UNIVERSIDADE FEDERAL DE UBERLÂNDIA

JOÃO PAULO RODRIGUES BUENO

PHYSIOLOGICAL RESPONSE, ENDOCRINE AND PERFORMANCE OF BROILERS UNDER CYCLIC HEAT STRESS

[Respostas fisiológica, endócrina e desempenho de frangos sob estresse cíclico de calor]

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Tese apresentada ao Programa de Pós-Graduação em Ciências Veterinárias/Doutorado da Faculdade de Medicina Veterinária da Universidade Federal de Uberlândia, como requisito para obtenção do Título de Doutor em Ciências Veterinárias.

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UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Secretaria da Coordenação do Programa de Pós-Graduação em Ciências Veterinárias
BR 050, Km 78, Campus Glória , Uberlândia-MG, CEP 38400-902
Telefone: (34) 2512-6811 - www.ppgcv.famev.ufu.br - mesvet@ufu.br

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Ata da defesa de **TESE DE DOUTORADO** junto ao Programa de Pós-Graduação em Ciências Veterinárias da Faculdade de Medicina Veterinária da Universidade Federal de Uberlândia.

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Reuni-se na sala 216, bloco 1CCG - Campus Glória da Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Ciências Veterinárias, assim composta: Professores(as) Doutores(as): **EVANDRO DE ABREU FERNANDES** – UNIVERSIDADE FEDERAL DE UBERLÂNDIA; **ROBSON CARLOS ANTUNES** – UNIVERSIDADE FEDERAL DE UBERLÂNDIA, **ANA CAROLINA PORTELLA SILVEIRA** - INSTITUTO FEDERAL DO TRIÂNGULO MINEIRO, **CRISTIANE FERREIRA PRAZERES MARCHINI** - UNIVERSIDADE DE FRANCA e **MARA REGINA BUENO DE MATTOS NASCIMENTO** orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da comissão Dr./Dra. MARA REGINA BUENO DE MATTOS NASCIMENTO concedeu a palavra ao(a) candidato(a) para uma exposição do seu trabalho, contando com o tempo máximo de 50 minutos. A seguir o(a) senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores, que passaram a arguir o(a) candidato(a), durante o prazo máximo de (30) minutos, assegurando-se ao mesmo igual prazo para resposta. Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Comissão Julgadora, em sessão secreta, considerou o(a) candidato(a):

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PROF. DR. EVANDRO DE ABREU FERNANDES

PROF. DR. ROBSON CARLOS ANTUNES

PROFA. DRA. ANA CAROLINA PORTELLA SILVEIRA

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familiares e amigos.
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para minha formação profissional e pessoal.

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“Duas estradas se bifurcaram no meio da minha vida.
Ouvi um sábio dizer.
Peguei a estrada menos usada.
Se eu tivesse tomado um atalho, uma rua estreita qualquer.
Que tipo de pessoa eu teria me tornado? Não sei.
Mas [não] gostaria de saber.
Pelo retrovisor, vejo todas as pessoas que eu poderia ter sido.
E não fui.
E isso fez toda a diferença cada noite e cada dia.”

(Larry Norman; O Teatro Mágico).

ABSTRACT

The objective of this study was to evaluate the effects of cyclic heat stress on the serum levels of thyroid hormones (T_3 and T_4), the thyroid histology, the performance, the temperatures (skin, cloaca, and litter), the feathering and the incidence of pododermatites in two broiler strains (Cobb Slow™ and Hubbard Flex™), exposed to different heat protocols. It is possible to conclude that exposure to cyclic heat stress for 3 h from day 14 to day 42 did not affect performance, thyroid histology or the serum levels of T_3 and T_4 and there is no change in feathering and incidence of pododermatites. Cobb Slow™ broilers had the best live weight values at slaughter and higher cloacal temperature. With increasing age, T_3 levels decrease and T_4 levels increase. The temperatures of the broilers and litter increase as the ambient temperature increases.

Keywords: Feathering. Pododermatites. Poultry. Skin temperature. Thyroidal hormones.

RESUMO

Avaliou-se os efeitos do estresse cíclico de calor sobre a concentração dos hormônios tireoidianos (T_3 e T_4), a histologia da tireoide, o desempenho, as temperaturas (superficial, cloacal e de cama), o empenamento e a incidência de pododermatites em frangos de corte, de duas linhagens (Cobb Slow® e Hubbard Flex®), mantidos em diferentes tempos de exposição ao calor. Concluiu-se que o estresse cíclico por calor por até 3 horas diárias dos 14 aos 42 dias de idade não altera a histologia da tireoide nem a concentração dos hormônios tireoidianos das aves, além disso, não prejudica o seu desempenho e não há alteração no empenamento e incidência de pododermatites. Ao abate, frangos Cobb apresentaram melhor peso vivo que frangos Hubbard e maior temperatura cloacal. Com a idade ocorre redução da concentração de T_3 e aumento de T_4. As temperaturas da ave e da cama aumentam conforme aumenta a temperatura do ambiente.

Palavras-chave: Avicultura. Empenamento. Temperatura Superficial. Hormônios tireoidianos. Pododermatites.

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SUMMARY

Chapter 1 – GENERAL CONSIDERATIONS

(Wrote in accordance with the rules of Biblioteca-UFU)

1 INTRODUCTION

Despite the crisis due to the political and economic scenarios in Brazil, the importance of the poultry industry in the country continues to increase. In 2017, the broiler chicken production reached 13.05 million tons, while *per capita* consumption was 42.07 kg and exports were 4.3 million tons. Currently Brazil holds the second place as producer and first as exporter of broilers in the world (ABPA, 2018).

However, the significant metabolism of these birds means that thermoregulation is affected when they are exposed to high temperatures and humidity levels (BOSCHINI et al., 2011). The optimal temperature to raise broilers varies with the age of birds. For instance, in the first week of life, the best results are reached farming broilers at 32-35 ºC. In the second, third, fourth, and fifth weeks, the ideal temperature ranges are 29-32 ºC, 26-29 ºC, 23-26 ºC, and 20-23 ºC, respectively. In the 6^{th} week of age, the best temperature to farm broilers is 20 ºC. The relative humidity throughout the broiler farming cycle is in the 60-70 % range. Due to the characteristics of climate in Brazil, these conditions are hard to observe (ABREU and ABREU, 2011). Moreover, although most studies have revealed that heat stress is observed in broilers raised under constantly high temperatures, it has been shown that the phenomenon acquires a cyclic character when broilers are exposed to natural conditions. While daytime temperatures are high, values decrease at night, ultimately influencing the levels of heat stress in these broilers (MARCHINI et al. 2007).

Other important variables that have to be taken into account are age of broilers and their genetic constitution. With age, broilers become more sensitive to heat due to the sharp increase in body weight. In addition, different broiler strains have different responses to heat stress, as observed for the most commercially interesting strains, namely Cobb and Hubbard (MARTINS et al., 2014; NASCIMENTO et al., 2014, API et al., 2017).

In the effort to maintain heat homeostasis, a number of strategies are at play in broilers under heat stress. One of such strategies is the changes in thyroid morphology and production of thyroidal hormones (PARCHAMI and FATAHIAN DEHKORDI, 2012; GONZÁLEZ and SILVA, 2017). Performance and feathering are affected (NASCIMENTO et al., 2011; OBA et al., 2012), and skin and cloacal temperatures have been shown to rise (NASCIMENTO et al., 2015). In addition, high environment temperatures may lower the quality of the litter, increasing the risk of pododermatitis (SHEPHERD and FAIRCHILD, 2010).

2 THE EFFECTS OF HEAT STRESS ON THE THYROID GLAND, THYROIDAL HORMONES, PERFORMANCE, TEMPERATURES OF SKIN, CLOACA, AND LITTER, AND FEATHERING AND INCIDENCE OF PODODERMATITIS IN BROILERS

2.1 THERMOREGULATION IN BROILERS

The maintenance of the temperature of the birds is a function of mechanisms of production and loss of heat. During heat stress, for example, the temperature of the broiler increases and physiological processes are activated for two main purposes: increase the heat dissipation to the environment and reduce the production of metabolic heat (FURLAN, 2006).

