, eye, comb, breast, back, wings, and leg) and squares for areas (average of the head area or body area).

The effects of phase I temperature treatment were significant only for back surface temperature (P < 0.05) for the 17th wk of age of phase II, Table 3. Pullets subjected to SeHs ($35^{\circ}C/20^{\circ}C$) and MoHs ($30^{\circ}C/20^{\circ}C$) had higher surface temperatures compared with the other 2 treatments (P < 0.05). No statistical difference was observed between the treatments TC and MiHs. The leg was the body part that had the highest surface temperature in all treatments, and the chest the lowest.

Pearson correlation coefficients between each body part temperature and cloacal temperature at the 7th and 17th wk of age are presented in Table 4. In all cases, there was a strong correlation (P < 0.001). Comparing areas, the body area had the highest correlation, r = 0.83 and 0.82 for the 7th and 17th wk of life, respectively. Regarding the body parts temperatures, the chest and wing had the highest correlation, r = 0.85 and 0.81 for the 7th and 17th wk of life, respectively, and the lowest correlation was at the head, r = 0.72 at the seventh week and the comb, r = 0.70, at the 17th wk of age.

Linear regression coefficients and goodness-of-fit statistics for cloacal temperature vs. each body part temperature of pullets at 17 wk are presented in Table 5. Standard errors of regression (Se) are related to the predictive accuracy of these models, and in all cases, an approximate 95% confidence interval (± 2 Se) was less than 0.4°C.

DISCUSSION

The main goal of phase I was to acclimate the pullets to mild cold stress, TC, or MiHs conditions during the first 6 wk of age, seeking to evaluate whether these temperature thresholds affected HS response later in life, during the phase II. Further information about phase I rearing is available from Andrade et al. (2017).

Acclimation promotes behavioral and physiological alterations to compensate for the negative effects of a single stressor acting alone. For example, if birds are raised at high temperatures during early periods of life, they may be able to better cope with HS later in life (Curtis, 1983; Yahav and Hurwitz, 1996).

	$Thermal environments^1$						
	$TC (20^{\circ}C/20^{\circ}C)$	MiHs $(25/20^{\circ}C)$	MoHs $(30/20^{\circ}C)$	SeHs $(35/20^{\circ}C)$			
Age (week)	Cloacal temperature (°C)						
7	41.5°	$41.6^{\rm c}$	41.9^{b}	$42.3^{\rm a}$	0.16		
8	$41.6^{ m c}$	41.5°	42.0^{b}	42.3^{a}	0.12		
9	$41.4^{\rm d}$	$41.6^{\rm c}$	41.9^{b}	42.2^{a}	0.14		
10	41.5^{d}	$41.6^{\rm c}$	41.8^{b}	$42.1^{\rm a}$	0.13		
11	41.5°	$41.5^{\rm c}$	41.7^{b}	$42.1^{\rm a}$	0.10		
12	$41.4^{\rm c}$	41.3°	41.6^{b}	41.8^{a}	0.11		
13	$41.4^{\rm c}$	$41.4^{\rm c}$	41.6^{b}	41.8^{a}	0.12		
14	41.5^{bc}	$41.4^{\rm c}$	41.6^{b}	42.0^{a}	0.10		
15	41.4^{d}	41.3°	41.7^{b}	42.0^{a}	0.11		
16	$41.1^{\rm c}$	41.2°	$41.5^{\rm b}$	42.0^{a}	0.12		
17	$41.1^{\rm c}$	$41.1^{\rm c}$	41.4^{b}	41.8^{a}	0.08		

Table 2. Average values of cloacal temperature for pullets from the 7th to 17th wk of age subjected to 4 different thermal environments.

^{a-d}Means within a row with different superscripts differ significantly (P < 0.05) by the Tukey test.

¹TC: Thermal comfort—20°C/20°C; MiHs: Mild heat stress—25°C/20°C; MoHs: Moderate heat stress—30°C/20°C; SeHs: Severe heat stress—35°C/20°C.

²SEM: Standard error of mean.

