

MÁRCIA GABRIELLE LIMA CÂNDIDO

**THERMAL TOLERANCE, PERFORMANCE, AND PHYSIOLOGY OF
EGG-TYPE PULLETS WHEN SUBJECTED TO DIFFERENT LEVELS OF
HEAT STRESS DURING THE BROODING AND GROWING PHASES
SEQUENTIALLY**

Thesis presented to the Federal University of Viçosa, as partial fulfillment of the requirements from the Graduate Program in Agricultural Engineering for attaining the degree of Doctor Scientiae.

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APPROVED: February 26, 2019.



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To my parents Ana Rochelle and Alípio (in memoriam), for the all the love, patience and support provided to me throughout my life.

To my brothers, nephews, and family.

To my husband, for all the support and great encouragement to my work.

And also, to all the animals who gave me the opportunity to work with them and for them.

I dedicate!!!

BIOGRAPHY

Márcia Gabrielle Lima Cândido, daughter of Ana Rochelle Lima and Alípio Cândido Filho (in memoriam), was born in Belo Horizonte, Minas Gerais state – Brazil, on February 29th of 1988.

In March of 2006, she started to pursue a degree in Veterinary Medicine at the Pontifical Catholic University of Minas Gerais (Betim, Minas Gerais state, Brazil), graduating in July of 2011. In August of 2013, started the Master's degree in Agricultural Engineering, in the area of Rural Buildings and Animals Ambience at the Federal University of Viçosa (Viçosa, Minas Gerais State, Brazil) and defended her dissertation in February 2015.

In March 2015, she became a doctoral student at the Graduate Program of Agricultural Engineering, conducting part of her research study at the University of Illinois at Urbana-Champaign (Urbana, Illinois state, United States of America). In February of 2019, she applied to defend her Doctoral Thesis.

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ABSTRACT

CÂNDIDO, Márcia Gabrielle Lima, D.Sc., Universidade Federal de Viçosa, February, 2019. **Thermal tolerance, performance, and physiology of egg-type pullets when subjected to different levels of heat stress during the brooding and growing phases sequentially.** Adviser: Ilda de Fátima Ferreira Tinôco. Co-advisers: Richard Stephen Gates and Luiz Fernando Teixeira Albino.

Brazil is a prominent producer of animal protein, especially regarding the poultry sector, being the largest egg producer in South America. As a tropical and subtropical country, Brazil is characterized by its low annual thermal amplitude and high temperatures during the year, across most of its territory. Thus, the thermal comfort of hens, especially regarding heat stress, has great influence on poultry performance. Heat stress can lead to a reduction in bird productivity, affecting their productive and physiological performance and behavior; in extreme cases it may cause death. Therefore, it is very important to know and to keep updated the zone of thermal comfort of birds, since this zone varies according to, for example, the age of the bird, productive phase, breeding environment, sex, genetics and previous exposure to hot or cold. In view of the above, this research was conducted with the aim to determine the thermal comfort zone for laying pullets during the growing phase based on physiological parameters and performance. Also evaluated was the effects of acclimatization of chicks during the brooding phase (first 6 weeks of life) when exposed to different thermal environments during the growing phase (7th to 17th week of life). This work was carried out in climatic chambers located in AMBIAGRO - DEA / UFV, with Lohmann LSL Lite laying pullets. It was conducted in two phases: Phase I with 648 birds from 1 day to 6 weeks of age and Phase II with 418 birds from 7 to 17 weeks of age. During Phase I the birds were subjected to one of three thermal environments, characterized as cold stress, comfort, and heat stress. Phase I aimed to prepare the birds for Phase II. At the beginning of the 7th week of age, birds from Phase I were randomly redistributed in four climatic chambers with different temperatures (thermal comfort, 20/20 °C and three levels of heat stress: mild, 25/20 °C, moderate, 30/20 °C, severe, 35/20 °C), each climatic chamber of Phase II received 5 groups of birds coming from each thermal environment of Phase I. During Phase II, during the night period all birds were kept in a thermal comfort environment (20/20 °C). Performance parameters (feed intake, feed conversion, body weight) and physiological parameters (cloacal and body surface temperature, T₃ and T₄ hormone concentrations

of blood plasma) were analyzed. No influence of Phase I on the performance of birds during Phase II was observed. In general, birds maintained at temperatures of 20/20 °C and 25/20 °C presented better performance than those exposed to 30/20 °C and 35/20 °C, indicating that this would be the desired environmental temperature range for the growing environment of pullets, from 7 to 17 weeks of age.

RESUMO

CÂNDIDO, Márcia Gabrielle Lima, D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Tolerância térmica, desempenho produtivo e fisiológico de frangas de postura quando submetidas a diferentes níveis de estresse por calor durante as fases sequenciais de cria e recria.** Orientadora: Ilda de Fátima Ferreira Tinôco. Coorientadores: Richard Stephen Gates e Luiz Fernando Teixeira Albino.

O Brasil é um país de destaque na produção de proteína animal, especialmente em relação a atividade avícola, sendo o maior produtor de ovos da América do Sul. Por ser um país de clima tropical e subtropical, o Brasil tem como característica baixa amplitude térmica média anual e altas temperaturas durante o ano na maior parte do seu território. Sendo assim, o conforto térmico, principalmente no que se refere ao estresse por calor, tem grande influência na atividade avícola. O estresse por calor pode levar a redução da produtividade das aves afetando seu desempenho zootécnico, fisiológico e pode levar a alterações comportamentais; em casos extremos pode a morte da ave. Sendo assim, é muito importante conhecer e manter atualizado a zona de conforto térmico das aves, uma vez que esta zona varia de acordo com, por exemplo, a idade da ave, fase produtiva, ambiente de criação, sexo, genética e exposição prévia ao calor ou frio. Tendo em vista o exposto, realizou-se esta pesquisa, com o objetivo de determinar a zona de conforto térmico para frangas de postura na fase de crescimento, por meio de parâmetros fisiológicos e desempenho zootécnico. Além do exposto, avaliar também os efeitos da aclimatização de frangas de postura na fase inicial de criação (primeiras 6 semanas de vida) quando expostas a diferentes ambientes térmicos durante a fase de crescimento (7^a a 17^a semana de vida). Para isto, o trabalho foi realizado em câmaras climáticas situadas no AMBIAGRO – DEA/UFV, com frangas de postura Lohmann LSL Lite. O experimento foi conduzido em duas fases: Fase I com 648 aves de 1 dia a 6 semanas de idade e Fase II com 418 aves de 7 a 17 semanas de idade. Durante a Fase I as aves foram submetidas a três ambientes térmicos, caracterizados como estresse por frio, conforto e estresse por calor. A Fase I teve como objetivo preparar as aves para a Fase II. No início da 7^a semana de idade as frangas da Fase I foram randomicamente redistribuídas em quatro câmaras climáticas com temperaturas distintas (conforto térmico, 20/20 °C e três níveis de estresse por calor: leve, 25/20 °C; moderado, 30/20 °C; severo, 35/20 °C) de forma que em cada câmara climática na Fase II, recebesse 5 grupos de aves provenientes de cada ambiente térmico da Fase I. Na Fase II, durante o período noturno todas as aves foram mantidas em ambiente de conforto térmico. Foram analisados parâmetros de desempenho zootécnico (consumo de ração, conversão alimentar, peso corporal) e fisiológicos (temperatura retal e superficial, e concentrações de hormônios T₃ e T₄ no plasma

sanguíneo). Não foi observado influência da Fase I no desempenho das aves durante a Fase II. De forma geral, as aves mantidas nas temperaturas de 20/20 °C e 25/20 °C, apresentaram melhor desempenho que aquelas expostas a 30/20 °C e 35/20 °C, indicando que esta (20/20 °C e 25/20 °C) seria a faixa de temperatura ambiental desejável ao ambiente de criação de frangas de postura na fase de crescimento, de 7 a 17 semanas de vida.

GENERAL INTRODUCTION

Brazil is one of the main producers of animal protein in the world, being the largest producer of eggs in South America, and the second in Latin America, after only Mexico, having produced almost 40 billion units in 2017 (ABPA, 2018). This substantial market means that the country has a great commitment to issues related to the sustainability of the activity, and the increasing demands of the internal and foreign markets regarding egg quality and efficient production. Brazil is a predominantly tropical and subtropical country, characterized by high daily average temperature during most of the year, requiring special care for poultry production regarding thermal stress, to which the birds are frequently subjected.

Among other factors the thermal environment, as represented by temperature, humidity, air velocity and radiation, is the aspect that most affects egg-type chickens, since it is responsible for the definition of the degree of comfort and level of stress (Arcila et al., 2018; Baêta and Souza, 2010; Cassuce et al., 2013; Kuczynski et al., 2011; Tinôco, 2001). A comfortable thermal environment is essential to reach high levels of productivity and welfare of the birds.

Significant physiological changes occur when birds are exposed to stressful environments. These include increased corticosterone, changes in thyroidal hormones triiodothyronine (T_3) and thyroxine (T_4), immunosuppression, and elevation of pulse and respiratory rates (Donkoh, 1989; Junqueira et al., 2000; Mack et al., 2013; Sahin et al., 2002). Other physiological effects of stress are behavioral changes of the birds, which can be reflected in anomalous behaviors, such as feather pecking and increased vocalization (Gates and Xin, 2001; Moura et al., 2008; Xie et al., 2015; Zimmerman et al., 2000). The effect of stress is decreased performance, with reduced body weight gain, feed intake, feed conversion efficiency, and egg quality and increased mortality (Freitas et al., 2017; Mashaly et al., 2004; Ruzal et al., 2011; Souza et al., 2016; Sterling et al., 2003). Heat stress occurs at thermal environments above the thermoneutral zone, whose upper boundary may be altered by several factors, such as genetics, age, sex, weight, physiological state and also, previous exposure to heat or cold (Cassuce et al., 2013; Cordeiro et al., 2011; Tinôco, 2001).

Chicks in the brooding phase, between 1 and 6 weeks of age, have not completely developed their thermoregulatory system; consequently, there is a need to warm up the housing facilities to avoid cold stress. Subsequently, during the growing period, from 7 to 16 weeks of age, pullets can be challenged by heat stress, since at this stage their thermoregulatory system is fully developed and the environment regularly exceeds comfortable thermal conditions of 18 °C to 20 °C and relative humidity of 50 to 70% (Albino and Carvalho, 2014; Lohmann., 2016).

The pullet growing phase is a critical period, because during this period, the main development of muscles and bones occurs, with skeletal development completed by the 14th week of age, and 70% of the mature hen weight reached by the 15th. Thus, it is important to avoid thermal stress of pullets by providing an environment which allows them to reach the egg-laying period with adequate body structure for adequate and long-lasting egg production (Kwakkel et al., 1993; Silva et al., 2000).

There is a lack of conclusive data for the appropriate thermal range for pullets raised in a tropical climate. The industry currently works with the thermal ranges recommended by international suppliers of the lineages which are predominantly from temperate climates.

However, it is inferred that by adapting to the Brazilian climate, the physiological responses of pullets may change, and thus the thermal zones currently recommended may not be applicable to hotter climates. This change in effective thermal comfort can occur due to changes in genetic and nutritional standards, management, and acclimatization to the elevated temperatures experienced (Arcila et al., 2018; Cassuce et al., 2013; Tinôco, 2001). Therefore, it is important to evaluate the temperature limits tolerated by pullets, and the temperature ranges that are considered ideal (i.e. the zone of thermal comfort) for each age and physiological state of birds acclimatized in warmer regions.