To increase the heat dissipation of the tissues, where it is produced, to the surface of the body, where it is dissipated, the bird uses mechanisms of sensible and latent heat loss. Sensitive heat dissipation occurs through non-evaporative mechanisms, i.e: radiation, convection and conduction. Thus, the broiler to increase the heat dissipation, seeks to maximize the body surface area by squatting, keeping the wings away from the body, induces feathers erection and increased blood flow to peripheral tissues not covered with feathers. In this way, the bird causes a sensible heat exchange for the environment, because the blood has, in a similar way to water, a great capacity to carry heat, from the tissues to the body surface, so that there is heat exchange with the environment (MACARI e FURLAN, 2001; FURLAN, 2006).

Respiratory evaporative cooling is one of the most important means of heat loss of broilers at high temperatures. This is because, broilers have the ability to increase respiratory rate by up to 10 times and thus increase heat loss in the respiratory tract. However, the increase in respiratory rate generates more energy by contracting the muscles, producing more heat, being able to determine severe hyperthermia frames for broiler chickens. In addition, as a consequence of the high respiratory rate, the chicken can develop disturbances of the acid-base balance called respiratory alkalosis (increased blood pH). Thus, heat loss through the respiratory evaporative process represents an important path of heat dissipation at high ambient temperatures, because the sensitive loss is greatly reduced; however, it may generate undesirable frames (BORGES et al., 2003; FURLAN, 2006)

Therefore the environmental condition should be managed, as far as possible, to avoid a negative effect on the productive performance of broilers; since they can affect the metabolism (production of body heat in low temperatures and dissipation of body heat in high temperatures), with a consequent effect on animal production and the incidence of metabolic diseases (SEVEGNANI et al., 2005; FURLAN, 2006; OBA et al., 2012; GONZÁLEZ and SILVA, 2017).

2.2 THE THYROID GLAND

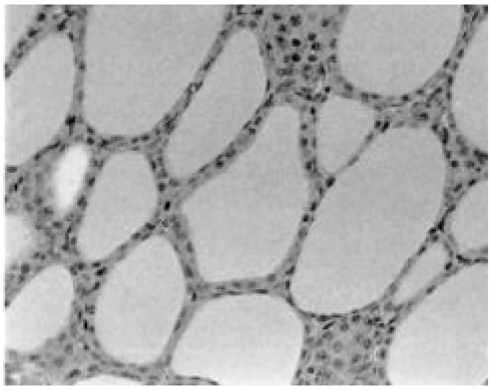
Present in all vertebrates and formed by two brownish red lobes, the thyroid is located on the floor of the pharynx laterally on the trachea, below the larynx. The thyroidal follicles are the gland's functional unit, and are surrounded by epithelial or follicular cells. The follicles store the colloid, that is, the secretion containing the thyroglobulins, or thyroidal hormones 3,5,3'-triiodothyronine (T_3) and 3,5,3',5'-tetraiodothyronine (T_4) and their precursors, monoiodotyrosine (MIT) and diiodotyrosine (DIT) (LOPES, 2002; MARIETTO-GONÇALVES et al., 2006; GONZÁLEZ and SILVA, 2017).

Under normal conditions, the lining epithelium of the thyroid is cuboidal in shape. Nevertheless, it takes on a columnar structure when the gland's activity is high. In addition to the parenchyma, the parafollicular cells are also located between follicles. These cells are also called C cells, and produce calcitonin. The colloid is produced by the follicular cells (GONZÁLEZ and SILVA, 2017).

Figure 1 shows the thyroid structures of a healthy broiler raised under thermal comfort conditions. Approximately spherical follicles of different sizes are highlighted, lined with cuboidal cells containing colloid (RACHID et al., 2001).

The thyroid is the only gland in the organism that stores iodine, since it can uptake large amounts of the element, incorporating it to the hormones it produces. Iodine uptake by the gland is promoted by the thyroid-stimulating hormone (TSH) and may be inhibited when levels of the element are high (GONZÁLEZ and SILVA, 2017).

Figure 1: Histology of the thyroid gland of broilers, HE, 100x



Source: Rachid et al. (2001)

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In the production of thyroidal hormones T_3 and T_4, iodine first forms MIT and DIT. Briefly, T_4 former is generated by the bonding of two DIT molecules, while T_3 is formed by the bonding of one DIT and one MIT molecule. The activity of TSH induces the secretion of these hormones into circulation (LOPES, 2002; GONZÁLEZ and SILVA, 2017).

Also called thyrotropin, TSH is secreted by the adenohypophysis. However, this phenomenon occurs only through the mediation of the thyrotropin-releasing hormone (TRH), which is produced in the hypothalamus and transported by the hypothalamic-hypophyseal portal circulation (GONZÁLEZ and SILVA, 2017).

It is known that thyroidal activity is associated with the temperature which broilers are exposed to in the environment. Environment temperature has been shown to alter the histological structure of the gland and, therefore, the secretion of the main hormones it produces (RACHID et al., 2001; MARIETTO-GONÇALVES et al., 2006; GONZÁLEZ and SILVA 2017).

According to Parchami and Fatahian Dehkordi (2012), histological changes in the thyroid in broilers are easily detectable, since the structure of the gland in these animals is very similar to that of other vertebrates in homeostasis. Marietto-Gonçalves et al. (2006) demonstrated that changes in the thyroid occur in animals given low iodine diets, not only environment temperature.

2.3 THE THYROIDAL HORMONES

In numerous animal species, two systems are responsible for the integration of metabolism in different organs, namely the nervous system, which operates through neurotransmitters, and the endocrinal system, whose messengers are the hormones transported by blood to the respective active sites. Of the four main groups of hormones (peptides, steroids, amines, and eikosanoids), the amines group includes iodothyronines, which are derived from the amino acid tyrosine and produced by the thyroid. However, their action mechanism is similar to that of steroid hormones (Table 1) (GONZÁLEZ and SILVA, 2017).

Table 1: Summary of thyroidal hormones

Hormone	Secreting organ	Target organ	Main role
Thyroxine	Thyroid	All cells	Increase metabolism
Triiodothyronine	Thyroid	All cells	Increase metabolism
Calcitonin	Thyroid	Bones	Reduce bone resorption
Calcitonin	Thyroid	Kidney	Increase Ca excretion

Characteristics	Thyroidal
Feedback	Yes
Biosynthesis	Post-translation change
Storage	Weeks
Secretion	Proteolysis
Binding proteins (plasma)	Yes
Half-life	Days
Receptors	Nucleus
Action mechanism	Transcription regulation

Source: Adapted from González and Silva (2017).

T_3 and T_4 are highly important in the control of metabolic processes in broilers, influencing thermogenesis and body constitution. As a response to high temperatures, broilers reduce the generation of metabolic heat resorting to endocrinal mechanisms such as the decrease in circulating thyroidal hormones, which eventually improves heat tolerance (DAHLKE et al., 2005).

Thyroidal hormones, whose composition is lipophilic, are transported in blood by specific proteins, such as thyroxine-binding globulin (TBG). This binding prevents the diffusion of the hormones through tissues, protecting these hormones from enzyme action. For this reason, the free form and the bound form of the hormone have to be in equilibrium, since, in order to enter target cells, they have to be in the free form. In broilers, the half-life of thyroxine is shorter than in mammals, while TBG exhibits comparatively poor binding potential. This phenomenon is mediated by albumin, which has lower binding potential, meaning that thyroxine is deployed more quickly (LOPES 2002; GONZÁLEZ and SILVA, 2017).

Like steroids, thyroidal hormones can cross the cytoplasmic membrane, and its receptors are located in the cell nucleus. For this reason, the interaction between the hormone and its receptor alters gene transcription. Since these hormones have to cross the membrane, the cytosol and then reach the nucleus, their action mechanism is slow. Another factor is the time required to synthesize mRNA in the nucleus and the subsequent synthesis of proteins in ribosomes (GONZÁLEZ and SILVA, 2017).

It should be stressed that T_4 accounts for 90% of the amount of hormones secreted (which works as some kind of reserve), while 10% is T_3, which is biologically active. Therefore, T_4 has to undergo deiodination to form T_3, in a process that takes place in most peripheral tissues. The half-life of T_4 is in the order of days, while that of T_3 is estimated at a few hours, which explains why T_3 titers are lower in plasma, compared with T_4 (LOPES, 2002; AZEREDO, 2004; GONZÁLEZ and SILVA, 2017).