There were no effects of phase I temperatures or their interaction with phase II temperatures on either cloacal temperature (Table 2) or body part surface temperature, with the exception of a single body part (back) at the 17th wk of age (Table 3). This could have happened because the lack of severity for the hot environment and also the humidity range applied during phase I. Sykes and Fataftah (1986) were successful acclimatizing laying hens after submitting them to 38°C, and according to Arjona et al. (1990) and Yahav and Hurwitz (1996), the acclimation can be reached when the humidity is set at 70 to 80%. Also, in a study with broilers conducted by Sykes and Fataftah (1986), it was observed that heat tolerance may be reduced over time postexposure. The heat tolerance was reduced at 19 D and even more at 47 D compared with the initial heat tolerance observed at 5 D, using a 42°C challenge. Abdelgader and Al-Fataftah (2014) concluded that the responses of acclimatized birds to HS can be affected by the length of the acclimation period; in their experiment, broilers were acclimated for 1, 2, 3, and 4 h of HS (38° C) daily for 14 D and then subjected to 4 h of 43°C at 36 D of life. In the present study, it may be that phase I temperatures were not hot nor cold enough to induce the acclimation, as demonstrated by the lack of significant interactions between phase I and II treatments, both for cloacal and body parts temperatures.

The results for cloacal temperature (Table 2) tend to be close with those for layers presented by Chang et al. (2018), who reported that core temperature increased to 42.4°C, when laying hens were subjected to 35° C, although the hens were exposed for only for 3 D. Giloh et al. (2012) raised broilers in a thermoneutral environment, and the average cloacal temperature was 41.3° C,



Figure 3. The effect of environmental air temperature on cloacal temperature at the 17th wk of age. Different symbols represent conditions for chicks raised through week 6 (Table 1). Linear and quadratic regression models between cloacal temperature and environmental air temperature, and the standard error of regression was 0.16°C and 0.11°C for linear and quadratic regressions, respectively. Abbreviation: T_{air} = environmental air temperature; all coefficients included in the equations are significant at P < 0.001.

Table 3. Average body parts surface temperature (head, eye, comb, chest, back, wing, and leg), areas (head area and body area), and results of analysis of variance for laying pullets during the 17th wk of age subjected to 4 different thermal environments.

	Body part surface temperature (°C)								
Thermal environments ¹	Head	Eye	Comb	Chest	Back	Wing	Leg	Head area	Body area
TC ($20^{\circ}C/20^{\circ}C$) MiHs ($25^{\circ}C/20^{\circ}C$) MoHs ($30^{\circ}C/20^{\circ}C$) SeHs ($35^{\circ}C/20^{\circ}C$) SEM ² R-value (phase II)	$\begin{array}{r} 39.4^{\rm b} \\ 40.1^{\rm b} \\ 46.4^{\rm a} \\ 47.0^{\rm a} \\ 1.54 \\ *** \end{array}$	$\begin{array}{c} 40.7^{\rm b} \\ 41.0^{\rm b} \\ 45.8^{\rm a} \\ 46.0^{\rm a} \\ 0.72 \\ *** \end{array}$	$\begin{array}{c} 42.5^{\rm b} \\ 43.4^{\rm b} \\ 47.6^{\rm a} \\ 47.5^{\rm a} \\ 1.09 \\ *** \end{array}$	34.1^{b} 35.1^{b} 43.5^{a} 44.1^{a} 0.71 ***	$\begin{array}{r} 34.7^{\rm b} \\ 36.0^{\rm b} \\ 44.1^{\rm a} \\ 45.1^{\rm a} \\ 0.62 \\ *** \end{array}$	$35.0^{ m b}$ $36.0^{ m b}$ $43.6^{ m a}$ $44.7^{ m a}$ 0.69 ***	$\begin{array}{c} 43.9^{\rm b} \\ 43.5^{\rm b} \\ 47.9^{\rm a} \\ 48.1^{\rm a} \\ 0.85 \\ *** \end{array}$	$\begin{array}{c} 41.9^{\rm b} \\ 42.4^{\rm b} \\ 47.0^{\rm a} \\ 47.4^{\rm a} \\ 0.73 \\ *** \end{array}$	35.2^{b} 35.8^{b} 43.7^{a} 45.0^{a} 0.58 ***
P-value (phase I) P-value (interactions)	ns ns	ns ns	ns ns	ns ns	* ns	ns ns	ns ns	ns ns	ns ns

^{a–b}Means within a column with different superscripts differ significantly (P < 0.05).