The approach used in this research was to assess the comfort zone is for pullets utilized for egg production in hot climates such as Brazil. The aim of this work was to determine the thermal comfort zone for laying pullets in the growing phase; to evaluate the effect of different thermal environments on the thyroid hormone concentration, cloacal and surface temperature during the growing phase; and also, to evaluate the performance of chicks that were previously acclimatized to different conditions when subjected to thermal stress during the growing phase.

This thesis has been prepared in journal manuscript format and includes three manuscripts that together help to answer the questions raised above. Chapter 1 consists of a literature review, and Chapters 2 and 3 are based on research studies which were conducted at the experimental area from the Center for Research in Ambience and Agroindustrial Engineering - AMBIAGRO, based in the Department of Agricultural Engineering, Federal University of Viçosa.

Chapter 2 is entitled “Effects of environment air temperature on pullet performance”; Chapter 3 is entitled “Effects of heat stress on pullet cloacal and body temperature”. A brief summary of the main findings on this research is provided in Chapter 4.

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CHAPTER 1. HEAT STRESS IN POULTRY - LITERATURE REVIEW

INTRODUCTION

Birds are homeothermic animals; homeothermy is the ability to maintain body temperature within its normal range despite variations in environmental temperature (Bligh and Johnson, 1973; Curtis, 1983; Etches et al., 2000; Renaudeau et al., 2012; Taylor et al., 2014; Wilson, 1948a). When exposed to high ambient temperature environments, birds tend to reduce their heat production and increase their heat loss. Additionally, they tend to change behavior, such as reducing movement and increasing water consumption (Fuquay, 1981). When subjected to high ambient temperature, bird body temperature may rise by 1 or 2 °C (Etches et al., 2000). In extreme high-temperature situations when birds cannot thermoregulate, or maintain their normal body temperature of 41 to 42 °C, it can rise to 46 °C and lead to death (Etches et al., 2000; Renaudeau et al., 2012).

The body temperature of birds is a result of the balance between the heat produced by metabolism and heat lost to the environment (Sturkie, 1986). The energy expenditure for keeping this balance is minimal when the birds are in their thermal comfort (or thermoneutral) zone (Etches et al., 2000). In the thermoneutral zone, animals are able to deliver their maximum productive capacity realized from genetics, nutrition, and rearing technologies. However, when the ambient temperature increases to a point at which the birds cannot compensate for thermal challenge by their natural coping strategies (behavior, reduction in feed intake, postural changes), a thermal imbalance occurs and heat stress (HS) is established (Farg and Alagawany, 2018). Comparatively, birds have a higher normal body temperature than mammals of the same weight (Mcnabb, 1966). This is because domestic chickens, such as hens and broilers, have a higher specific metabolic rate (kW per kg of liveweight) and also lower rates of heat loss than mammals of the same weight (Mcnabb, 1966).

HS can be classified into two categories which differ by the duration of the applied stress. Acute heat stress is more punctual, the birds are subjected to high environmental temperatures for a short period of time, within hours (Curtis, 1983) and is characterized by a rapid body temperature increase. On the other hand, in chronic HS the environmental temperature stays high for a long period and can be either continuous or cyclic, and the environmental temperature may increase slowly. Also, chronic stress may lead to acclimatization. However, in both cases, the birds present changes in behavior, performance and physiological parameters (Curtis, 1983; Lara and Rostagno, 2013; Loyau et al., 2015).

The physiological and behavioral mechanisms which birds use to try to cope with heat stress is a topic of interest, due to the negative effects of HS in animal performance. Research regarding the effects of high ambient temperature environments in poultry production systems, such as broilers, turkeys, quails, laying hens and embryos of those species continues due to evolving changes in the environmental temperature requirements for those species as genetic improvements continue (Arcila et al., 2018; Cândido et al., 2016; Cassuce et al., 2013; Freitas et al., 2017; Hamidu et al., 2018; Santos et al., 2017; van den Brand et al., 2019). The objective of this review is to compile the current knowledge about heat stress focusing on the physiology and thermal tolerance of domestic poultry, especially broilers and hens during the initial stages of life.

Thermal tolerance

When birds are exposed to high environmental temperatures or excessive metabolic heat production, they may not control their body temperature and it rises to the lethal levels of 46 to 47 °C (Dawson and Whittow, 2000; Yahav, 2009). However, death can be avoided by three thermal tolerance paths: rapid thermal stress response (RTSR), acclimation/acclimatization, and embryonic or post-hatch thermal manipulation (Piestun et al., 2008b; Yahav, 2009).

During the RTSR the initial body response is to increase the blood flow to the skin to improve heat removal from the viscera. This vasodilation action seeks to lose sensible heat by radiation, convection and conduction by the temperature gradient between the environment and the skin (Etches et al., 2000; Yahav, 2015; Yahav et al., 2004, 1998). This heat loss is more efficient in featherless body parts when compared with feathered parts as described in studies from Nääs et al. (2010), Nascimento et al. (2011), Souza Jr et al. (2013), Zhao et al. (2013), Mayes et al. (2015) and Al-Ramamneh et al. (2016). To increase the blood flow, it is necessary to increase the cardiac rate and also promote vasoconstriction in visceral organs combined with peripheral vasodilatation. However, RTSR may not always be successful because severe hyperthermia leads to decreased blood pressure, brain hypoxia (which can cause neuronal dysfunction), cell fatigue, and also metabolic alkalosis and death (Bogin et al., 1996; Etches et al., 2000; Whittow, 1986; Yahav, 2015, 2009). Thus, the length and severity of the heat stress episode can be a determining factor for determining whether or not birds cope with HS during the RTSR (Bogin et al., 1996; Yahav, 2009).

Acclimation is defined as alterations to compensate for the effects of a single stressor acting alone, such as high environmental temperature (Curtis, 1983). This induced thermal tolerance occurs usually in experimental or artificial situations. Once in a relatively thermoneutral environment, the birds usually are subjected to changes in several variables simultaneously

(Bligh and Johnson, 1973; Curtis, 1983; Yahav, 2009). Acclimatization, similar to acclimation, is also related to the alterations with the purpose to compensate for the effects of a stressor (Curtis, 1983). However, in acclimatization, the stressor agent can be simultaneous environmental factors and not just one acting alone (Bligh and Johnson, 1973; Curtis, 1983). For example, birds can be acclimatized when they are naturally exposed to seasonal changes when the temperature slowly changes in a cyclic pattern (Yahav et al., 1997b).

The induced thermal conditioning is dependent primarily on age and the temperature used (Tanizawa et al., 2014; Yahav and McMurtry, 2001). The main goal of thermal conditioning is to change the threshold temperature limit for the birds to cope with HS later in life, and reduce mortality (Basilio et al., 2003; Tanizawa et al., 2014; Yahav, 2009; Yahav and McMurtry, 2001). This method uses the immaturity of the thermoregulation system, exposing young chicks to high temperature levels (Arjona, 1988; Modrey and Nichelmann, 1992; Yahav et al., 1997a; Yahav and Hurwitz, 1996). Basilio et al. (2003) reported significant improvement in chicks that were thermal conditioned at 5 days of age, with reduced mortality, lower body temperature and also lower heat mass at 34 days of age when subjected to 32 to 34 °C. Other authors also reported similarly positive results for thermal conditioning from 3 to 5 days of age, such as Arjona (1988), Yahav and Hurwitz (1996), Yahav and McMurtry (2001) and Basilio et al. (2003).

Induced thermal tolerance also can be reached when birds are exposed to high temperature during embryogenesis, the egg incubation period. In an experiment with fertile broiler eggs, Piestun et al. (2008a), Piestun et al. (2008b) and Piestun et al. (2009) stated that the incubation period for induced thermotolerance is between day 7 to day 16 of incubation, for 12h.day⁻¹ with a temperature of 39.5 °C. The results were based on the maturation of the hypothalamus-hypophysis-thyroid axis, indicated by T₃ and T₄ levels, and also the corticosterone plasma concentration. Later in life, the conditioned-embryo broilers showed improved sensible heat loss, and significantly lower stress response and mortality in high environmental temperatures. These findings highlight the importance and the options the producer has to reduce production losses caused by HS.

Behavioral changes during heat stress

One of the ways for birds to dissipate heat during HS, and also to measure their welfare, is through behavioral changes (Etches et al., 2000; Mack et al., 2013; Modrey and Nichelmann, 1992; Pereira et al., 2015; Renaudeau et al., 2012; Santos et al., 2017). When subjected to high environmental temperatures birds tend to be more disperse, increase drinking frequency, reduce

feed intake, lift wings, ruffle feathers, reduce movements, and panting (Barbosa Filho et al., 2007; Gerken et al., 2006; Lara and Rostagno, 2013).

In a study with two strains of laying hens, Barbosa Filho et al. (2007) observed that under heat stress both strains did not take sand baths, beat and stretch their wings, or shake their feathers, but did increase drinking frequency and the time seated and with no movement, compared with birds in a thermoneutral environment. Mack et al. (2013) evaluated the behavior of two strains of laying hens. Those subjected to hot environments spent less time feeding and walking than the control group; the hens in a hot environment also spent more time with their wings elevated, drinking water and resting; panting behavior was only present for hens in hot environments. The thermal conditioned birds may have behavioral changes when exposed to HS. Tanizawa et al. (2014), working with thermal conditioned chicks, demonstrated delays in the onset of panting and wing-drop behaviors compared to the control group (non-conditioned).

The behavioral patterns made by birds during HS have specific functions. Panting, among all behaviors caused by HS, is the more visible one. The objective of panting is to dissipate the heat through evaporative (latent) cooling (Etches et al., 2000; Richards, 1971). The increase of drinking frequency has the purpose of compensating for the water lost through evaporative cooling. This behavior occurs faster than feed intake reduction (Etches et al., 2000). Also, birds may use water for evaporative cooling by splashing it in the comb and wattles (Etches et al., 2000). The wing lifted movement is a way to increase the sensible heat loss, by exposing the featherless body part under the wings where the body has near-surface blood vessels. Thus, the wing lifted movement promotes sensible heat transfer by convection from the body to the environment (Gerken et al., 2006; Mack et al., 2013). Like wing lifting, preening is also a way to dissipate heat through sensible heat loss (Gerken et al., 2006).

Hormonal role in heat stress

The physiology of the bird's response to HS implicates the joint function of several hormone secretions seeking to dissipate heat and maintain homeostasis. Among the hormones involved in the heat stressed bird, the thyroidal hormones can act as stress markers and also help the birds to control their body temperature.

The thyroid gland is located ventrolaterally to the trachea and is responsible for hormone production and its release in the blood (McNabb and Darras, 2015). This gland can change size and weight as a result of external factors, such as environmental temperature. Under HS a decrease in the gland dimensions may occur (McNabb and Darras, 2015). Thus, although thyroid is not considered a vital organ, it has an important role in HS. Thyroid hormones,

triiodothyronine (T₃) and thyroxine (T₄) are important metabolic mediators and are considered key factors in the control of homeothermy, and their concentrations are altered in thermal stress (McNabb, 1995; Swenson and Reece, 1996).