T_3 is the metabolically active thyroidal hormone, and plays an essential role in energy metabolism. Any marked change in thyroidal activity, whether it is hyperthyroidism or hypothyroidism, affects metabolic rate (ABDEL-FATTAH et al., 2008). In young animals, T_4 levels are higher than in adults, suggesting that they are functionally hyperactive, since thyroidal hormones are associated with growth and development (LOPES, 2002; GONZÁLEZ and SILVA, 2017).

Carew et al. (2003) obtained T_3 and T_4 levels of 2.82 ng/mL and 3.98 μg/mL, respectively, in 22-day-old Hubbard broilers given feed *ad libitum*. Similar results were reported by Carew et al. (2005) in an experiment carried out under the same conditions (T_3 = 2.65 ng/mL and T_4 = 3.21 μg/mL).

As a rule, T_4 levels vary less than T_3, as shown by Abdel-Fattah et al. (2008). The authors used diets supplemented with organic acids and observed high levels of T_3, while those of T_4 did not vary. The reason for these results is the fact that T_4 is converted into T_3 and that, the higher the conversion rate, the better the broiler immune status. In addition, fat deposition is lower using these diets.

In a study with Cobb broilers of different ages (1-21 days, ± 35 ºC and 22-42, ±32 ºC) raised at constantly high temperatures, Souza et al. (2011) observed T_3 values on days 21 and 42 of 0.31 ng/mL and 0.32 ng/mL, respectively.

In a study with fast and slow growth rate broilers on days 21, 35, and 42 of age, Dahlke et al. (2005) observed T_3 were lower at high temperatures compared to low ones on days 21 and 42. On day 42, the slow growth strain had higher levels of T_3. In turn, T_4 levels were lower in the fast growth strain at high temperature, compared with the slow growth strain. Broilers raised at intermediate and low temperatures had higher T_4 levels.

When broilers are exposed to low temperatures, which may occur in the first week of age, T_4 levels decrease, while T_3 levels rise. Levels of both hormones drop with increasing age of broilers (LUGER et al., 2001).

Sohail et al. (2010) exposed 22- and 42-day-old at 35 ºC for 8 h a day. Levels of T_3 and T_4 decreased in association with higher cortisol levels. Independently, T_4 levels were always higher than those of T_3, and the decrease in T_3 levels was more pronounced than the drop in T_4 levels.

As a rule, any stressing situation, whether associated with the environment or not, alters the response of the thyroid in different ways, since glucocorticoids that inhibit thyroid activity are released, affecting hormone production (GONZÁLEZ and SILVA, 2017).

It is also known that, since T_3 and T_4 are associated with growth of broilers, the factors that affect levels of these hormones also influence performance, since these two characteristics are closely associated (OSORIO et al., 2011).

2.4 PERFORMANCE OF BROILERS

Heat stress is one of the main factors behind economic losses in intensive broiler farming, especially in the last growth stage (FURLAN, 2006). It is known that these broilers adapt to high temperatures when the maximum temperature at night is 25ºC, since it enables them to recover from the heat stress faced during the day (BALNAVE, 2004).

Oba et al. (2012) discovered that environment temperature strongly influences the production characteristics of broilers. The worst values of weight gain, feed intake, feed conversion, and viability were observed when broilers were raised at high temperatures, between 32 ºC and 35 ºC. This temperature setting induce broilers to consume less feed in the effort to decrease the metabolic heat generation and maintain thermal balance, which translates as lower weight gain, poor feed conversion, and higher mortality rates (SARTORI et al., 2001; TINÔCO, 2001; MEDEIROS et al., 2005).

Oliveira Neto et al. (2000) observed that environment temperature affects weight gain in Hubbard broilers on days 22 to 42 of age. Weight gain was 16% lower in broilers raised under heat stress (mean temperature of 32.3 ± 0.31 ºC, relative humidity of 60 ± 1.59%, and black globe humidity index (BGHI) of 71 ± 1.01). The authors also reported poor feed conversion values.

Medeiros et al. (2005) observed that in hot environments, that is, those with BGHI between 78 and 88, increasing the temperature from 26 ºC to 36 ºC and relative humidity from 34% to 76% at the same time that air circulation is decreased from 2.4 m/s to 0.6 m/s makes the environment less comfortable to broilers between days 22 and 42 of age. Under these conditions, feed intake decreases by 43.37 g/day, from 123.15 g/day to 79.78 g/day, to reduce metabolic heat generation, which consequently decreases weight gain by 40.01 g/day (from 65.10 g/day to 25.00 g/day).

Another study discovered that Avian Farms of 22 to 42 days of age had increased weigh gain when farmed at temperatures of up to 24.4 ºC. Nevertheless, when these broilers were raised at 32 ºC, weight gain is 21.3% lower than that of broilers grown at 25 ºC (OLIVEIRA et al., 2006a).

For Lana et al. (2000), temperature influences feed intake and live weight of Hubbard broilers, though feed conversion remained unaffected. The authors also observed that, when the strain was farmed at high temperature (between 25.9 ºC and 36 ºC, mean temperature of 31ºC) live weight of these broilers from days 1 to 42 of age was 15% lower than that of the control group (between 20.1 ºC and 30.2 ºC, mean temperature of 25.1 ºC). Feed intake decreased by a similar value (15%), which meant that feed conversion did not vary across the two treatments.

Oliveira et al. (2006b) observed that high temperatures affect performance of broilers from day 1 to 49 of age, and that this effect is more pronounced when relative humidity values rise. Lana et al. (2000) added that, when temperatures are equal to or below 35 ºC and relative humidity is below 55%, the effect on body temperature is not significant. In other words, when environment temperature is high, relative humidity values should be kept low.

Marchini et al. (2018) reported that 1-h heat stress cycles do not affect performance of broilers, independently of age of animals and times of the day cycles are deployed. In addition, this exposure time may alter only the weight of breast at the end of the production cycle.

Medeiros et al. (2005) add that, as heat stress increases, broilers detect life hazard and reduce energy accumulation for production and reproduction. In this scenario, these broilers resort to a survival strategy, which results in poor performance.

Cobb broilers generally exhibit better performance in the early stages of life compared with Hubbard broilers. Hubbard is a fast growth strain, whose performance usually improves at the end of the life cycle (API et al., 2017). Similarly, Vieira et al. (2007) reported that 21-day-old Cobb females have better performance, compared with Ross counterparts.

Recent studies compared fast growth broiler strains and did not report any difference in overall performance (MARTINS et al., 2014; API et al., 2017). The main difference between these strains is carcass yield, mobility problems, and sudden death, which may become worse when these broilers are slaughtered after day 42 of age.

2.5 TEMPERATURES OF SKIN, CLOACA, AND LITTER

Although body temperature is the best parameter to assess the thermal condition of broilers, the practical and physiological obstacles at play in the interpretation of data may undermine the importance of body temperature as a source of information on the thermal condition of commercial broilers (GILOH et al., 2012).

Changes are observed in broiler production as a response to heat stress. Cloacal temperature is a measure of the body temperature of broilers, and may be used as a parameter to assess heat stress (BROWN-BRANDL et al., 2003).

Under thermal comfort, cloacal temperature should be approximately 41 ºC, and it is measured using a mercury thermometer (BUENO et al., 2014). High cloacal temperature indicates heat stress and challenge to the environment. Marchini et al.

(2007) adds that one hour into exposure to high temperature, broilers express thermal discomfort, increasing body temperature to approximately 42.7 ºC.

Mean skin temperature (MST) of broilers responds more quickly to changes in the environment (NÄÄS et al., 2010). Nascimento et al. (2014) maintain that, independently of age, the contribution of all surfaces (except the wattles, which are still growing) has to be taken into account in the calculation of MST. Even though the thermoregulation system of broilers is not fully developed and feathering is incomplete, there will always be differences in temperature between areas with and without feathers.

Nääs et al. (2010) add that there is a more robust correlation between featherless areas in broilers (wattles and feet) and environment temperature. These regions have a faster response to environment changes compared with the parts of the body covered in feathers (head, back, and wings), since blood flow in these parts is higher. The authors observed a difference of as much as 6 ºC between a featherless part compared with one that is covered in feathers.