* = significant at (P < 0.05); *** = significant at (P < 0.001); ns = non-significant.

¹TC: Thermal comfort—20°C/20°C; MiHs: Mild heat stress—25°C/20°C; MoHs: Moderate heat stress—30°C/20°C; SeHs: Severe heat stress—35°C/20°C.

 2 SEM: Standard error of mean.

similar to this experiment. At week 17 of this study, the increase in cloacal temperature above TC was 0° C, 0.3° C, and 0.7° C for MiHs, MiHs, and SeHs, respectively.

In an experiment with broilers raised in the same temperatures as used in this study (20°C, 25°C, 30°C, and 35°C), Donkoh (1989) found a similar pattern in the cloacal temperature; the environments TC and MiHs were not different from each other, and the treatments MoHs and SeHs were different from each other and from TC and MiHs (Table 2). Also, the broilers showed a gradual increase in cloacal temperature when subjected to an environmental air temperature higher than 25°C. However, at 35°C, the average broiler cloacal temperature was 42.9°C, slightly higher than the 42.2°C found in this experiment. This difference can be related to higher broiler metabolism when compared with pullets, making it more challenging for them to lose heat.

The regression models for cloacal temperature vs. phase II environmental air temperature were similar (P > 0.05) for birds coming from any of the 3 phase I treatments. Thus, regardless of the environmental air temperature to which they were acclimatized, they had a similar pattern of cloacal temperature when exposed to the different temperature treatments in phase II. The linear regression shows an increase in cloacal

Table 4. Pearson correlation coefficients between cloacal temperature and the body surface temperature parts (head, eye, comb, chest, back, wing, and leg) and areas (head area and body area) of pullets at the 7th and 17th wk of age subjected to 4 different thermal environments, n = 60 and P < 0.001.

Body part	7th week	17th week		
Head	0.723	0.755		
Eye	0.780	0.754		
Comb	0.754	0.700		
Chest	0.854	0.808		
Back	0.823	0.808		
Wing	0.829	0.811		
Leg	0.814	0.766		
Head area	0.820	0.786		
Body area	0.831	0.823		

temperature with increasing environmental air temperature $(0.042^{\circ}C/^{\circ}C)$. However, when evaluated by a quadratic regression, it can be inferred that cloacal temperatures were similar for the environmental air temperatures of 20°C and 25°C. This behavior was noted for cloacal temperature measurements from week 7 to 17 (Table 2). Similar results can be observed in Chang et al. (2018) in which laying hens showed an accelerating increase surface temperature with the increase in environmental air temperature, especially for temperatures above 25°C. Sensible heat loss in the thermoneutral zone is the predominant mechanism for maintaining core temperature. However, as air temperatures increase above the thermoneutral zone, sensible heat loss cannot maintain BT in the normal range. Thus, it is necessary to use other pathways, being either evaporative (latent) heat loss or changes in metabolic rates by hormonal changes, for example changes in thyroidal hormones concentration. As environmental temperature increases further, birds may not be able to fully control their BT, and it increases. This BT pattern described is noted in the quadratic effect between the environmental temperature and the cloacal temperature. In Figure 3, it is possible to observe a relatively flat line until the environmental temperature reaches about 25°C, above which the cloacal temperature initiated a rapid increase.

Body surface temperature measurement can be a useful tool for estimating the cloacal temperature and thus serve as an indicator of HS. One of the primary mechanisms for decreasing BT is vasodilatation, which increases the blood flow to the skin, moving the heat from the viscera to the periphery (Nascimento et al., 2013:Chang et al., 2018). This mechanism, consequently, increases the skin temperature and can be better observed in featherless parts (Chang et al., 2018). In the present study, among all the body parts observed, the comb and the leg had the highest temperatures (Table 3). This result supports the importance of these featherless body parts in thermal control of the pullets during HS. Al-Ramamneh et al. (2016) trimmed the comb and wattle of White Leghorn and found higher mortality and increased BT in birds without the comb

Table 5. Statistical summary of the linear regression equations, for the predictive model of cloacal temperature from various body part temperatures for pullets at the 17th wk of age.