These hormones act to increase basal metabolism, with the availability of glucose to the cells, thus providing a stimulus for protein synthesis, increased neuronal and cardiac functions, and increasing the consumption of tissue oxygen (Mack et al., 2013; McNabb, 1995). Several studies reported a correlation between reduced T₃ plasmatic concentration with HS and its effects on feed intake (Elnagar et al., 2010; Star et al., 2008; Yahav and Hurwitz, 1996). Williamson et al. (1985) in an experiment with chickens subjected to 40 °C found reduced T₃ in plasma related to decreased feed consumption. These authors also associated the decrease in skeletal growth to low levels of T₃.

However, the role of these hormones is not fully determined, especially for T₄. Thus in the literature it is possible to find results showing its increase, decrease or stabilized concentration when poultry are subjected to HS (Bobek et al., 1980; Cogburn and Freeman, 1987; Elnagar et al., 2010; Hahn et al., 1965; Mack et al., 2013; May, 1978; May et al., 1986; Sinurat et al., 1987). This variability can be also due to experimental differences, such as length of heat stress applied (weeks, days, hours, acute, chronic or cyclic), the temperature applied, and feeding status, fasting or fed ad libitum.

Stress in the initial phases

Egg production usually starts at about 18 weeks of age, however, for the hen to achieve maximum production and a long lay period provided by, among other factors, genetics and nutrition, it is important to utilize good management during the brooding and growing phases. The environment during the initial rearing stages is crucial for standard development. Ericsson et al. (2016), in an experiment with Hy-Line chicks exposed to three different types of stress (food frustration, physical restraint and social isolation) at the 2nd, 8th and 17th week of age, found long-term stress effects after sexual maturity, especially in the birds which were stressed at the 2nd and 8th weeks. During the initial rearing phase stress can cause persistent negative effects on physiology, behavior, immune system as also body development (Ericsson et al., 2016; Farnell et al., 2001; Franco-Jimenez et al., 2007; Salvatierra et al., 2009).

The importance of the first stages of life is also correlated with body development. During the firsts 5 weeks of age the chick has a faster development of viscera, bone structure, and feathers (Alves et al., 2019; Silva et al., 2015). Bone development, either for weight (shank and tibia) or length (keel) is completed at 12 weeks (Kwakkel et al., 1993). Bruno et al. (2000) in

an experiment with broilers subjected to different environmental temperatures and food restriction found reduced bone length and width in the birds raised in the HS environment. Also, the authors reported that heat exposure affected the index of bone density.

The growing phase, usually from 7 to 17 weeks of age, is a crucial period for body composition development and higher body weight gain. According to Kwakkel et al. (1993) in research with White Leghorns, almost 70% of the mature body weight is gained by 14 weeks of age, in contrast with only 8% gained after 22 weeks of age. According to Kwakkel et al. (1993) a pullet needs a minimum of 82% of mature weight before her sexual maturity spurt starts. In a modern pullet this threshold must be achieved before 17 weeks age. This body weight threshold may change according to the genetic strain; the Lohmann LSL-Lite manual shows, for example, that 82% of the mature body weight is reached by the 17th week of age, whereas for the Hy-Line W36 this threshold is achieved one week earlier (Hy-Line, 2016; Lohmann., 2016). Similar results were found by Alves et al. (2019) working with four different strains of pullets. Besides body weight gain, body changes for sexual maturity also occur at the end of the growing phase, such as the growth of the reproductive tract, an increase in comb and wattle size, and abdominal fat deposition (Albino and Carvalho, 2014; Kwakkel et al., 1993)

Chicks and pullets subjected to a sub-optimal environment, such as HS, may exhibit behavior and hormonal imbalance, leading to decreased feed intake and subsequent reduction in body weight gain. Thus, if pullets do not reach a minimal body weight, which varies between the strains, there may be a delay in sexual maturity and a negative influence on efficiency of egg production (Bish et al., 1985; Dunnington and Siegel, 1984). Hens with lower body weight produce lighter eggs in comparison with heavier birds (Bish et al., 1985; Kamanli et al., 2015; Sterling et al., 2003).

Thus, for hens to start the laying period and deliver expected performance it is important they have a standard body composition (Galeano-vasco and Cerón-muñoz, 2013). To achieve this goal, HS must be managed, especially during the initial stages of development. Factors including genetics, nutrition, the rate of metabolism, population density, and new technologies applied in the facilities are constantly changing, which require continuous efforts to adjust the birds' needs to the environment.

Final Considerations

Heat stress is an important stressor for poultry, with effects both production and bird welfare. To avoid production losses and mortality, HS needs to be managed with tools such as

induced thermal tolerance, adequate environment and also knowledge about comfort thermal zones.

Henceforth it is necessary to continue investigation about the deleterious effects of HS on poultry production, as the actualization of thermal comfort zones and methods to help the birds to cope with the HS especially during the early periods of life.

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CHAPTER 2. EFFECTS OF ENVIRONMENT AIR TEMPERATURE ON PULLET PERFORMANCE

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ABSTRACT The thermal environment may affect the performance and physiology of poultry, especially when subjected to high temperatures. One mean of reducing deleterious thermal effects during production is to acclimatize birds during their initial weeks. The objectives of this study were to evaluate the impact of heat stress during the pullet growing phase and to determine whether exposing chicks to one of three different temperatures during the brooding phase served to acclimate pullets to subsequent heat stress. Additionally, verifies the effects of different air temperature levels, during the growing phase, on performance seeking to identify the ideal environmental temperature for pullets during this life stage. A total of 648 chicks (Lohmann LSL Lite), with 1 day of age and uniform weights, from the same hatchery, were used in two different phases, namely Phase I - initial (day 1 to 6 weeks of age – brooding phase) and Phase II (weeks 7 through 17 – growing phase). During the Phase I chicks were randomly divided in 3 groups of 216 chicks, and each group was subjected to one of three different environmental temperatures: thermal comfort, hot or cold. From the 7th week through the 17th week of age, each group of 54 pullets from each Phase I environment was subjected to one of four day-time thermal treatments: Thermal Comfort (20/20 °C), Mild Heat Stress (25/20 °C); Moderate Heat Stress (30/20 °C); Severe Heat Stress (35/20 °C), with the purpose of evaluating the influence of the different thermal environments to which the birds were subjected during Phase I, on the development and performance on Phase II. During Phase II for all treatments the night temperature was 20/20 °C. Performance measures, including body

weight, feed intake, feed conversion, and mortality were measured, as also cloacal and body temperatures and T₃ and T₄ hormones. There were no effects of Phase I temperature or its interaction with Phase II temperatures ($P < 0.05$) noted for all variables evaluated at the 17th week of age. The pullets subjected to 20/20 °C and 25/20 °C had better physiological and productive performance, regardless of thermal environments they were subjected to during the Phase I. Pullets subjected to 35 °C gained less weight, had lower feed intake, and higher feed conversion compared with pullets in the other three treatments. T₃ hormone concentration decreased with increasing air temperature ($P < 0.05$). Cloacal and body temperature increased with the increasing environmental temperature ($P < 0.05$). In conclusion, the air temperatures applied for the first six weeks did not affect pullet development after the growing phase. Pullets subjected to 35/20 °C during Phase II had their performance and physiological status negatively affected, and pullets subjected to 20/20 °C and 25/20 °C showed the best performance among the other two treatments.

Key words: acclimation, heat stress, poultry, pullet, thermoregulation

INTRODUCTION

Heat stress (**HS**) is one of the factors that can negatively affect the performance response of poultry production, either for egg or meat production. The negative effects of HS on birds are well known, and include decreased productive performance as measured by body weight, feed intake and feed conversion (Arcila et al., 2018; Azad et al., 2010; Felver-Gant et al., 2012; Fouad et al., 2016; Mahmoud et al., 1996; Mashaly et al., 2004). HS also reduces production and quality of eggshells and increases bird mortality (Felver-Gant et al., 2012; Mashaly et al., 2004). Furthermore, HS can also change physiological metrics such as core temperature, behavior, and thyroid hormonal concentration (Bobek et al., 1980; Dahlke et al., 2005; Lara and Rostagno, 2013). The thyroidal hormones triiodothyronine (**T₃**) and thyroxine (**T₄**) have a key role in metabolism, affecting growth, feed efficiency, feed intake, carbohydrates metabolism, proteins and lipids, thermogenesis and body composition (Bobek et al., 1980; Cogburn and Freeman, 1987; Dahlke et al., 2005; Elnagar et al., 2010).

The occurrence and severity of HS is affected by the type of production system, bird initial thermal acclimation/acclimatization, the genetic strain, nutrition, management, and the intensity, rate and duration of the HS (Cassuce et al., 2013; Lin et al., 2006). For example, cloacal temperature can be stable when the air temperature increases slowly, up to about 33 °C (depending on air velocity), but if it increases rapidly to only 30 °C, non-acclimated birds will have more difficulty maintaining homeothermy (Boone and Hughes, 1971; J.S. Welker et al., 2008).

To reduce the deleterious effects of HS and increase animal welfare inside the facilities, strategies used include appropriate genetic selection, reduced energy density feed, diurnal feeding pattern adjustment, addition of key environmental control features such as evaporative cooling, and also acclimation (Lin et al., 2006). Acclimation to HS is described as the ability of the bird to better deal with this stressor by prior exposure (Mahmoud and Yaseen, 2005). This method consists to expose chicks at an early age to nonlethal heat stress, taking advantage of the fact that their thermoregulatory system is not fully developed (Yahav and Hurwitz, 1996). According to Yahav and McMurtry, (2001), and Azad et al. (2010), this practice can increase the temperature threshold for onset of HS and provide poultry with an ability to better cope when exposed to high temperatures.

To avoid economic losses during hot periods, Yahav and McMurtry (2001), and Azad et al. (2010) indicated that the bird's thermoregulation system must be prepared for exposure to high environmental temperatures. This type of management would be very interesting,

especially in countries and locals where poultry facilities do not have total thermal environmental control. Brazil is one of the largest world egg producers and has a tropical and subtropical climate, with small thermal annual amplitude. Priority has been given to facilities with a hybrid ventilation and thermal conditioning system (facilities with some degree of mechanical ventilation and cooling systems, but allowing full lateral opening and consequent natural ventilation during periods in which natural thermal conditions are compatible with the thermal requirements of poultry in their different stages of life). However, the environmental temperatures used for laying hens follow international standards of the lineage, and it is not known if such thermal zones are really adequate to the acclimatized birds in the country. This makes it difficult to understand if the management is properly established in favor of energy saving and animal welfare in each stage of the birds' life.

Therefore, it is of interest to determine whether pullet chicks acclimatized to higher temperature in the first six weeks (brooding phase), can better cope with HS during the subsequent pullet development phase. The aim of this study was to evaluate the effect of different levels of HS during the growing phase, and determine whether exposing chicks to different temperatures during the brooding phase served to acclimate pullets to subsequent heat stress. Additionally, the study was designed to evaluate the effects of different air temperature levels, during the growing phase, on pullet performance.

MATERIAL AND METHODS

Experimental Design

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

A total of 648 day-old commercial egg-type chicks (Lohmann LSL Lite), uniform weights from the same hatchery, were randomly allocated into three controlled-environment chambers. Each chamber measured 3.20 m x 2.44 m x 2.38 m (LxWxH). Chicks were placed inside cages measuring 0.50 m x 0.50 m x 0.50 m (LxWxH). Following the industry guidelines (Lohmann, 2016), chick placement density was 140 cm² chick⁻¹ for the first four weeks (17 chicks cage⁻¹), 285 cm² chick⁻¹ (9 chicks cage⁻¹) for the fifth and sixth weeks, and 357 cm² chick⁻¹ (7 pullets) for the 7th through 17th week. For density adjustments throughout the experiment the pullets were randomly culled. Also following the Lohmann (2016) guidelines, the cages were equipped with 0.5m of linear feeder at the cage front, and one nipple drinker placed on a side midway between the front and back. Journals and jug waterers were provided during the first week to assist chick starting, and an additional nipple drinker was placed until the 6th week per

industry guidelines (Lohmann, 2016). The feed provided during the experiment was formulated according to the composition and nutritional requirements indicated for Rostagno et al. (2011), Attachment I shows the formulation for Phase II.