In a study with Cobb broilers, Nascimento et al. (2015) observed that MST increases by ± 1 ºC on day 41 of age when broilers are exposed to a 1-h heat cycle and ±3 ºC when exposure lasts 2 h and 3 h. The authors also noted that cloacal temperature rises more by at least 0.5 ºC after exposure to heat for 1 h.

Under thermal comfort conditions, MST values vary considerably in broilers raised in conventional barns, since the parameter depends on the skin regions it is measured, season of the year, and thermal control system. These temperatures may vary between 30 ºC and 35 ºC (BUENO et al., 2014; MARTINS et al., 2015). In turn, cloacal temperature is more constant, at between 41 ºC and 41.5 ºC in broilers nearing slaughter, since the parameter responds less quickly to changes in environment temperatures, compared with MST.

The cloacal temperature and MST gives the mean body temperature (MBT) of broilers. Welker et al. (2008) discovered that MBT may vary between 38 ºC and 39.8 ºC using different settings and cooling systems in conventional barns. Temperature values above those indicate heat stress, since cloacal temperature increases with MST, increasing MBT.

Cangar et al. (2008) and Bueno et al. (2014) also observed a decrease in skin temperature with growing age. For the authors, this drop is due to the less strict temperature requirements as broilers grow. In another study, Marchini et al. (2007)

observed that cloacal temperature increases from 40 ºC on day 1 to 41.2 ºC on day 42 in broilers farmed at normal temperatures. For Martins et al. (2015) also reported high cloacal temperature with age concomitant with high MBT values, while Bueno et al. (2014) observed a decrease in MBT.

Studies have demonstrated the correlation between skin temperature and cloacal temperature of broilers, when increasing environment temperatures lead to a rise in physiological temperatures (GILOH et al., 2012). It was shown that high environment temperatures increase skin temperature of broilers due to peripheral vasodilation, which is a physiological response in the effort to improve heat dissipation.

Concerning litter temperature, Carvalho et al. (2011) observed that the parameter is directly proportional to the temperature inside the barn. Bueno et al. (2014) add that temperature is higher at the edge of litter, compared to middle sections, since broilers resort to the edge of the pen to rest and the central part of it to feed, since feeders and drinkers are usually installed there. Mendes et al. (2010) claim that there will be no harm to broilers as long as litter temperature remains bellow 36ºC.

Bueno et al. (2014) also reported that, with age, litter temperature increases, since body size of broilers rises while the actual physical room in the barn remains unchanged, promoting the heat exchange between broilers and litter.

2.6 FEATHERING AND INCIDENCE OF PODODERMATITIS

According to Nascimento et al. (2011), the amount of feathers in broilers reflects an adaptive response to high environment temperatures as a strategy to dissipate heat. Mendes (2001) add that, despite the fact that low feathering development helps dissipate heat in broilers, good feathering cover is an essential factor in carcass quality. Feathers act as a barrier against lesions such as woody breast, scratches, lacerations, cuts, and other skin problems that are observed mostly in confined broilers.

Fukuyama et al. (2005) explain that broilers tend to reduce feather cover with rising environment temperatures as a heat dissipation strategy, which also improves performance. Edens et al. (2001) observed the opposite in cold environments: in the effort to maintain metabolic heat, broilers tend to develop a more efficient feather cover as adaptive response.

The decrease in feathering was also observed by Dahlke et al. (2005) in a study with Cobb broilers. The authors reported that, in addition to an overall decrease in feathering, the feather cover diminishes mostly on the back. Another important factor is that the authors believe that the decrease in feathering due to exposure to heat may be directly correlated with the drop in plasmatic T_3 levels, since this hormone plays a role in metabolic processes, possibly acting in feather development.

The thermal conditions of the environment may also play a direct role in the litter, inducing the emergence of pododermatitis due to the poor quality of litter (MENDES and KOMIYAMA, 2011).

Sheperd and Fairchild (2010) claim that humidity levels of the litter and the temperature of the barn are the main factors behind the emergence of calluses on feet of broilers, and add that genetic constitution may also promote the problem: heavier broilers tend to present larger numbers of these lesions, compared to light strains.

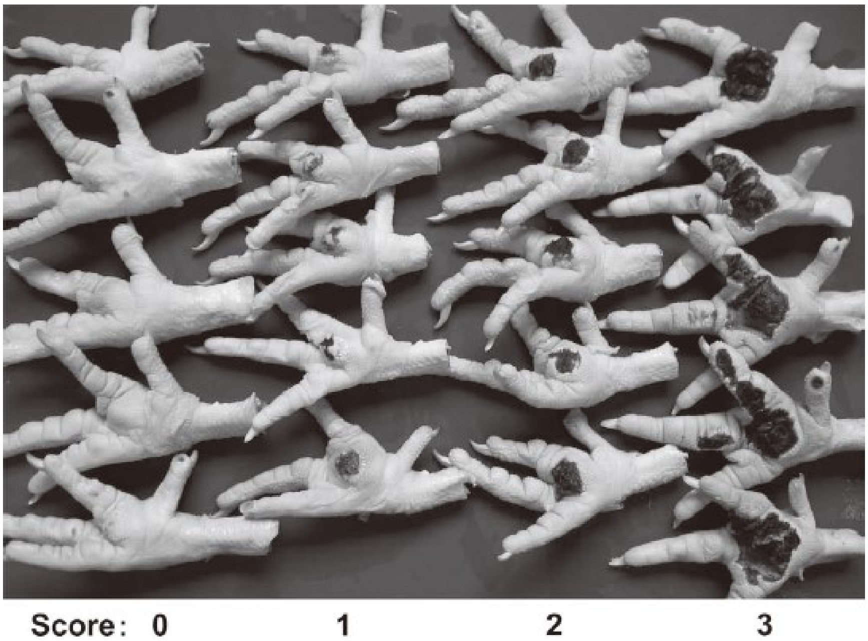
Mendes and Komiyama (2011) observed that pododermatitis is more common when wood shavings is used as litter, compared with the use of rice husk. Also, depending on the season of the year, incidence of these lesions increases due to the higher humidity in the rainy season, for instance.

It should also be remembered that high humidity levels or inappropriate litter condition may promote the compaction of the material due to the drying and dampening cycles. This induces the emergence of pododermatitis in broilers, which has been reported to be the reason behind economic losses in the industrial scale production of broilers and turkeys (MEDEIROS et al., 2008).

The severity of pododermatitis has been described by Hashimoto et al. (2011). The authors developed a scoring system to classify these lesions on the plantar surface of broilers' feet. According to the system, score 0 indicates no lesion, and scores 1, 2, and 3 signal lesions affecting less than 50%, between 50% and 100%, and 100% of the plantar surface, respectively (Figure 2).

It is also known that, in addition to thermal settings, nutrition plays a crucial role in the quality of litter and, therefore, the incidence of foot lesions. This is observed mainly when the feed given to broilers reduces the viscosity and increases the acidity of feces or makes them sticky, as observed with diets containing soy meal and high levels of carbohydrates (MENDES and KOMIYAMA, 2011; CARVALHO et al., 2014). This explains the need for studies that establish the contribution of each factor for the emergence of pododermatitis.

Figure 2: Pododermatitis scoring in broilers



Source: Hashimoto et al. (2011)

3 OBJECTIVE

The aim of this research was to assess the effects of exposure to heat cycles on thyroidal hormones, histology of the thyroid, performance, feathering and temperatures of cloaca, skin, and body of two strains of broilers, and to evaluate the temperature of the litter in pens, looking into the incidence of pododermatitis.

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CHAPTER 2

(Wrote in accordance with the rules of International Journal of Biometeorology)

EFFECT OF HEAT STRESS ON THYROIDAL HORMONES, THYROID HISTOLOGY, AND PERFORMANCE OF TWO BROILER STRAINS

João Paulo Rodrigues Bueno[1]*; Mara Regina Bueno de Mattos Nascimento[1]; Luciana Ruggeri Menezes Gotardo[2]; Aline Monteiro dos Santos[1]; Fernanda Heloisa Litz[1]; Otávio Cintra Lemos Olivieri[1]; Rodrigo Lemos Olivieri Rodrigues Alves[3]; Cíntia Amaral Moraes[1]

[1]Programa de Pós-Graduação em Ciências Veterinárias, Universidade Federal de Uberlândia, Campus Glória – BR 050, km 78 - Sala 209B – CEP 38410-337, Uberlândia, MG, Brazil.