	Equation: $CT = a$ surface ter	a + b *(body part nperature)			\mathbf{R}^2	
Body part	$a~(Se_a)~^\circ C$	$b (Se_b)$	Se (regression) $^{\circ}\mathrm{C}$	Adj. \mathbb{R}^2	Cross validation	
Head (HE)	38.957(0.273)	0.0552(0.006)	0.187	0.56	0.74	
Eye (EY)	37.894(0.396)	0.0795(0.009)	0.187	0.56	0.79	
Comb (CO)	37.961(0.453)	0.0747(0.010)	0.203	0.48	0.85	
Chest (CH)	39.508(0.177)	0.0467(0.005)	0.168	0.65	0.77	
Back (BK)	39.434(0.184)	0.0478(0.005)	0.168	0.65	0.80	
Wing (WI)	39.364(0.189)	0.0498(0.004)	0.167	0.65	0.76	
Leg (LE)	37.137(0.464)	0.0917(0.010)	0.183	0.57	0.70	
Body area (BA)	39.303(0.189)	0.0512(0.005)	0.162	0.67	0.75	
Head area (HA)	37.622(0.385)	0.0833(0.009)	0.176	0.61	0.79	

All coefficients included in the equations are significant at P < 0.001.

Abbreviations: CT, cloacal temperature; Se, standard error of the coefficients (Se_a, Se_b) and regression, Se (regression).

and wattle compared with a control group when both were heat stressed. The authors attributed this result to the reduced capacity of hens to thermoregulate after the trim procedure.

Nääs et al. (2010) also found higher surface temperatures of featherless areas of laying hens compared with feathered areas. The leg, wattle, and comb were observed to have the highest temperature among the observed parts, based on measurements of broilers at 42 D of age for 6 times a day. Also, Nääs et al. (2010) found a high correlation (0.8) between featherless areas and environmental temperature and attributed this result to the increased blood flow in these areas.

The different results between the temperatures in the body parts found in this experiment also was described in other poultry studies, such as in broilers by Nääs et al. (2010) and Nascimento et al. (2011), in layer hens by Zhao et al. (2013), in pullets by Hester et al. (2015), in quails by Santos et al. (2019), and by Mayes et al. (2015) working with turkeys. This diversity in temperature for the body parts can be attributed to variations in the insulation cover, both for the presence and absence of feathers, as well as for density of feathers and the peripheral blood circulation (Nääs et al., 2010; Zhao et al., 2013).

According to several studies, the core temperature and heat production can be predicted using thermal camera measurements for different body parts in farm animals (Montanholi et al., 2008; Johnson et al., 2011; Nascimento et al., 2013; Barros et al., 2016; McManus et al., 2016). This prediction is a useful alternative to cloacal temperature measurement, which is stressful and invasive (Vicente-Pérez et al., 2016). The core temperature is a useful parameter to assess the TC of the animals inside facilities, so a noninvasive measure is useful.

In this study, all Pearson's correlations (Table 4) between the body parts (head, eye, comb, chest, back, wing, leg, head area, and body area) and cloacal temperature were positive and significant (P < 0.001). This finding agrees with Vicente-Pérez et al. (2016) for ewes, George et al. (2014) for ewes and cattle, as well as Giloh et al. (2012) for broiler chickens, who compared cloacal temperature with facial surface temperature. The highest correlation coefficient at the 17th wk was for the body area, 0.82 to 0.83, which can be attributed to the higher representative area of temperature collected when compared with the head area. The correlation between eye and cloacal temperature in this study was 0.77 to 0.75, but this correlation value can vary depending on the species; Vicente-Pérez et al. (2016) found different correlations when studying ewes, 0.76 to 0.72, and cows, 0.58; Barros et al. (2016) found 0.51 for buffaloes. Giloh et al. (2012) found that a strong correlation between facial temperature and BT in broilers and point out this correlation sometimes was better than blood hormones concentration measurements, such as corticosterone, thyroxine, and triiodothyronine when broilers are submitted to HS.