The light program used was that recommended by the industry guidelines (Lohmann, 2016). The Light:Dark (**L:D**) hourly schedule was 24L:0D and 16L:8D, for days 1-2, and 3-6 respectively. It was then reduced by 1 hour per week, until the 6th week, finishing with 10L:14D applied for the remainder of the experiment.

Chamber temperatures were individually controlled with a microcontroller (model MT-531R Plus, Full Gauge Controls, Canoas/RS, Brazil), connected to an air heater (Model AB Split 1, Britania Eletrodomesticos S.A. Pirabeiraba, SC, Brazil) and an air conditioner (Model ABS 12FC 2LX, Komeco, Manaus, AM, Brazil). Relative humidity was maintained in the range of 40–60% with an ultrasonic humidifier (Model HUL535W, Kaz USA, Inc., Marlborough, MA) and the air conditioner. Ventilation was provided by two 10 cm axial fans (model FD08025 S1M, Ambition Technology Company, Guangdong, China), providing approximately $1.08 \text{ m}^3 \text{ min}^{-1}$ (3.6 air changes hr^{-1}). Air quality was monitored daily for ammonia (Gas Alert Extreme NH_3 Detector, BW Technologies®, Oxfordshire, UK) and carbon dioxide (AZ 77535, AZ Instrument Corp., Taichung City, Taiwan). Chamber temperature and relative humidity were recorded by a datalogger each five minutes for the entire experiment (Model HOBO U14-001, Onset, USA), for relative humidity the measurement range was from 0 to 100%, accuracy of $\pm 2.5\%$ from 10% to 90%, and resolution of 0.05%; for temperature the measurement range was from -20°C to 50°C , accuracy of $\pm 0.21^\circ\text{C}$ from 0° to 50°C and resolution of 0.02°C .

The research was conducted in two phases, named Phase I (from day 1 until the end of the 6th week of the bird's life) and Phase II (started at the 7th week of bird's life until the end of the 17th week).

Phase I. During Phase I (brooding phase – first six weeks of age), chicks were divided randomly into groups of 216 and placed into one of three environmental chambers, each one with different weekly air temperatures (mild cold stress, thermal comfort, mild heat stress) as shown in Table 1. The thermal comfort treatment was estimated from the literature and the breeding manual, in which for the first week the environmental temperature started with 35°C , and reducing weekly until reaching 31°C at the end of the first week (Albino and Carvalho, 2014; Lohmann, 2016). The mild cold and mild heat stress conditions were created by reducing 3°C or adding 3°C to the thermal comfort treatment values, respectively, for each week. The chicks were subjected to the Phase I temperatures 24h/day. Evaluation of performance and

physiological responses of these chicks at the end of the Phase I are reported separately in Andrade (2017) and Andrade et al. (2017). Of note for this study, the body weight of pullets at the end of Phase I were similar, averaging 418 g per bird. At the end of Phase I the birds were then randomly reassigned (with identify noted for subsequent evaluation) to different HS levels during the Phase II.

Table 1. Air temperatures during Phase I

Thermal Environment	1 st week	2 nd week	3 rd week	4 th week	5 th and 6 th week
Mild cold stress	28	25	23	20	17
Thermal comfort ¹	31-35	28	26	23	19
Mild heat stress	38	31	29	26	22

¹Per Lohmann (2016); Albino and Carvalho, 2014

Phase II. During Phase II (growing phase - from the 7th through the 17th week of age), a total of 420 pullets, 140 of which were obtained randomly from each of the three Phase I temperatures and divided into four groups of 35 pullets (five cages) were placed into one of four independent environmental controlled chambers. Temperature treatments were denoted as: Thermal Comfort (**TC, 20/20 °C**); presumed Mild Heat Stress (**MiHs, 25/20 °C**); presumed Moderate Heat Stress (**MoHs, 30/20 °C**); presumed Severe Heat Stress (**SeHs, 35/20 °C**). During the night the air temperature was set to 20 °C, from 7:00 p.m. to 7:00 a.m. for all chambers. Of note for this study the birds were randomly selected from Phase I to Phase II

Productive Performance and Physiological Responses

Productive performance assessment consisted of weekly determination of body weight, feed intake, feed conversion (feed consumption:body weight), and mortality rate. Pullets were individually weighed at the end of each week. Feed intake was measured daily as the difference between feed provided and leftover, with each cage measured separately, and results tabulated weekly. Feed conversion was also determined on a weekly basis. Mortality was recorded daily.

Physiological responses included weekly measures of cloacal temperature and mean body temperature computed from surface temperature measurements, as well as thyroid hormones thyroxine (T₄) and triiodothyronine (T₃) obtained from blood samples collected from one bird per cage at the end of the experiment. Cloacal temperature was measured with a clinical thermometer (Incoterm, Porto Alegre/RS, Brazil) which was inserted approximately 1cm into the cloaca and was read after temperature stabilization. The body parts surface temperature was

collected with an infrared thermometer (model TI-860, Instrutherm, São Paulo, Brazil) with 0.95 of emissivity. The mean body temperature (T_b) was calculated using the mean surface temperature ($\overline{T_s}$) and the cloacal temperature as shown in Equations 1 and 2, according to Dahlke et al. (2005):

$$T_b = 0.3 \overline{T_s} + 0.7 T_c \quad (1)$$

$$\overline{T_s} = 0.12 T_w + 0.03 T_h + 0.15 T_l + 0.7 T_{ba} \quad (2)$$

where T_c is the cloacal temperature; T_w , wing temperature; T_h , head temperature; T_l , leg temperature; T_{ba} , back temperature, as adapted from Dahlke et al., 2005; Richards, 1971.

Cloacal and mean body surface temperatures were collected weekly from 4 pullets per cage (240 birds). Blood tests were collected from the brachial vein (5 mL per sample) and analyzed for T_3 and T_4 concentrations by chemiluminescence method (Abbott Laboratories, Longford, Ireland).

Statistical Analysis

The experimental design was a completely randomized in a split-plot arrangement with four treatments (Phase II temperatures - TC; MiHs; MoHs; SeHs) in the plots and three subplots (Phase I temperatures). Were analyzed their interactions, being five replicate cages per treatment x sub-plot combination. The quantitative analysis of body weight, feed intake, feed conversion, cloacal temperature, mean body temperature, and hormones was performed using analysis of variance (SAEG, 2007). Differences between group means were compared by Tukey's test, with a 5% confidence level ($P < 0.05$) for significance of treatment effects, interactions and differences between means.

RESULTS

The mean target air temperature and humidity maintained from the 7th through the 17th week for each treatment during Phase II are presented in Table 2; the air temperatures and humidity for Phase I are reported separately in Andrade (2017) and Andrade et al. (2017).

Table 2. Mean and standard deviations (between brackets) of measured air temperature values, relative air humidity for each climatic condition accumulated from 7th throughout the 17th week of age

Thermal Environment	Air Temperature (°C)	Relative Air Humidity (%)
Thermal Comfort (20/20 °C)	20.4 (0.9)	63.4 (6.6)
Presumed Mild Heat Stress (25/20 °C)	25.2 (0.9)	68.9 (6.6)
Presumed Moderate Heat Stress (30/20 °C)	30.6 (0.8)	68.0 (8.3)
Presumed Severe Heat Stress (35/20 °C)	35.3 (0.7)	64.5 (6.3)

Final mean body weight, feed intake and feed conversion of pullets subjected to the 4 different Phase II temperatures during the growing phase (TC, 20/20 °C; MiHs, 25/20 °C; MoHs, 30/20 °C; SeHs, 35/20 °C) are presented in Table 3. Effects of Phase I temperature treatment and its interaction with Phase II temperatures were not significant ($P > 0.05$). Pullets subjected to 35/20 °C gained less weight, had lower feed intake and feed conversion compared with pullets subjected to 20/20 °C, 25/20 °C and 30/20 °C treatments ($P < 0.001$). Only two pullets died, and no significant mortality differences between treatments were found. Both feed intake and feed conversion were similar for TC and MiHs, and for MiHs and MoHs. Pullets subject to SeHs and MoHs were not different from each other for feed conversion.

Table 3. Effects of Phase II temperature, Phase I temperature, and interactions on the performance of pullets at 17 weeks of age.

Thermal Environments	Performance Parameters		
	Body weight (g)	Feed intake (g pullet ⁻¹)	Feed conversion (g feed:g body weight)
Thermal Comfort (TC, 20/20 °C)	1194.5 ^a	5178.1 ^a	4.34 ^a
Mild Heat Stress (MiHs, 25/20 °C)	1193.1 ^a	4992.3 ^{ab}	4.18 ^{ab}
Moderate Heat Stress (MoHs, 30/20 °C)	1165.5 ^a	4602.9 ^b	3.95 ^{bc}
Severe Heat Stress (SeHs, 35/20 °C)	1063.4 ^b	3858.9 ^c	3.63 ^c
SEM	31.64	400.22	0.35
P-value (Phase II)	<0.001	<0.001	<0.001
P-value (Phase I) ¹	ns	ns	ns
P-value (Interactions)	ns	ns	ns

^{a-c}Means within a column with different superscripts differ significantly ($P < 0.05$).

¹ Temperatures used in the Phase I are listed in Table 1.

Abbreviation: SEM: Standard error of mean

Weekly mean body weights for the four Phase II temperatures were also consistently less for SeHs compared to TC, MiHs, and MoHs ($P < 0.001$, Figure 1). An initial effect of Phase I temperature was observed for weeks seven and eight ($P < 0.001$) and week nine ($P < 0.05$), but it disappeared by week 10 and remained insignificant for the remainder of the experiment.