[2]Programa de Pós-Graduação em Engenharia de Alimentos, Universidade de São Paulo, Campus Fernando Costa - Av. Duque de Caxias Norte, 225 – CEP 13635-900, Pirassununga, SP, Brazil.

[3]Programa de Pós-Graduação Ciência Animal e Pastagens, ESALQ-USP, Avenida Pádua Dias, 11 – CEP 13418-900, Piracicaba, SP, Brazil.

*E-mail: jprbueno@hotmail.com; Phone: +5534988662398

ABSTRACT: This study determined the serum levels of thyroid hormones (T_3 and T_4) and investigated the histology of the thyroid gland and the performance of two broiler strains exposed to different heat protocols. Broilers (N = 1120, males, 560 Cobb Slow™ and 560 Hubbard Flex™) were distributed in 32 boxes. Each thermal environment included eight boxes, four for each strain, and 35 birds were placed in each box. Broilers in the control environment (0) were reared following the thermal recommendations for each strain from day 1 to day 42. In the other environments (1, 2, and 3), broilers were exposed to high temperatures for 1, 2, and 3 h a day, respectively from day 14 to the day they were slaughtered. On days 21, 28, 35, and 42, six birds of each strain in each environment were chosen to provide blood samples collected to determine serum levels of T_3 and T_4. The same broilers were slaughtered on day 42, and thyroid glands were removed for histological analyses. Weight gain, feed consumption, feed conversion ratio, live weight, and viability were calculated once a week. All data were submitted to statistical analysis. It is possible to conclude that exposure to cyclic heat stress for 3 h from day 14 to day 42 did not affect performance, thyroid histology or the serum levels of T_3 and T_4. Cobb Slow™ broilers had the best live weight values at slaughter. With increasing age, T_3 levels decrease and T_4 levels increase in both strains.

KEYWORDS: cyclic heat stress, broiler, thyroid histology, thyroxin, triiodothyronine.

INTRODUCTION

Due to the economic importance of the poultry industry in Brazil, genetic improvement has become a tool to increase productivity. However, the high metabolism of broilers affects thermoregulation capacity when they are exposed to high temperature and humidity levels (Boschini et al. 2011). Under these circumstances broilers are more sensitive to heat stress, which may reduce performance (Uzum and Oral Toplu 2013).

The ideal temperature to breed broilers varies with the age of birds. On the 1st week, this temperature is in the 32-35 ºC range. On the 2nd, 3rd, 4th, and 5th weeks, these intervals are 29-32 ºC, 26-29 ºC, 23-26 ºC, and 20-23 ºC. On the 6th week, the ideal temperature to breed broilers is 20 ºC. These temperatures have been stipulated for relative humidity values between 60% and 70% (Abreu and Abreu 2011). However, these conditions are difficult to maintain in a country like Brazil. Moreover, although most studies have shown that heat stress is observed in broilers under constantly high temperatures, it has been shown that the phenomenon acquires a cyclic character when broilers are exposed to natural conditions. While daytime temperatures are high, values decrease at night, ultimately influencing the levels of heat stress in these birds (Marchini et al. 2007).

Another important factor is that the weight of broilers increases considerably with time, raising concerns with heat stress before slaughter, when these broilers are more sensitive to high temperatures (Nascimento et al. 2014). When exposed to stress, especially the heat levels typical of tropical environments, broilers resort to several strategies to maintain thermal homeostasis, such as behavioral, physiological, biochemical, and hormonal adaptation (Kadim et al. 2008; Oguntunji and Alabi 2010).

In addition, the genetic structure may also influence thermophysiological reactions of broilers. This underscores the importance of considering the commercial broiler strains, among which Cobb Slow™ and Hubbard Flex™. It is often necessary to investigate the main economically important aspects of these strains, which may vary due to heat stress and management practices. In this sense, genetic investigations are important in the choice of the most appropriate broiler strain in the effort to ensure the best production numbers (Martins et al. 2014; Api et al. 2017).

The activity of the thyroid is influenced by temperature, which may alter the histology of the gland and the secretion of its main hormones (Rachid et al. 2001; Marietto-Gonçalves et al. 2006; González and Silva 2017). In this sense, T_3 and T_4 are considered to play the most important roles in metabolic processes, since they direct nutritional efficiency, catabolism, anabolism, and thermogenesis. Nevertheless, these hormones function differently, depending on the growth stage of broilers and environmental conditions (Dahlke et al. 2005; Tao et al. 2006; Melesse et al. 2011).

Moreover, T_4 is the main hormone released by the thyroid as a "storage hormone". It is converted into T_3, which is the biologically active form of the hormone. This also explains the fact that the half-life of T_4 is longer than that of T_3 (Azeredo 2004; González and Silva 2017). These hormones are associated with the development of the animal, meaning that the factors that reduce T_3 and T_4 levels in broilers also affect the performance of these animals (Osorio et al. 2011).

The present study evaluated the serum levels of T_3 and T_4, the histology of the thyroid, and the performance of broilers of two commercial strains exposed to cyclic heat during different times.

MATERIALS AND METHODS

The experiment was conducted from April to May 2015 in the Poultry Farm "Glória", Federal University of Uberlândia. Briefly, the barn used was built in brick and mortar, steel structures, fiber cement roofing, concrete floor, wire walls, double lining (inside and outside), and plastic lining ceiling. Temperature and humidity are controlled using fans and humidification nozzles. Infrared bulbs are used in week 1. Shavings are used as litter.

In total 1,120 male chicks (560 Cobb Slow™ and 560 Hubbard Flex™ individuals, mean weight of 44 g) were purchased from a commercial incubation company. All chicks were bred from the same breeding stock and incubated in the same apparatus under identical conditions. The broilers were allocated following a randomized block design with space and time blocks (to analyze thyroid hormones) and space blocks (for the performance analysis and thyroid histology) in 32 boxes. Each box measured 1.90 x 1.50 m and was equipped with a tubular feeder and an automatic nursery drinker in the 1st week of age. This drinker was replaced by a hanging poultry drinker on week 2.

All birds were given feed formulated based on Rostagno et al. (2011) (Table 1). Bromatological analyses of raw materials used were carried out. The feeding regimen included a prestarter (days 1 to 7), a starter (days 8 to 21), a grower (days 22 to 33), and a finisher feed (days 34 to 42). Feed and water (3 – 5 mg/mL chlorine) were offered *ad libitum*. The lighting regimen was 2 h, 4 h, and 2 h in the dark for days 1 to 7, 8 to 21, and 22 to 42, respectively.

Table 1. Ingredients and nutritional levels of prestarter (days 1 to 7), starter (days 8 to 21), grower (days 22 to 33), and finisher (days 34 to 42) feeds given to broilers in the experiment

Ingredients (%)	Prestarter	Starter	Grower	Finisher
Ground sorghum 8.5	52.87	56.94	58.51	63.35
Soybean meal 46.0	40.69	36.68	34.04	29.07
Soybean oil	2.71	2.85	4.17	4.61
Calcitic limestone 36%	1.36	1.38	1.16	1.45
Dicalcium phosphate 18.5	1.03	0.90	0.82	0.32
Ground table salt	0.50	0.45	0.38	0.36
DL-Methionine 98%	0.08	0.04	0.14	0.09
L-Threonine 98%	0.04	0.03	0.05	0.01
Premix VMA FC	0.70[1]	0.70[1]	0.70[2]	0.70[3]
TOTAL	100	100	100	100
Nutritional levels				
Apparent metabolizable energy (kcal/kg)	3.050	3.099	3.219	3.299
Total protein (%)	23.53	22.00	21.00	19.00
Linoleic acid (%)	2.24	2.32	2.99	3.23
Available calcium (%)	1.03	1.00	0.90	0.90
Available phosphor (%)	0.48	0.45	0.43	0.33
Potassium (%)	0.97	0.90	0.85	0.77
Sodium (%)	0.23	0.21	0.18	0.17
Digestible lysine (%)	1.28	1.18	1.16	1.00
Digestible methionine (%)	0.65	0.59	0.59	0.49
Digestible methionine +cystine (%)	0.96	0.88	0.87	0.75
Digestible threonine (%)	0.81	0.75	0.74	0.64