In this study, the adjusted R^2 values of the regression models to predict the cloacal temperature from body parts temperature were between 0.48 to 0.65 (Table 5). Vicente-Pérez et al. (2016) used linear regression models to predict cloacal temperature from surface temperatures (head, rump right flank, shoulder, belly) and respiratory frequency in pregnant ewes subjected to natural HS. Their adjusted R^2 varied from 0.43 to a maximum of 0.56, lower than the results in this experiment. These authors classified the results as moderate. However, other authors found greater values for R² when including additional variables in the regression. Ponciano et al. (2012), working with broilers and using combined variables such as temperature–humidity index, black globe humidity index, humidity, air temperature, and age achieved a value of 0.73. Also, Nascimento et al. (2013) showed an R² of 0.74 to 0.72 to predict featherless surface temperature in broilers, and an R^2 of 0.68 to 0.67 for feathered surface temperature. The surface body part temperature can be used as an alternative for cloacal temperature measurement. From the standard errors of regression for the present study, an approximate 95% confidence value for predictive accuracy is better than 0.4°C. This may be useful in future studies when comparing effects of different air temperatures on groups of birds without direct cloacal temperature measurement. Among the tested regression models, all had similar predictive power (SE_{regression} $< ^{\circ}$ C). The body

area regression had the highest R^2 (0.67) combined with a relatively low standard error of the regression of 0.162°C. However, through cross validation, the highest R^2 was for comb, back, eye, and head area; combined with SE_{regression} results, the back, eye, and head areas were most useful for predict the cloacal temperature.

CONCLUSION

Exposing chicks during brooding to mild cold stress, TC, or MiHs had no effect on the subsequent cloacal and body parts temperatures (with exception of back temperature) during the growing phase from week 7 through 17 of life. The results of this study suggest that pullets are able to maintain their cloacal and body parts temperatures in the normal range when subjected to environmental air temperatures from 20° C to 25° C for 12 h/D during growout.

Thermal imaging of body parts temperature (back, eye, or head area) appears to offer a simple alternative for estimating cloacal temperature, given the positive linear relationship between cloacal temperature and body part temperature found in this study. The regression models provided are able to predict cloacal temperature with reasonable accuracy.

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REFERENCES

- Abdelqader, A., and A. R. Al-Fataftah. 2014. Thermal acclimation of broiler birds by intermittent heat exposure. J. Therm. Biol. 39:1–5.
- Abreu, L. H. P., T. Yanagi Junior, A. T. Campos, M. Bahuti, and É. J. Fassani. 2017. Cloacal and surface temperatures of broilers subject to thermal stress. Eng. Agríc. Jaboticabal 37:877–886.
- Al-Ramamneh, D. S., M. M. Makagon, and P. Y. Hester. 2016. The ability of White Leghorn hens with trimmed comb and wattles to thermoregulate. Poult. Sci. 95:1726–1735.
- Andrade, R. R., I. de F. F. Tinôco, F. C. Baêta, M. Barbari, L. Conti, P. R. Cecon, M. G. L. Cândido, I. T. A. Martins, and C. G. S. T. Junior. 2017. Evaluation of the surface temperature of laying hens in different thermal environments during the initial stage of age based on thermographic images. Agron. Res. 15:629– 638.
- Arcila, J. C. P., I. de F. F. Tinôco, J. A. O. Saraz, K. S. O. Rocha, and M. G. L. Cândido. 2018. Zootechnical and physiological performance of broilers in the final stage of growth subjected to different levels of heat stress. Rev. Fac. Nac. Agron. 71:1–8.