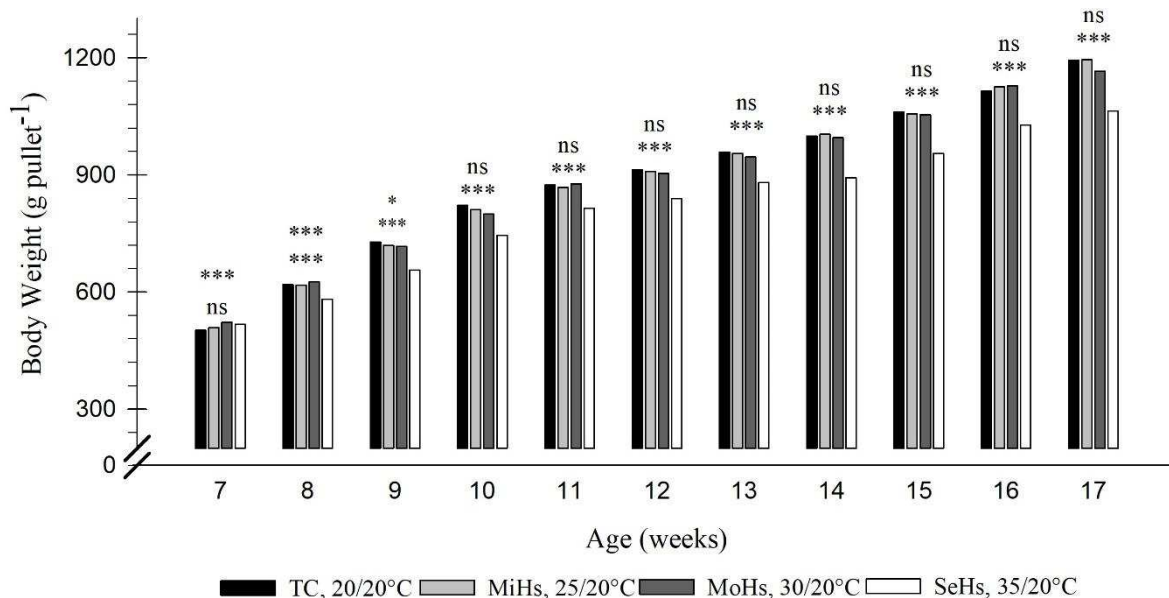


Figure 1. Weekly mean body weight of pullets subjected to four different Phase II temperatures during the growing phase. Treatments were: Thermal Comfort – 20/20 °C (TC); Mild Heat Stress – 25/20 °C (MiHs); Moderate Heat Stress – 30/20 °C (MoHs); and Severe Heat Stress – 35/20 °C (SeHs). Pullets were subjected to three different thermal environments during the brooding phase (Table 1), denoted as Phase I. Results for testing the effects of pre-experiment temperature and Phase II temperatures are provided above the bars for each week, with the top line for the Phase I and bottom line for the Phase II.

ns = not significant; * = ($P < 0.05$); *** = ($P < 0.001$).

As shown in Figure 1, the effects of Phase I temperatures were present during Phase II in the weeks 7, 8 and 9 of age. Pullets that have been subjected to thermal comfort during Phase I were statistically heavier than the pullets with were exposed to heat stress during the firsts 6 weeks of age, at the weeks 7, 8 and 9 of age, Table 4.

Table 4. Effects of Phase I temperature in body weight of pullets at the 7 to 9th weeks of age

Thermal Environments Phase I ¹	Body weight (g)		
	7 th Week	8 th Week	9 th Week
Cold Stress	512.0 ^{ab}	609.6 ^{ab}	706.9 ^{ab}
Thermal Comfort	529.1 ^a	629.4 ^a	720.1 ^a
Heat Stress	496.6 ^b	593.8 ^b	688.6 ^b

^{a-d}Means within a column with different superscripts differ significantly ($P < 0.05$)

¹ Temperature from Table 1

The hormonal concentration of T₃ and T₄, and mean cloacal and body temperature (T_b) of pullets subjected to four different air temperatures, at the 17 weeks of age, are presented in Table 5. Effects of Phase I temperature, and its interaction with Phase II temperatures were not significant (P < 0.05) for all the physiological variables evaluated, Table 5. The T₃ hormone concentration decreased with increasing temperature, pullets subjected to TC and MiHs having higher T₃ than pullets at MoHs and SeHs environments (P < 0.05). For the T₄ concentrations, the only difference found was between MiHs and MoHs treatments.

Table 5. Physiological response of pullets subjected to 4 levels of Phase II temperature, Phase I temperature, and interactions on the performance of pullets at 17 weeks of age

Thermal Environments	Physiological Parameters			
	T ₃ (ng dL ⁻¹)	T ₄ (μg dL ⁻¹)	Cloacal Temperature (°C)	Mean Body Temperature ¹ (°C)
Thermal Comfort (TC, 20/20 °C)	183.2 ^a	2.13 ^{ab}	41.1 ^c	38.7 ^a
Mild Heat Stress (MiHs, 25/20 °C)	181.0 ^a	2.04 ^b	41.1 ^c	40.1 ^b
Moderate Heat Stress (MoHs, 30/20 °C)	125.3 ^b	2.36 ^a	41.3 ^b	42.5 ^c
Severe Heat Stress (SeHs, 35/20 °C)	118.2 ^b	2.31 ^{ab}	41.7 ^a	43.6 ^d
SEM	28.67	0.30	0.08	0.24
P-value (Phase II)	<0.001	<0.01	<0.001	<0.001
P-value (Phase I)	ns	ns	ns	ns
P-value (Interactions)	ns	ns	ns	ns

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

¹ From equation (1)

Abbreviation: SEM: Standard error of mean

Cloacal and mean body temperature increased with environmental temperature, Table 5. Cloacal temperature of pullets in treatments TC and MiHs were similar (P < 0.05), and different for the two hotter conditions (P < 0.001). All the treatments were different (P < 0.05) for T_b at 17th week of age. Mean weekly T_b for the four treatments for weeks seven through 17 are plotted in Figure 2.

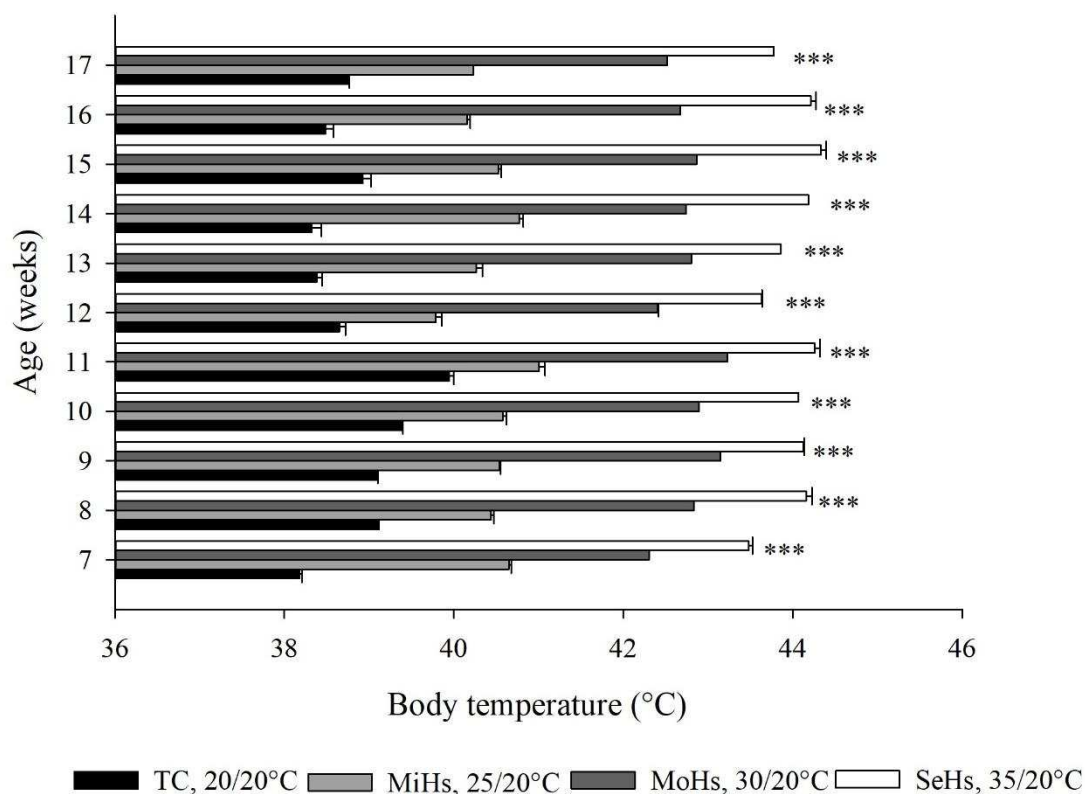


Figure 2. Body temperature (T_b) of pullets on different ages subjected to 4 different thermal environments, Thermal Comfort - 20/20 °C (TC); Mild Heat Stress - 25/20 °C (MiHs); Moderate Heat Stress - 30/20 °C (MoHs); and Severe Heat Stress - 35/20 °C (SeHs). Means and standard error bars within each age differ significantly, for all the weeks and treatments *** = significant at ($P < 0.001$) per Tukey test.

DISCUSSION

HS is may cause mortality and performance decrease (Calefi et al., 2017; Rath et al., 2015; Yahav, 1999). However, that was not the case in this study. Cooper and Washburn (1998) tested temperatures between 21 and 32 °C on broilers and also did not find a difference in mortality rates. Yahav and Hurwitz (1996) concluded that temperatures higher than 38 °C lead to increased mortality rates in broilers. In this study, the highest temperature tested was 35 °C.

Thermal tolerance of broiler chicks has been shown to be induced by exposure to nonlethal heat stress in the first days of life (Yahav and Hurwitz, 1996; Yahav and McMurtry, 2001; Abdelqader and Al-Fataftah, 2014). These studies were done with broilers, which have faster development and metabolism than pullets. By contrast, in this study, there were no differences between pullets maintained at different temperatures during the Phase I when subjected to other thermal environments during the growing phase with respect to final body weight, cumulative

feed intake and feed conversion at the end of the 17th week. The lack of effect of Phase I temperature on final performance parameters may be due to the Phase I air temperatures and humidity being insufficiently high for acclimation. Arjona et al. (1990) and Yahav and Hurwitz (1996) hypothesized that the resistance of young broiler chicks to high temperature can be due to the temperature itself plus high humidity (70-80%). In this study relative humidity was maintained in a lower range (Table 1), thus the overall thermal challenge for pullets was not severe. Yahav (2009) in a review about thermal tolerance, attributed a long-lasting effect of the thermal modulation to the modulation done at the pre-hatch period, due to the alteration in the hypothalamic threshold during the embryogenesis.

Final body weight of pullets was affected by the Phase II temperatures during the growing phase ($P < 0.001$) and decreased with the increased environmental temperature (Table 3). This pattern has been reported in several studies for broilers and laying hens (Cândido et al., 2016; Cassuce et al., 2013; Cooper and Washburn, 1998; Star et al., 2008). Pullets subjected to treatments TC, MiHs and MoHs had similar body weight, higher than those subjected to 35/20 °C. In this study, daily air temperature cycled between the treatment level during the day, and 20/20 °C at night, which could help the birds in all treatments except 35/20 °C recover from the stress to which they were exposed. Wolfenson et al. (1979), found no significant difference in hens egg production when subjected to heat stress only during the day.

As noted, the treatments TC and MiHs were similar in their effect on body weight, feed intake and feed conversion at the end of 17 weeks. This suggests that pullets from newer genetic lines may be more resistant to higher temperatures than generally accepted. However, no data were collected after this period, and thus conclusions regarding the impact of this higher temperature for pullets on subsequent egg production, egg quality, and other measures cannot be made. By contrast, the treatment SeHs resulted in the lowest feed intake of the treatments ($P < 0.001$), suggesting that pullets subjected to 35/20 °C could not recover and were heat stressed.

Although there was no effect of the Phase I temperatures on the performance of birds at the 17th week of age, this effect it was observed for the first three weeks of the Phase II and then disappeared. The more pronounced effect during this first three weeks were the highest body weight in pullets raised in thermal comfort when compared to the ones subjected to heat stress (Table 4). There was no difference in body weights amongst the treatments after the first week of the Phase II. In contrast, Cooper and Washburn (1998), which subjected broilers to two different temperatures (21 and 32 °C), found a difference ($P < 0.05$) in body weight gain after one week. However, broilers have increased metabolism and body weight gain, which can

result in a faster response to the treatments (Buzala et al., 2015). Body weights at the end of the 8th and 9th weeks were affected by both phases (Phase I and Phase II). Starting from the 8th through the 17th week, the Phase II affected body weight, and specifically for the SeHs (35/20 °C) treatment. Abdelqader and Al-Fataftah (2014) showed in their experiment that thermotolerance can be affected by the duration of heat exposure, and Sykes and Fataftah (1986) indicated that the acclimation response for domestic fowls starts as a flat line with a decline over time, agreeing with the body weight pattern in this experiment.