[1]**Starter (kg/product):** Lysine 110 g, methionine 350g, vitA 1,000,000 IU, vitD3 285,700 IU, vitE 1.571 IU, vitK3 214 mg, vitB1 257 mg, vitB2 714 mg, vitB6 343 mg, vitB12 1,428.50 mcg, niacin 5,000mg, pantothenic acid 1,643 mg, folic acid 114.27 mg, biotin 5.70 mg, choline 42.85 g, manganese 8,570 mg, zinc 7,140 mg, iron 5.714 mg, copper 1,142.86 mg, iodine 114.30 mg, selenium 42.86 mg, phytase 71.429 un., protease 53.571 un., amylase 53.571 un., B-glucanase 44.643 un., xylanase 89.286 un., cellulase 80.357 un., ethoxyquin 9.524 mg, virginamycin 2,358 mg, nicarbazin+maduramicin 6,250 mg. [2]**Grower (kg/product):** Lysin 170 g, methionin 230 g, vitA 785,000 IU, vitD3 171,000 IU, vitE 1,428 IU, vitK3 171 mg, vitB1 171 mg, vitB2 571 mg, vitB6 271 mg, vitB12 1,142 mcg, niacin 4,000 mg, panthotenic acid 1,285 mg, folic acid 85.70 mg, choline 37.19 g, manganese 8,500 mg, zinc 7,100 mg, iron 5,700 mg, copper 1,142 mg, iodine 114 mg, selenium 35,70 mg, ethoxyquin 9,430 mg, phytase 71,429 un., protease 53,571 un., amylase 53,571 un., B-glucanase 44,643 un., xylanase 89,286 un., cellulase 80,357 un., virginamycin 2,357 mg, salinomycin 9,428.57mg. [3]**Finisher (kg/product):** Lysin

114 g, methionin 187 g, vitA 285,714 IU, vitD3 71,429 IU, vitE 785.71 IU, vitK3 78.56 mg, vitB2 285.70 mg, vitB12 714.28 mcg, niacin 2,857 mg, pantothenic acid 928.60 mg, choline 17.10g, manganese 8,570mg, zinc 7,143 mg, iron 5,714mg, copper 1,142.86mg, iodine 114.30mg, selenium 28.57mg, phytase 71,430 un., protease 53,571 un., amylase 53,571 un., B-glucanase 44,643 un., xylanase 89,286 un., cellulase 80,357un., ethoxyquin 9,524mg.

From the 1st to the 13th day of age, broilers were kept under the heat regimen recommended for each strain. On day 14, the barn was split into four parallel chambers (5.60 x 10.20 x 2.8 m) using double-face plastic lining along the width of the barn suspended so as to show the white side of the material. This allowed obtaining four thermal environments, each with eight boxes, of which four were used to house each strain (Cobb Slow™ and Hubbard Flex™). Thirty-five birds were placed in each box. In the control environment (0), broilers were reared according to the recommendations for each strain throughout the experiment (1st to 42nd day). In the other environments (1, 2, and 3), broilers were exposed to high temperatures for 1 h, 2 h, and 3 h daily, respectively, starting at 11h00, that is, the heat peak in the region, from day 14 to slaughter day.

During heat stress, temperature and relative humidity were monitored using data loggers (HOMIS 404A) installed in three locations in the barn 30 cm above the litter. The temperature-humidity index (THI) was calculated using the equation

$$THI = 0.8T + [H(T-14.3)/100] + 46.3$$

T: temperature

H: humidity

Values of THI above 79.92 indicate that birds are exposed to heat stress (Yakubu et al. 2018) (Table 2). Thermal comfort was provided using fans and humidification nozzles considering the environment temperatures.

Cyclic stress was induced using infrared light fixtures, with mean temperatures set at 36 ºC, 35 ºC, 34 ºC, and 33ºC on days 14 to 20, 21 to 27, 28 to 34, and 35 to 42, respectively (Table 2). Simultaneously to heat exposure, fans were kept on to maintain environmental homogeneity. Also, CO_2 levels did not exceed recommended values: maximum 3,000 ppm (Equipment: Li-cor™ LI-8100).

Table 2. THI and mean temperature and humidity values (with standard deviation) broilers were exposed to in Uberlândia, MG, Brazil

	Control			
	14-20 days	21-27 days	28-34 days	35-41days
Temperature (°C)	26.6±1.9	25.9±1.7	24.8±2.2	27.0±1.6
Humidity (%)	63.9±5.5	63.4±2.1	67.9±6.3	60.0±2.8
THI	75.43	74.37	73.26	75.5
	Stress (1 h, 2 h, and 3 h)			
	14-20 days	21-27 days	28-34 days	35-41days
Temperature (°C)	35.1±1.1	34.2±0.9	33.2±0.9	32.3±0.8
Humidity (%)	65.2±2.2	55.3±1.5	58.7±1.1	60.3±2.0
THI	87.9	84.66	83.95	82.99

From the beginning (11h00) to the end (14h00) of artificial heating, measurements were conducted every 10 min in the four environment to prevent temperature variation.

At the same time on the 21st, 28th, 35th, and 42nd days of age, six animals of each strain were removed from each environment (totaling 48 birds per week) and blood was collected from the ulnar vein using plastic tubes with no anticoagulant agent and centrifuged to obtain serum, which was frozen and stored at -20 ºC. Before analysis, serum samples were thawed and the levels of T_3 and T_4 were determined using the enzyme-linked immunosorbent assay (ELISA) and specific kits (Interkit™) at 37 ºC in a multichannel automatic analyzer (ChemWell) in the Laboratory of Animal Clinical Pathology, Federal University of Uberlândia.

On the 42nd day of age, the same broilers used to collect blood samples for T_3 and T_4 analyses were slaughtered following official Brazilian regulations (Brasil 1998). Upon necropsy, thyroids were excised and fixed in buffered formalin 10% (formaldehyde 37 – 40% in distilled water with monobasic sodium phosphate and anhydrous dibasic sodium phosphate). Next, glands were paraffin-embedded and stained by hematoxylin-eosin in the Laboratory of Animal Clinical Pathology, Federal University of Uberlândia. The 48 histological sections (six slides from birds housed in each environment and for each strain) were inspected using an optical microscope (10x objective), photographed using an adapter (Microcel™), and submitted to morphological analyses. Results were compared considering the presence of follicles, follicle lining (follicular or epithelial cells) and follicle filled with colloid.

Once a week, all broilers were weighed on a digital balance to measure weight gain and live weight. Feed consumption was calculated based on the difference between the amount of feed offered and the leftovers. Feed conversion was calculated using the ratio of feed consumption to weight gain considering the weight of slaughtered broilers. Viability was obtained using the difference between the number of birds at the beginning of each stage and the number of birds that died, multiplied by 100.

All data were tested for normality (Kolmogorov-Smirnov test, number of blocks > 50) and homogeneity (Levene test) followed by an analysis of variance in the software SISVAR™. Means were compared using the Tukey test (5% probability). Results of the histological analysis of the thyroid gland were analyzed by descriptive and comparative analyses.

RESULTS

Levels of T_3 and T_4 did not vary across strains and thermal regimens (Table 3).

Concerning age, T_3 levels decreased with time, while T_4 levels on day 42 did not differ from the values observed on day 21, though they were higher compared with days 28 and 35.