- Arjona, A. A., D. M. Denbow, and W.D. Weaver, Jr. 1990. Neonatally-induced thermotolerance: physiological responses. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 95:393–399.
- Barros, D. V., L. K. X. Silva, P. R. Kahwage, J. B. Lourenço Júnior, J. S. Sousa, A. G. M. Silva, I. M. Franco, L. G. Martorano, and A. R. Garcia. 2016. Assessment of surface temperatures of buffalo bulls (Bubalus bubalis) raised under tropical conditions using infrared thermography. Arq. Bras. Med. Vet. Zootec. 68:422–430.
- Chang, Y., X. J. Wang, J. H. Feng, M. H. Zhang, H. J. Diao, S. S. Zhang, Q. Q. Peng, Y. Zhou, M. Li, and X. Li. 2018. Real-time variations in body temperature of laying hens with increasing ambient temperature at different relative humidity levels. Poult. Sci. 97:3119–3125.
- Curtis, S. E. 1983. Environmental Management in Animal Agriculture. Iowa State University Press, Ames, IA. 410 (viii).
- Donkoh, A. 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. Int. J. Biometeorol. 33:259–265.
- Eddy, A. L., L. M. Van Hoogmoed, and J. R. Snyder. 2001. The role of thermography in the management of equine lameness. Vet. J. 162:172–181.
- Edgar, J. L., C. J. Nicol, C. A. Pugh, and E. S. Paul. 2013. Surface temperature changes in response to handling in domestic chickens. Physiol. Behav. 119:195–200.
- George, W. D., R. W. Godfrey, R. C. Ketring, M. C. Vinson, and S. T. Willard. 2014. Relationship among eye and muzzle temperatures measured using digital infrared thermal imaging and vaginal and rectal temperatures in hair sheep and cattle. J. Anim. Sci. 92:4949–4955.
- Giloh, M., D. Shinder, and S. Yahav. 2012. Skin surface temperature of broiler chickens is correlated to body core temperature and is indicative of their thermoregulatory status. Poult. Sci. 91:175–188.
- Hester, P. Y., D. S. Al-Ramamneh, M. M. Makagon, and H. W. Cheng. 2015. Effect of partial comb and wattle trim on pullet behavior and thermoregulation. Poult. Sci. 94:860–866.
- Johnson, S. R., S. Rao, S. B. Hussey, P. S. Morley, and J. L. Traub-Dargatz. 2011. Thermographic eye temperature as an index to body temperature in ponies. J. Equine Vet. Sci. 31:63–66.
- Lohmann. 2016. LSL-lite Commercial Management Guide. Lohmann Tierzucht. Cuxhaven, Germany.
- Mayes, S. L., M. L. Strawford, S. D. Noble, H. L. Classen, and T. G. Crowe. 2015. Cloacal and surface temperatures of tom turkeys exposed to different rearing temperature regimes during the first 12 weeks of growth. Poult. Sci. 94:1105–1114.
- McManus, C., C. B. Tanure, V. Peripolli, L. Seixas, V. Fischer, A. M. Gabbi, S. R. O. Menegassi, M. T. Stumpf, G. J. Kolling, E. Dias, and J. B. G. Costa. 2016. Infrared thermography in animal production: an overview. Comput. Electron. Agric. 123:10–16.
- Metzner, M., C. Sauter-Louis, A. Seemueller, W. Petzl, and W. Klee. 2014. Infrared thermography of the udder surface of dairy cattle: characteristics, methods, and correlation with rectal temperature. Vet. J. 199:57–62.
- Moe, R. O., J. Bohlin, A. Flø, G. Vasdal, and S. M. Stubsjøen. 2017. Hot chicks, cold feet. Physiol. Behav. 179:42–48.
- Montanholi, Y. R., N. E. Odongo, K. C. Swanson, F. S. Schenkel, B. W. McBride, and S. P. Miller. 2008. Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (Bos taurus). J. Therm. Biol. 33:468–475.
- Nääs, I. A., C. E. B. Romanini, D. P. Neves, G. R. do Nascimento, and R. do A. Vercellino. 2010. Broiler surface temperature distribution of 42 day old chickens. Sci. Agric. 67:497–502.
- Nascimento, G. R., I. A. Nääs, D. F. Pereira, M. S. Baracho, and R. Garcia. 2011. Assessment of broiler surface temperature variation when exposed to different air temperatures. Braz. J. Poult. Sci. 13:259–263.
- Nascimento, S. T., I. J. O. da Silva, A. S. C. Maia, A. C. de Castro, and F. M. C. Vieira. 2013. Mean surface temperature prediction models for broiler chickens - a study of sensible heat flow. Int. J. Biometeorol. 58:195–201.
- Ponciano, P. F., T. Yanagi Junior, R. R. D. Lima, L. Schiassi, and V. H. Teixeira. 2012. Adjust of regression models to estimate the rectal temperature of broilers for the first 14 days of life. Eng. Agríc. 32:10–20.

- Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. de Oliveira, D. C. Lopes, A. S. Ferreira, and S. L. de T. Barreto. 2011. In Tabelas Brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. H. S. Rostagno ed. Universidade Federal de Viçosa, Viçosa, MG, Brazil.
- SAEG. 2007. Sistemas de Análises Estatísticas, Version 9.1. Fundação Arthur Bernanrdes - UFV, Viçosa.
- Sahin, K., N. Sahin, O. Kucuk, A. Hayirli, and A. S. Prasad. 2009. Role of dietary zinc in heat-stressed poultry: a review. Poult. Sci. 88:2176–2183.
- Santos, T. C., R. S. Gates, I. F. F. Tinôco, S. Zolnier, K. S. O. Rocha, and L. C. S. R. Freitas. 2019. Productive performance and surface temperatures of Japanese quail exposed to different environment conditions at start of lay. Poult. Sci. 98:2830–2839.
- Sturkie, P. D. 1986. Avian Physiology. Fourth. Springer, New York, NY.
- Sykes, A. H., and A. R. A. Fataftah. 1986. Acclimatisation of the fowl to intermittent acute heat stress. Br. Poult. Sci. 27:289–300.
- Tao, X., and H. Xin. 2003. Surface wetting and its optimization to cool broiler chickens. Trans. ASABE 46:483–490.
- Taylor, N. A. S., M. J. Tipton, and G. P. Kenny. 2014. Considerations for the measurement of core, skin and mean body temperatures. J. Therm. Biol. 46:72–101.
- Unruh, E. M., M. E. Theurer, B. J. White, R. L. Larson, J. S. Drouillard, and N. Schrag. 2017. Evaluation of infrared thermography as a diagnostic tool to predict heat stress events in feedlot cattle. Am. J. Vet. Res. 78:771–777.
- Vicente-Pérez, R., L. Avendaño-Reyes, Á. Mejía-Vázquez, F. D. Álvarez-Valenzuela, A. Correa-Calderón, M. Mellado,

C. A. Meza-Herrera, J. E. Guerra-Liera, P. H. Robinson, and U. Macías-Cruz. 2016. Prediction of rectal temperature using non-invasive physiologic variable measurements in hair pregnant ewes subjected to natural conditions of heat stress. J. Therm. Biol. 55:1–6.

- Welker, J. S., A. P. Rosa, D. J. de Moura, L. P. Machado, F. Catelan, and R. Uttpatel. 2008. Broiler body surface temperature in function of different thermal control systems in broiler housing. Rev. Bras. Zootcnia 37:1463–1467.
- Wilson, W. O. 1948. Some effects of increasing environmental temperatures on pullets. Poult. Sci. 27:813–817.
- Yahav, S., and S. Hurwitz. 1996. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poult. Sci. 75:402–406.
- Yahav, S., and J. P. McMurtry. 2001. Thermotolerance acquisition in broiler chickens by temperature conditioning early in life—the effect of timing and ambient temperature. Poult. Sci. 80:1662– 1666.
- Yahav, S., A. Shamai, A. Haberfeld, G. Horev, S. Hurwitz, and M. Einat. 1997. Induction of thermotolerance in chickens by temperature conditioning: heat shock protein expression. N. Y. Acad. Sci. 813:628–636.
- Yanagi Junior, T., H. Xin, and R. S. Gates. 2002. Optimization of partial surface wetting to cool caged laying hens. Trans. ASABE 45:1091–1100.
- Zhao, Y., H. Xin, and B. Dong. 2013. Use of infrared thermography to assess laying-hen feather coverage. Poult. Sci. 92:295– 302.