The primary response to heat stress in poultry is reduced feed intake, which is directly associated with reduced body weight or weight gain (Wolfenson et al., 1979; Xie et al., 2015). In this study, feed intake was significantly reduced for the SeHs treatment, as was T₃ in both SeHs and MoHs, suggesting that chronic heat stress was induced. Also, the reduced T₃ in pullets subjected to 35/20 °C, and 30/20 °C may have reduced the metabolism, consequently, reducing the maintenance energy expenditure, and thus, increased the relative gain, with could be observed in the feed conversion values.

In this experiment, T₃ levels were reduced significantly ($P < 0.001$) with the increase of the temperature (Table 5). In a study with quails subjected to cold (-1 to -6 °C) and warm (34 and 35 °C) environments, Bobek et al. (1980) also reported decrease T₃ with heat stress. Other studies have also shown that T₃ consistently reduces with heat stress, for quails (Bobek et al., 1980), domestic chickens (Bogin et al., 1996), young chickens (Cogburn and Freeman, 1987), broilers (Dahlke et al., 2005; Xie et al., 2015), and hens (Elnagar et al., 2010). In a study with broilers, Arjona et al. (1990) found that T₃ concentration was reduced when birds were subjected to HS later in life independently of previous exposure to high temperature.

In this experiment, concentrations of T₄ did not change significantly (Table 5). A previous study conducted by Mack et al. (2013) reported that neither high ambient temperature (35 °C) nor control (23 °C) affected T₄ concentration in two strains of laying hens. Cogburn and Freeman (1987), reported changes in T₄ blood concentrations in cockerels only after exposing them to 38 °C. Elnagar et al., (2010) reported increased T₄ concentrations for laying hens subjected to HS (35 °C). In contrast, other studies reported changes in T₄ plasma concentrations for young chickens and hens subjected to cold stress (Cogburn and Freeman, 1987; Hahn et al., 1965). Therefore, the T₄ concentration is more inconclusive for interpretation of HS results than is T₃.

Cloacal temperature and body temperature increases, until certain levels, as the environmental temperature increases (Andrade et al., 2017; Dahlke et al., 2005; Loh et al., 2004; Wilson, 1948). The ability of pullets to control and maintain thermogenesis will vary

depending on a few variables, such as environmental temperature, duration of heat exposure, genetics, and also metabolic rate (Cassuce et al., 2013; Dahlke et al., 2005; Meltzer, 1987). In an experiment, Dahlke et al. (2005), tested the resistance to heat stress between fast and slow-weight gain naked neck gene broilers, with the second group showing greater tolerance to HS and better performance when compared with fast weight gain group.

The cloacal temperatures of the pullets subject to the four environmental temperatures remained within a standard range of 41 to 42 °C (Meltzer, 1987; Wilson, 1948). In an experiment, Wilson (1948) found that cloacal temperature increases markedly when environment temperature exceeded 40.5 °C, but the cloacal temperature started to vary when the pullets were subjected to environments of 29.5 °C. These results agree with the findings in this experiment, in which cloacal temperature for the treatments TC and MiHs were similar, but the treatments at which the environmental temperatures were higher than 30 °C the birds had showed increased cloacal temperature ($P < 0.001$) (Table 5).

The cloacal temperature and T_b followed a pattern, as the environmental temperature increased the cloacal and T_b increased as well (Figure 2 and Table 4). The treatments TC and MiHs were similar for cloacal temperature, although these treatments were different from the other two treatments, MoHs and SeHs ($P < 0.001$). However, for body temperature, all treatments were different among them ($P < 0.001$) being pullets at SeHs with the highest values and the TC the lowest.

The differences found between cloacal and mean surface temperature can be due to the featherless body parts, such as comb and leg, which have more pronounced vasoconstriction and vasodilation, leading to more temperature variations (Richards, 1971; Cangar et al., 2008; Nascimento et al., 2011). According to literature the temperature variation in birds extremities, such as legs and comb, can vary up to 20 °C (Cangar et al., 2008; Richards, 1971), being the body temperature a more sensitive parameter. Also when birds are subject to high environmental temperatures one way to help heat dissipation is vasodilatation, removing the heat from the viscera to the body periphery (Souza Jr et al., 2013). In a study with laying hens, subjected to different combinations of temperature and humidity, it was concluded that the inflection temperature point, temperature in which the hens can start to increase their own temperature, was 23.89 °C for skin temperature (collected from the back) and 25.89 °C for core temperature (collected from the gizzard) both with 50% of relative humidity (Chang et al., 2018). This result agrees with the differences between cloacal and body temperature in this study and also corroborate with the insight that the core/cloacal temperature is less sensitive than the body/skin temperature. This also corroborates with the findings from Giloh et al.

(2012), who observed a stronger response for facial temperature than cloacal temperatures of male broilers subjected to 3 different environments.

In an experiment with pullets from one to 42 days of age subjected to four different thermal environments, a difference of as much as 3.3 °C between body and shank temperature was noted (Andrade et al., 2017). Giloh et al. (2012), also found an average of 2 °C difference between cloacal temperature and facial surface temperature in male broilers subjected to different environmental temperatures. Under those circumstances, it is expected that body temperature shows more variation than cloacal temperature.

CONCLUSION

In conclusion, and according to the way in which this experiment was conducted, acclimating chicks with reduced or elevated air temperatures during the brooding phase did not affect pullet productive performance (body weight, feed intake and feed conversion) or physiological response (hormonal responses, T₃ and T₄, and cloacal and body temperature) at the 17th week of age. However, the body weight of pullets during the first three weeks of growing phase were influenced by the temperatures during the brooding phase, being the birds raised in the thermal comfort heavier when compared with the birds raised in heat stress.

Pullets exposed to thermal comfort conditions (20/20 °C) and presumed mild heat stress (25/20 °C) exhibited better physiological and productive performance compared with the higher HS treatments, regardless of the brooding phase acclimation conditions. Environmental temperatures of 30/20 °C affected negatively some of the performance parameters evaluated and physiological status. Environmental temperatures of 35/20 °C negatively affected performance and physiological status of pullets regardless of their previous acclimation.

These results suggest there is no difference between 20/20 and 25/20 °C daily temperature exposure for developing pullets, and there is no effect of mild deviations in temperature from thermoneutral conditions during brooding on subsequent pullet development. However, further studies are recommended to confirm that the comfort zone for pullet development temperatures are warranted.

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CHAPTER 3. 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 two different phases; Phase I (day 1 through 6 wk of age) and Phase II (wk 7 through 17). During Phase I chicks were reared at one of three different thermal environments: thermal comfort (35 to 19 °C), mild heat stress (38 to 22 °C) or mild cold stress (28 to 17 °C). In Phase II, pullets were randomly redistributed to one of four daytime temperature treatments: 20 °C, 25 °C; 30 °C; 35 °C, all with night time temperature of 20 °C. Cloacal temperature and body surface temperature for eight parts (head, eye, comb, chest, back, wing, leg, head area, and body area) were obtained weekly from four and two birds per treatment, respectively, during Phase II. There were no effects for the interactions between the two 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 35/20 °C and 30/20 °C had the highest body parts temperatures compared with the other two 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 zone for homeothermic animals is characterized by a range of environmental temperatures, within which animals have a minimal and nearly constant energy expenditure for maintaining body temperature (**BT**) (Chang et al., 2018; Curtis, 1983). BT for birds normally varies from 41 to 42°C (Welker et al., 2008; Wilson, 1948). To maintain BT in this range, the thermoregulatory system adjusts physiological responses to increase or decrease the body heat loss (Sturkie, 1986; Taylor et al., 2014). Outside of the thermal comfort zone in situations characterized by heat stress (**HS**) or cold stress, birds also adjust their metabolism to further compensate their energy balance (Arcila et al., 2018; Chang et al., 2018; Hester et al., 2015; Nascimento et al., 2014).

Poultry are adversely affected by HS, as it can reduce their productive performance, negatively affects the bird's wellbeing, and in severe HS situations can lead to death (Al-Ramamneh et al., 2016; Arcila et al., 2018; Tao and Xin, 2003; Yanagi Junior et al., 2002). To reduce the effects of HS it is important to understand how environmental temperature is related to thermal comfort. 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 (Abreu et al., 2017; Chang et al., 2018; Edgar et al., 2013). Measuring BT is a method to assess the severity of HS (Chang et al., 2018; Unruh et al., 2017).

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 to altered or misleading results (Andrade et al., 2017; Eddy et al., 2001; Edgar et al., 2013; McManus et al., 2016; Vicente-Pérez et al., 2016). Handling birds to measure temperature can cause a bias in reading since the induced stress can change BT. Moe et al. (2017) found a drop of 2°C in the comb and eye temperature after one minute of handling. One tool which can replace the cloacal thermometer is the thermographic camera (McManus et al., 2016). This non-invasive device utilizes infrared radiant emission from a surface, and can be used at a distance from the bird, thus removing the need to handle birds (Edgar et al., 2013; Giloh et al., 2012; Metzner et al.,

2014). However, the relation between bird body parts surface temperature and cloacal temperature, and the effect of different thermal environments on this relation, have not been established.

The objective of this study was to investigate the body temperature (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 four environmental controlled chambers. Each chamber measured 3.20 m length, 2.44 m width, 2.38 m high.

The control and real-time monitoring of the temperature in each chamber was through an electronic microcontroller (Model MT-531R Plus, Full Gauge Controls, Canoas/RS, Brazil), connected with air heater (Model AB Split 1, Britania Eletrodomesticos S.A. Pirabeiraba, SC, Brazil), and an air conditioner (Model ABS 12FC 2LX, Komeco, Manaus, AM, Brazil). The air temperature and relative humidity of each chamber were recorded by dataloggers (Model HOBO U14-001, Onset, USA; specifications: temperature accuracy of $\pm 0.21^{\circ}\text{C}$ from 0° to 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 40–60% by use of an ultrasonic air humidifier (Model HUL535W, Kaz USA, Inc., Marlborough, MA). Two continuously running axial exhaust fans (Model FD08025 AMB, Ambition Technology Company, Guangdong, China), were used for the renewal of the air inside the environmental controlled chambers during the whole experimental period.

In each environmental chamber, the birds were randomly allocated to cages with dimensions of 0.50 x 0.50 x 0.50 m (length \times width \times height). Chick placement density was $140\text{ cm}^2\text{ chick}^{-1}$ for the first four weeks ($17\text{ chicks cage}^{-1}$), 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 per cage); from the 6th week through the 17th week stocking density was $357\text{ cm}^2.\text{pullet}^{-1}$ (seven pullets per cage) per industry guidelines (Lohmann, 2016). Density adjustments were accomplished by random culling. Each cage was equipped with 0.5m of linear feeder at the cage front, and one nipple drinker placed on a side midway between the front and back.

Newspapers and jug waterers were provided during the first week to assist chick starting, and an additional nipple drinker was placed until the 6th week per industry guidelines (Lohmann, 2016). All birds received feed and water ad libitum. The birds were fed a starter diet until the 6th week, and thereafter a grower ration according to Rostagno (2011), Attachment I.