Table 3. Serum levels of T_3 and T_4 in Cobb Slow™ and Hubbard Flex™ broilers at different ages exposed to heat for different times

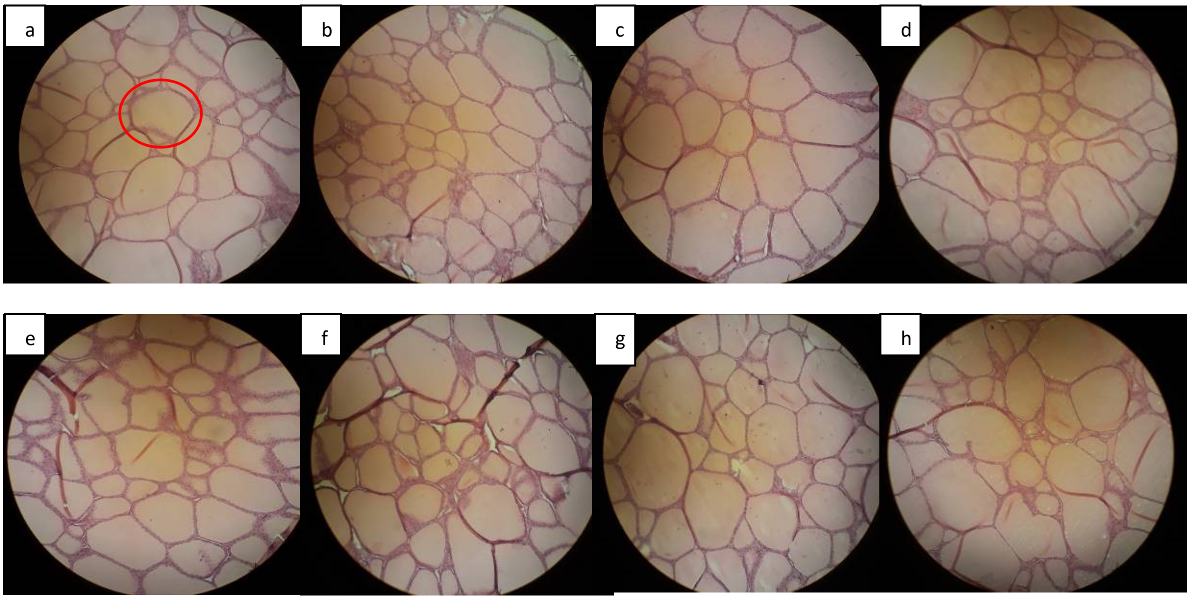
		T_3 (ng/mL)	T_4 (µg/dL)
Environment			
	0	2.15	1.442
	1	2.37	1.536
	2	2.02	1.665
	3	2.38	1.383
	CV%	28.66	24.85
Strain			
	Cobb Slow™	2.22	1.528
	Hubbard Flex™	2.24	1.485
	CV%	14.68	24.46
Age			
	21	4.17a	1.589ab
	28	2.35b	1.219c
	35	1.42c	1.409bc
	42	0.98d	1.808a
	CV%	24.74	26.19

P-value			
	Environment x Strain x Age	0.6615	0.4632
	Environment x Strain	0.0690	0.9776
	Environment x Age	0.2191	0.5193
	Strain x Age	0.4868	0.7637
	Environment	0.1326	0.0650
	Strain	0.7940	0.5211
	Age	0.0000*	0.000*

Means followed by different lowercase letters in columns differ in the Tukey test (5%).
* $P < 0.05$ indicates statistically significant difference; 0 (Control) = natural temperature and humidity conditions; 1 = exposure for 1 h (11h00-12h00); 2 = exposure for 2 h (11h00-13h00), and 3 = exposure for 3 h (11h00-14h00).

The thyroid preserved its morphology within histological patterns on day 42 of age of broilers, independently of heat exposure: follicles were approximately spherical and of various sizes, filled with colloid of different densities and lined with flat cuboidal cells (Fig. 1).

Fig. 1 Histology slides of the thyroid (10x magnification) of Cobb Slow™ and Hubbard Flex™ broilers at the age of 42 days submitted to different heat exposure times



Cobb Slow™: a) control environment, b) environment 1, c) environment 2, and d) environment 3.
Hubbard Flex™: e) control environment, f) environment 1, g) environment 2, and h) environment 3.
Red ellipsis indicates follicle surrounded by purple epithelial cells (follicular cells) filled by colloid (pink).

Also, exposure to heat for 1 h, 2 h, and 3 h did not affect broiler performance (Table 4).

Table 4. Performance of Cobb Slow™ and Hubbard Flex™ broilers on days 14 to 21, 22 to 28, 29 to 35, and 36 to 42 of age exposed to heat for 1 h, 2 h, and 3 h.

	Environment				Strain		P-value		
	0	1	2	3	Cobb Slow™	Hubbard Flex™	E x S	E	S
14-21 days									
FC (kg)	0.71	0.72	0.70	0.70	0.71	0.71	ns	ns	Ns
WG (kg)	0.44	0.44	0.44	0.45	0.45a	0.43b	ns	ns	0.0243
F_{Conv}	1.62	1.61	1.61	1.53	1.54a	1.64b	ns	ns	0.0208
LW (kg)	0.87	0.88	0.88	0.90	0.90a	0.86b	ns	ns	0.0013
V (%)	99.75	99.75	99.87	99.62	99.56	99.93	ns	ns	ns
22-28 days									
FC (kg)	1.01	1.00	1.02	1.02	1.03a	0.99b	ns	ns	0.0010
WG (kg)	0.65	0.64	0.64	0.64	0.64	0.65	ns	ns	ns
F_{Conv}	1.52	1.57	1.57	1.58	1.59b	1.53a	ns	ns	0.0483
LW (kg)	1.51	1.51	1.52	1.54	1.54a	1.51b	ns	ns	0.0485
V (%)	99.37	99.87	99.75	99.62	99.50	99.81	ns	ns	ns
29-35 days									
FC (kg)	1.25	1.28	1.26	1.27	1.28	1.25	ns	ns	ns
EG (kg)	0.78	0.75	0.78	0.76	0.77	0.76	ns	ns	ns
F_{Conv}	1.50	1.60	1.58	1.61	1.58	1.56	ns	ns	ns
LW (kg)	2.29	2.26	2.30	2.32	2.32a	2.27b	ns	ns	0.0167
V (%)	98.75	98.50	99.50	99.50	99.12	99.00	ns	ns	ns
36-42 days									
FC (kg)	1.45	1.48	1.39	1.45	1.46	1.43	ns	ns	ns
WG (kg)*	-	-	-	-	-	-	0.0307	ns	ns
F_{Conv}	2.02	1.90	2.16	2.00	2.04	2.00	ns	ns	ns
LW (kg)	2.96	2.95	2.92	2.97	2.97a	2.93b	ns	ns	0.0498
V (%)	98.87	98.00	99.25	98.50	98.56	98.75	ns	ns	ns

Means followed by different letters on the same line for each parameter environment (E) and strain (S) differ in the Tukey test at 5%.
0 (Control) = natural temperature and humidity conditions; 1 = exposure for 1 h (11h00-12h00); 2 = exposure for 2 h (11h00-13h00), and 3 = exposure for 3 h (11h00-14h00).
FC = feed consumption, WG = weight gain, F_{Conv} = feed conversion rate, LW = live weight, V = viability
*Table 5 describes the interaction environment x strain

Between days 14 and 21, Cobb Slow™ broilers presented the highest weight gain, the best live weight, and the best feed conversion rate. Between days 22 and 28, Cobb Slow™ broilers had the best live weight, though these animals also had the highest feed consumption rate and the lowest feed conversion rate. Live weight was the highest in Cobb Slow™ than Hubbard Flex™ broilers on days 29 to 35 and on days 36 to 42 (Table 4).

Also, interaction environment x strain was observed for weight gain on days 36 to 42 (Table 5). Hubbard Flex™ broilers had higher weight gain compared with Cobb Slow™ exposed for 1 h. In addition, Hubbard Flex™ broilers had the lowest weight gain when exposed to heat for 2 h.

Table 5: Interaction environment x strain for the variable weight gain on days 36 to 42 of age

	Environment			
Strain	0	1	2	3
Cobb Slow™	0.70	0.64B	0.61	0.66
Hubbard Flex™	0.64ab	0.74aA	0.61b	0.65ab

Means followed by different lowercase letters on a line and different uppercase letters on a column differed in the Tukey test at 5%.
0 (Control) = natural temperature and humidity conditions; 1 = heat exposure for 1 h (11h00-12h00); 2 = heat exposure for 2 h (11h00-13h00), and 3 = heat exposure for 3 h (11h00-14h00).

DISCUSSION

High temperature cycles did not affect T_3 and T_4 levels in broilers possibly due to the fact that thyroidal activity was not influenced, showing that broilers managed to maintain endocrinal activity when environment temperatures were within the comfort zone of each strain. Mello et al. (2018) likewise did not observe any change in T_3 and T_4 levels in broilers submitted to 32 ºC for as long as 72 h a week on days 21, 35, and 42 of age. The authors claimed that non-endocrinal mechanisms to control body heat are efficient to dissipate endogenous heat, cancelling off the influence of thermal stress on thyroidal activity.