The light program adopted was that recommended by the lineage manual (Lohmann., 2016). The Light (L):Dark (D) hourly schedule was 24L:0D, 16L:8D, for days 1-2, and 3-6 respectively. From the 2nd week, was reduced to 1 hour per week, until 10L:14D on the 6th week, which was maintained until the 17th week.

The research was conducted in two phases, Phase I (from day 1 until the end of the 6th week) and Phase II (from the 7th week until the end of the 17th week).

- **Phase I**

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

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

Thermal Environment	1 st week (°C)	2 nd week (°C)	3 rd week (°C)	4 th week (°C)	5 th - 6 th week (°C)
Mild cold stress	28	25	23	20	17
Literature thermal comfort	31-35	28	26	23	19
Mild heat stress	38	31	29	26	22

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

- **Phase II**

Birds from each thermal environment in Phase I were uniformly and randomly redistributed in four different environmental chambers, each one with a different thermal environment, as follows: Thermal Comfort (TC, 20/20°C); presumed Mild Heat Stress (MiHs, 25/20°C); presumed Moderate Heat Stress (MoHs, 30/20°C); presumed Severe Heat Stress (SeHs, 35/20°C). During the night (from 7:00 p.m to 7:00 a.m.) the air temperature was reduced to 20°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.

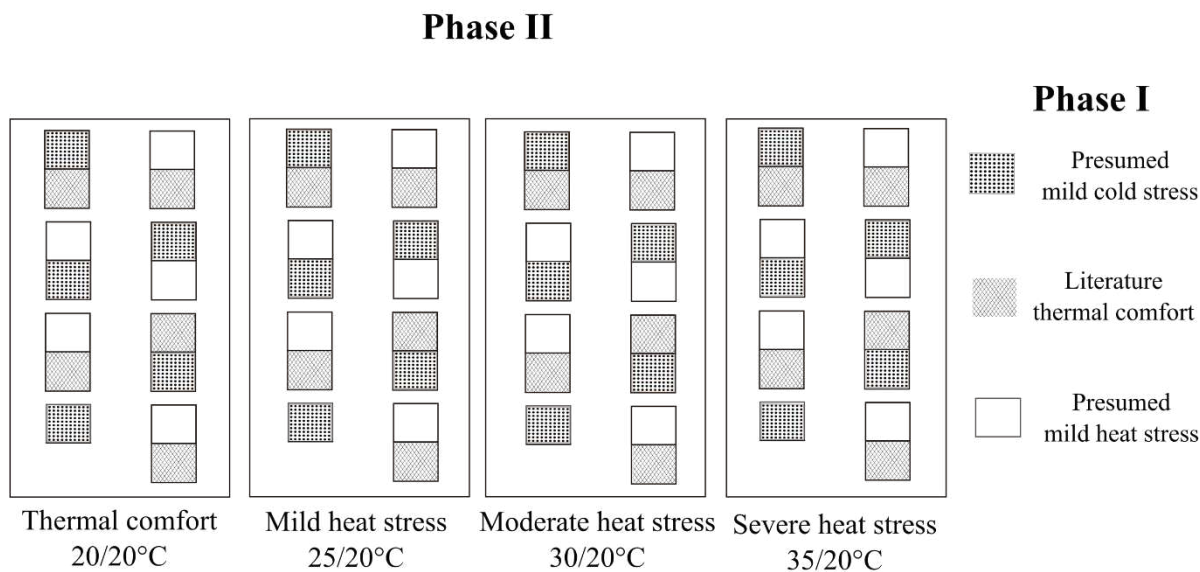


Figure 1. Experimental design of pullets' distribution in the environmentally controlled chambers during the Phase II phase, from the 7th through the 17th weeks of age.

Measurements

Cloacal temperature was measured with a clinical thermometer (Model Incoterm, Porto Alegre/RS, Brazil), with a temperature range of 32 to 43.9 °C and resolution of 0.1 °C. The thermometer was inserted into the cloaca and was read after temperature stabilization. Typically 45-60s. The cloacal temperature was collected once a week during the Phase II phase from 4 birds in each cage, corresponding to 57% of the birds in each replicate.

The infrared thermal camera (Model ThermaCAM@b60, FLIR Systems, Wilsonville, OR, USA) had a temperature range of -20 to 120°C, absolute accuracy of ± 2 °C, and resolution of 2048 x 1536 pixels. The coefficient of emissivity (ϵ) was 0.95 and kept constant based on recommendations of the manufacturer and Nääs et al., (2010). The points of interest for body temperature were analyzed with the software FLIR Tools (FLIR Systems, Inc., North Billerica, MA, USA). Pictures of two birds from each cage were taken once a week during Phase II. During the procedure, the camera was positioned 1.3 m from the pullets. From each image, the temperature of 8 distinct body parts was collected (Figure 2), divided into six points (head, comb, breast, back, wings, legs) and two area averages (head, and body without head, neck, and legs), 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.

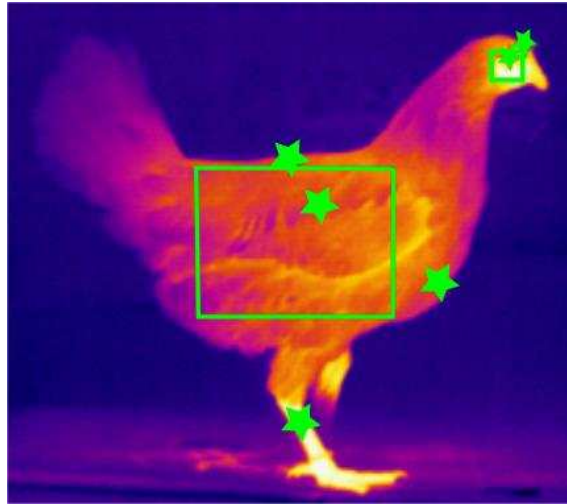


Figure 2. Side view of a pullet with the parts of interest identified with the green stars (head, comb, breast, back, wings and leg) and squares (average of the head area or body area).

Statistical Analyses

The Phase II experimental design was completely randomized in a split-plot arrangement with four treatments (Phase II temperatures - TC; MiHs; MoHs; SeHs) in the plots and three subplots (birds reared under Phase I phase temperatures). There were five replicates (cages) per treatment x subplot combination. The effect for each wk of Phase II treatments, Phase I treatment, and their interactions on cloacal and the body parts temperature was performed using analysis of variance (SAEG, 2007). Differences between group means were compared by Tukey's test, with a 5% confidence level ($P < 0.05$) for 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 7th through the 17th week for each chamber during Phase II ranged from 0.2 to 0.6 °C warmer than the nominal treatment values

(standard deviation 0.7 to 0.9 °C), and relative humidity varied between 63 and 69% (standard deviation 6 to 8%).

Weekly mean cloacal temperatures of pullets subjected to the four 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 wk 7 through wk 17 of age. Pullets subjected to 35/20 °C air temperature during the 7th through 17th wk had consistently higher cloacal temperature ($P < 0.05$), regardless of the previous temperature exposure, Table 2. Pullets subjected to 20/20 °C and 25/20 °C treatments had lower cloacal temperatures than the other two treatments ($P < 0.05$), and were similar for eight of the 11 wk evaluated ($P < 0.05$), Table 2.

Table 2. Average values of cloacal temperature for laying pullets from the 7th to 17th weeks of age submitted to four different thermal environments

Age (week)	Thermal Environments ¹				SEM ²
	TC	MiHs	MoHs	SeHs	
	Cloacal temperature (°C)				
7	41.5 ^c	41.6 ^c	41.9 ^b	42.3 ^a	0.16
8	41.6 ^c	41.5 ^c	42.0 ^b	42.3 ^a	0.12
9	41.4 ^d	41.6 ^c	41.9 ^b	42.2 ^a	0.14
10	41.5 ^d	41.6 ^c	41.8 ^b	42.1 ^a	0.13
11	41.5 ^c	41.5 ^c	41.7 ^b	42.1 ^a	0.10
12	41.4 ^c	41.3 ^c	41.6 ^b	41.8 ^a	0.11
13	41.4 ^c	41.4 ^c	41.6 ^b	41.8 ^a	0.12
14	41.5 ^{bc}	41.4 ^c	41.6 ^b	42.0 ^a	0.10
15	41.4 ^d	41.3 ^c	41.7 ^b	42.0 ^a	0.11
16	41.1 ^c	41.2 ^c	41.5 ^b	42.0 ^a	0.12
17	41.1 ^c	41.1 ^c	41.4 ^b	41.8 ^a	0.08

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

¹Abbreviations: TC: Thermal Comfort - 20/20 °C; MiHs: Mild Heat Stress - 25/20 °C; MoHs: Moderate Heat Stress - 30/20 °C; SeHs: Severe Heat Stress - 35/20 °C

²SEM: Standard error of mean

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 R^2 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 standard error of regression and R^2 coefficient.

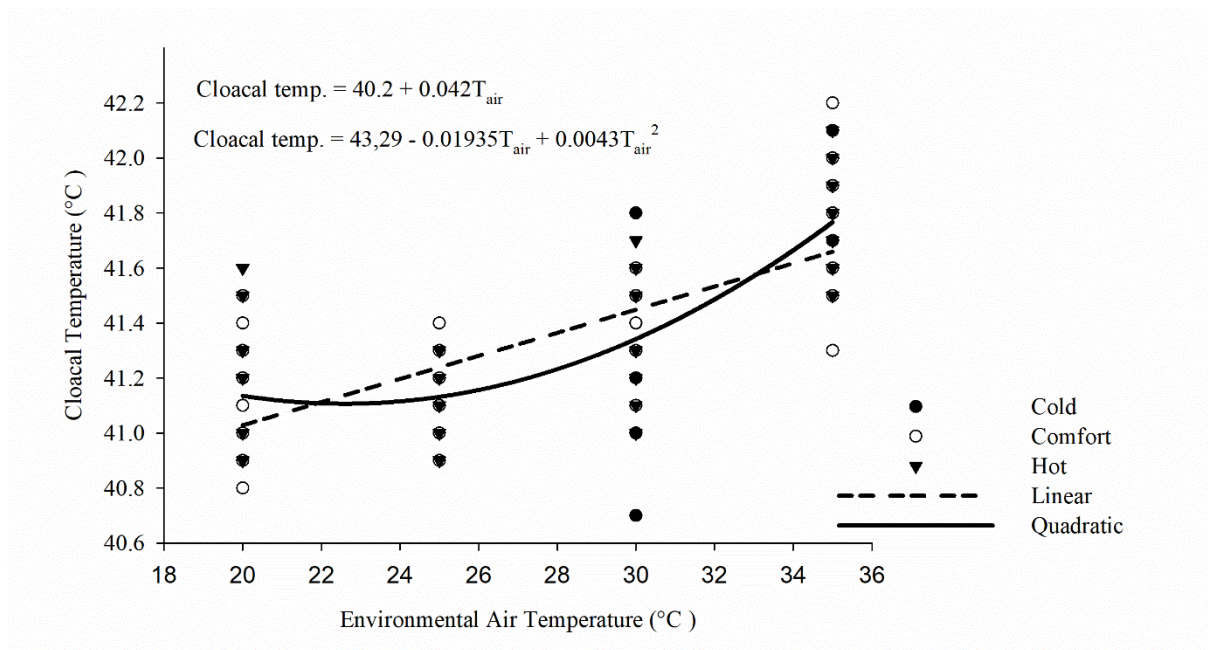


Figure 3. The effect of environmental air temperature on cloacal temperature at the 17th wk of age. Different symbols represent conditions for chicks raised through wk 6 (Table 1). Linear and quadratic regression models between cloacal temperature and environmental air temperature; the standard error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

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

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 (35/20 °C) and MoHs (30/20 °C) had higher surface temperatures compared to the other two treatments ($P < 0.05$). No statistical difference was observed between the treatments TC and MiHs. The leg was the body part which had the highest surface temperature in all treatments, and the chest the lowest.

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

Thermal Environments ¹	Body Part Surface Temperature Measurements (°C)								
	Head	Eye	Comb	Chest	Back	Wing	Leg	Head Area	Body Area
TC	39.4 ^b	40.7 ^b	42.5 ^b	34.1 ^b	34.7 ^b	35.0 ^b	43.9 ^b	41.9 ^b	35.2 ^b
MiHs	40.1 ^b	41.0 ^b	43.4 ^b	35.1 ^b	36.0 ^b	36.0 ^b	43.5 ^b	42.4 ^b	35.8 ^b
MoHs	46.4 ^a	45.8 ^a	47.6 ^a	43.5 ^a	44.1 ^a	43.6 ^a	47.9 ^a	47.0 ^a	43.7 ^a
SeHs	47.0 ^a	46.0 ^a	47.5 ^a	44.1 ^a	45.1 ^a	44.7 ^a	48.1 ^a	47.4 ^a	45.0 ^a
SEM ²	1.54	0.72	1.09	0.71	0.62	0.69	0.85	0.73	0.58
P-value (Phase II)	***	***	***	***	***	***	***	***	***
P-value (Phase I)	ns	ns	ns	ns	*	ns	ns	ns	ns
P-value (Interactions)	ns	ns	ns	ns	ns	ns	ns	ns	ns

^{a-c}Means within a column with different superscripts differ significantly ($p < 0.05$)

¹TC: Thermal Comfort - 20/20 °C; MiHs: Mild Heat Stress - 25/20 °C; MoHs: Moderate Heat Stress - 30/20 °C; SeHs: Severe Heat Stress - 35/20 °C

²SEM: Standard error of mean

* = significant at ($p \leq 0.05$); *** = significant at ($p \leq 0.001$); ns = non-significant

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 7th wk and the comb, $r = 0.70$, at the 17th wk of age.

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

Body Part	7 th week	17 th 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

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.

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 week of age

	Body part	Equation: CT = a + b *(body part surface temperature)		Se (regression) °C	adj. R ²
		a (Se _a) °C	b (Se _b)		
Phase I Presumed cold stress	Head (HE)	38.381 (0.469)	0.067 (0.010)	0.156	0.67
	Eye (EY)	37.840 (0.722)	0.0802 (0.016)	0.185	0.54
	Comb (CO)	38.444 (0.677)	0.0640 (0.015)	0.197	0.48
	Chest (CH)	39.410 (0.287)	0.0489 (0.007)	0.149	0.70
	Back (BK)	39.283 (0.281)	0.0510 (0.006)	0.140	0.73
	Wing (WI)	39.233 (0.298)	0.0527 (0.007)	0.144	0.72
	Leg (LE)	37.157 (0.775)	0.0909 (0.017)	0.173	0.60
	Body Area (BA)	39.174 (0.282)	0.0539 (0.007)	0.135	0.75
	Head Area (HA)	37.590 (0.659)	0.0837 (0.015)	0.167	0.62
Phase I Thermal Comfort	Head (HE)	39.439 (0.532)	0.0440 (0.012)	0.214	0.38
	Eye (EY)	38.337 (0.609)	0.0695 (0.014)	0.183	0.55
	Comb (CO)	37.225 (0.972)	0.0902 (0.021)	0.199	0.47
	Chest (CH)	39.763 (0.295)	0.0402 (0.008)	0.174	0.60
	Back (BK)	39.706 (0.328)	0.0411 (0.008)	0.181	0.56
	Wing (WI)	39.571 (0.334)	0.0445 (0.008)	0.175	0.59
	Leg (LE)	37.283 (0.757)	0.0887 (0.017)	0.174	0.59
	Body Area (BA)	39.545 (0.330)	0.0451 (0.008)	0.172	0.60
	Head Area (HA)	37.852 (0.739)	0.0780 (0.165)	0.187	0.53
Phase I Presumed heat stress	Head (HE)	38.863 (0.432)	0.0582 (0.010)	0.190	0.63
	Eye (EY)	37.320 (0.790)	0.09315 (0.018)	0.206	0.57
	Comb (CO)	37.717 (0.824)	0.0810 (0.18)	0.223	0.49
	Chest (CH)	39.293 (0.356)	0.0524 (0.009)	0.190	0.64
	Back (BK)	39.262 (0.358)	0.0524 (0.009)	0.188	0.64
	Wing (WI)	39.261 (0.361)	0.0530 (0.009)	0.190	0.63
	Leg (LE)	36.954 (0.945)	0.0961 (0.020)	0.217	0.52
	Body Area (BA)	39.159 (0.368)	0.0552 (0.009)	0.186	0.65
	Head Area (HA)	37.450 (0.665)	0.0878 (0.015)	0.189	0.64

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

CT = cloacal temperature

Se = standard error of the coefficients (Se_a, Se_b) and regression, Se (regression)

DISCUSSION

The main goal of Phase I was to acclimate the pullets to mild cold stress, thermal comfort, or mild heat stress conditions during the first six wk of age. Acclimation promotes behavioral

and physiological alterations to compensate for negative effects of a single stressor acting alone. For example, if birds are raised in high temperature during early periods of life they may be able to better cope with HS later in life (Curtis, 1983; Yahav and Hurwitz, 1996). However, the Phase I temperatures range may not have been great enough to acclimate the birds, as 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). In a study with broilers conducted by Sykes and Fataftah (1986), it was observed that heat tolerance may be reduced over time post-exposure. 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. Abdelqader 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 days. Giloh et al. (2012) raised broilers in a thermoneutral environment and the average cloacal temperature was 41.3 °C, similar to this experiment. At wk 17 of this study, the increase in cloacal temperature above TC was 0, 0.3 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, 25, 30 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 the higher broiler metabolism when compared to the 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 three 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 temperature with increasing environmental air temperature (0.042 °C/°C). However, when evaluated by the quadratic line, it is possible to infer that for the environmental air temperatures of 20 and 25 °C the cloacal temperature tended to be similar. This agrees with the results found for cloacal temperature measurements from wk 7 to 17 (Table 2). The same results can be observed in Chang et al. (2018) in which laying hens showed an increased surface temperature with the increase in environmental air temperature, especially for temperatures above 25 °C.

One of the primary mechanisms for decreasing body temperature is vasodilatation, which increases the blood flow to the skin, moving the heat from the viscera to the periphery (Chang et al., 2018; Nascimento et al., 2014; Souza Jr et al., 2013). 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 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.

Souza Jr et al. (2013) and Nääs et al. (2010) also found higher surface temperatures of featherless areas of laying hens compared with feathered areas. The leg temperature had the highest temperature among the observed parts. Souza Jr et al. (2013) attributed this result to the legs' role in the exchange of sensible heat. Also, Nääs et al. (2010) found a high correlation (0.8) between featherless areas with 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 broilers with Nääs et al. (2010) and Nascimento et al. (2011), Naked Neck layers with Souza Jr et al. (2013), layers with Zhao et al. (2013), pullets with Hester et al. (2015), quails with Santos et al. (2019) and 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 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 (Barros et al., 2016; McManus et al., 2016; Montanholi et al., 2008; Nascimento et al., 2014). 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 thermal comfort of the animals inside facilities, so a non-invasive 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, 0.82-0.83, 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 0.77-0.75, but this correlation value can vary depending on the species; Vicente-Pérez et al. (2016) found different correlations when studying ewes, 0.76-0.72, 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 of the regression models to predict the cloacal temperature from body parts temperature were between 0.38 to 0.75 (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 heat stress. 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 R^2 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. (2014) showed an R^2 of 0.74-0.72 to predict featherless surface temperature in broilers, and an R^2 of 0.68-0.67 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 temperature on groups of birds without direct cloacal temperature measurement.

CONCLUSION

High environmental air temperatures (30 and 35 °C for 12h/day) during wk 7 through 17 increased cloacal and body parts temperatures ($P < 0.05$ and $0 < 0.001$, respectively). A quadratic relation between cloacal and daily environmental air temperature was noted.

Brooding temperatures (mild cold stress, thermal comfort, or mild heat stress) had no effect on the subsequent cloacal and body parts temperatures (with exception of back temperature, $P < 0.05$) for any of wk 7 through 17 of growing phase.

A positive correlation between cloacal temperature and body part temperature was found ($0.70 \leq r \leq 0.82$ at wk 17), and regression models to predict cloacal temperature from different body parts temperatures (± 2 standard errors < 0.4 °C) can be used as a simple alternative for estimating cloacal temperature..

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CHAPTER 4. FINAL SUMMARY

This thesis attempts to cover concerns about thermal comfort zones and how the environment affects the birds during the pre-laying period. Also, it was important to know the effects of acclimation in the early rearing period on the growing period, when the egg-type chicks have their major body development. To answer these questions were used a combination of physiological and performance parameters.

According to the way in which this experiment was conducted, acclimating chicks with reduced or elevated air temperatures during the brooding phase did not affect pullet productive performance or physiological response.

The comfort zone for pullets during the 7th to 17th weeks of age was the thermal comfort conditions (20/20 °C) presumed by literature; but also, can be expanded to 25/20 °C, as the pullets exhibited better physiological and productive performance compared with the other two higher temperature treatments. Environmental temperatures of 30/20°C affected negatively some of the performance parameters evaluated and physiological status. Furthermore, temperatures of 35/20 °C negatively affected performance and physiological status of pullets regardless of their previous acclimation and can be characterized as causing heat stress in pullets during the growing phase.

Equally important, this study demonstrated the potential application of the use of thermal imaging in determining the thermal status of pullets in different thermal environments, by measuring pullets body parts.

Attachment I: Composition and nutritional values of the ingredients used in the formulation of the feed for pullets from the 7th through the 17th week of age

Ingredients	Quantities (%)
Corn	67.1896
Soybean meal (45%)	15.5390
Wheat bran.	14.1814
Limestone	1.1997
Dicalcium phosphate	1.1690
Iodized Salt	0.3264
Vitamin premix	0.1000
Choline chloride	0.1000
Mineral premix	0.1000
Anticoccidial	0.0550
Antibiotic	0.0300
Antioxidant ¹	0.0100
Calculated nutrient content	
Crude protein (%)	15.0000
Metabolizable energy (Mcal/kg)	2.9000
Linoleic acid (%)	1.6400
Calcium (%)	0.8160
Chlorine (%)	0.2444
Crude fiber (%)	3.4810
Available phosphorus (%)	0.3510
Fat (%)	3.0837
Potassium (%)	0.6336
Sodium (%)	0.1550
Digestible phenylalanine + thyroxine (%)	1.1101
Digestive arginine (%)	0.8670
Digestible histidine (%)	0.3849
Digestible isoleucine (%)	0.5255
Digestible leucine (%)	1.2496
Digestible lysine (%)	0.6024
Digestible methionine + cystine (%)	0.4549
Digestible threonine (%)	0.4805
Digestible tryptophan (%)	0.1520
Digestible valine (%)	0.6130
Digestible glycine + serine (%)	0.6906

¹Butyl Hydroxy Toluene- BHT