Oppositely, T_3 and T_4 levels generally decrease in broilers exposed to high temperatures. Also, Sohail et al. (2010) found that this is due to the increase in cortisol levels observed in broilers bred under stress for 8 h a day from days 22 to 42 of age. High cortisol levels inhibit thyroidal activity, negatively affecting metabolism. This was not observed in the present study, since exposure to heat for up to 3 h was not enough to influence T_3 and T_4 levels.

Dahlke et al. (2005) noted that exposure to high temperature oscillating between 3 ºC and 9 ºC above the recommended value for fast weight gain broilers decreases T_3 and T_4 levels on days 21 to 42. The authors also found that T_4 levels decrease on days 35 to 42. In a study with Cobb Slow™ broilers bred at high temperature for 24 h (35 ºC for days 1 to 21 and ± 32 ºC for days 22 to 42), Souza et al.

(2011) observed that T_3 values were low on days 21 (0.31 ng mL^{-1}) and 42 (0.32 ng mL^{-1}), and concluded that a sharp drop in T_3 levels takes place when these broilers are exposed to constant heat stress.

Also, Tao et al. (2006) obtained T_3 and T_4 levels 40% as low as the levels recorded in broilers at thermal comfort zone, with no variation in daily mean values during exposure at six sample collection times (0 h, 4 h, 8 h, 12 h, 16 h, and 20 h), indicating the possibility that half-life of these hormones is longer than as 20 h (Azeredo 2004; González and Silva 2017).

It is important to underscore that stress conditions have to be harsh to elicit a change in thyroidal response, to the point of inducing the release of glucocorticoids that in turn inhibit the activity of the gland (González and Silva 2017). This was not observed in the present study, possibly because the broilers recovered from the effects of stress during the warmest part of the day. This adaptation response of broilers to cyclic heat stress was also discussed by Bueno et al. (2017) and Balnave (2004), who observed a compensatory gain (in performance or in physiology) of broilers during periods when temperature draws near to thermal comfort, most often at nighttime.

As observed in previous research (Carew et al. 2003; Abdel-Fattah et al. 2008; Sohail et al. 2010), T_4 values were higher than T_3 ones in the present study. This is a result of the fact that T_4 is the main hormone released by the thyroid as a “storage hormone”. The biologically active T_3 accounts for a small quantity of thyroidal hormones (Abdel-Fattah et al. 2008; González & Silva 2017). Moreover, the order of magnitude of T_4 half-life is a few days, while the value observed for T_3 is a few hours, which explains the low levels of the latter (Azeredo 2004; González and Silva 2017).

The similar levels of T_3 and T_4 for the two strains used in the present study confirms the findings published by Api et al. (2017) and Martins et al. (2014), who discovered that, independently of strain, fast growth broilers share performance and behavioral traits, which indicates that they are physiologically and biochemically similar.

Concerning age, most studies published to date recorded a decrease in levels of both hormones with age, as confirmed by our results for T_3, but not T_4. Melesse et al. (2011) analyzed egg laying chicken breeds exposed to high temperatures (30 ºC to 32 ºC), observing that T_3 levels decrease under heat and that the concentration of both thyroidal hormones drop with age, indicating decreased activity of the gland. Luger et al. (2001) reported a decrease in both T_3 and T_4 levels with age in broilers.

The changes in behavior of thyroidal hormones with age may also be associated with carrier proteins. Azeredo (2004) found that albumin is the main carrier protein of thyroidal hormones. Based on the albumin levels found by Bueno et al. (2017) in a study that used broilers exposed to cyclic heat stress, the highest levels of albumin are observed in broilers on days 28 and 42 of age. This means that it is possible that T_4 was efficiently converted into T_3 on day 28, when metabolism is high, since the concentration of T_4 was the lowest and T_3 levels were still high.

Importantly, on day 42, when broilers have high levels of T_4 compared with the levels detected on days 28 to 35 of age, T_3 levels were the lowest. This may indicate that the conversion of T_4 into T_3 decreases, since the metabolism on day 42 is lower than the value observed in the time interval above. Another possibility is that, with the increase in albumin levels detected at this age (Bueno et al. 2017), its affinity for the hormone ensures that it is not degraded by enzymes, thus remaining longer in circulation.

Similarly to the effect of heat exposure and strain on serum levels of T_3 and T_4 on day 42 of age, thyroid morphology was in accordance with histological standards. Rachid et al. (2001) and Parchami and Fatahian Dehkordi (2012) observed that the thyroid is composed of almost spherical microfollicles and macrofollicles with small amounts of interstitial tissue. Each follicle is lined with a single layer of epithelial or interstitial cells on the follicular vesicle, which is filled with colloid, as observed in our study.

González and Silva (2017) and Azeredo (2004) discovered that thyroidal follicles are the functional unit constituting the thyroid. The authors also found that the lining epithelium is cuboidal in shape and assumes a columnar structure only when the gland's activity is very high, and that the secretion found inside the follicle, that is, the colloid produced by follicular cells, is the site T_3 and T_4 are stored. Marietto-Gonçalves et al. (2006) observed that changes in thyroid morphology occur when broilers are given low iodine feed. Parchami and Fatahian Dehkordi (2012) add that the structure of the thyroid is similar to that of the gland in other vertebrates in homeostasis.

High temperature cycles did not affect the performance of broilers, possibly because the broilers were capable of recovering during the night, when temperatures are lower and near the thermal comfort value. It has been shown that broilers adapt better to high temperatures when it drops to 25 ºC or less at night, since they can recover from the heat stress they are exposed to during the day (Balnave 2004). This is in agreement with the results obtained in the present study, showing that a 3-h stress exposure period was not long enough to affect the performance of broilers. On the other hand, Oba et al. (2012) observed that environment temperature strongly influences production parameters of broilers. The lowest levels of weight gain, feed consumption, feed conversion, and viability were observed in broilers kept at high temperatures, namely 32 ºC and 35 ºC. The broilers exposed to high environmental temperature consume less feed in the effort to reduce heat generation and maintain thermal balance. Therefore, weight gain is reduced, feed conversion is less effective, and mortality increases (Sartori et al. 2001; Tinôco 2001; Medeiros et al. 2005).

In a study with Hubbard Flex™ broilers bred at average (23.3 ºC) and high (32.3 ºC) temperatures, Oliveira Neto et al. (2000) observed low performance under heat stress. Similarly, Oba et al. (2012) reported low performance of Cobb Slow™ broilers bred at high temperatures for 24 h from days 1 to 47 of age had low performance compared with broilers bred in the heat comfort zone. Medeiros et al. (2005) noted that as heat stress increases, the animal senses death hazard and ceases to accumulate energy for production and reproduction, focusing on survival which in turn manifests as poor performance.

The better performance presented by the Cobb Slow™ strain in this study on days 14 to 21 may be explained based on the fact that this strain reaches maturity sooner than the Hubbard Flex™ strain, which generally attains compensatory growth at the end of the production cycle (Api et al. 2017). Vieira et al. (2017) also observed better performance of Cobb Slow™ hens on day 21 of age compared with Ross strain individuals.

Api et al. (2017) observed that Hubbard Flex™ broilers generally reach compensatory growth only from the 4th week onwards, which explains the better feed conversion rate observed for the strain at this age, even though final weight did not exceed the value recorded for the strain Cobb Slow™. Despite the poor conversion rates, the conservation of the best live weight values observed for Cobb Slow™ broilers on days 22 to 28 shows that weight of broilers on the first weeks of age is a very reliable indicator of the genetic potential concerning fast growth (Garcia Neto and Campos 2004), since the strain preserved the best values of live weight throughout the experimental period.

In the present study, final live weight (days 36 to 42 of age) of Cobb Slow™ broilers may be explained based on the fact that these broilers are more resistant, responding better to high densities and changes in environmental and nutritional environment, with overall better performance (Lara et al. 2008). Nevertheless, recent studies that compared commercial fast growth strains did not report any differences in performance of broilers at the end of the production period (Martins et al. 2014, Api et al. 2017), since these strains differ mostly in carcass yield as well as the likelihood of locomotor problems and the risk of sudden death, which may be observed if the broilers are slaughtered after day 42 of age.

Even though weight gain of Hubbard Flex™ birds in environment 1 on days 26 to 42 of age was higher than the value measured for Cobb Slow™ broilers and despite the fact that Hubbard Flex™ broilers had the lowest weight gain in environment 2 compared with environments 0, 1, and 3, final performance was not affected. Also, Cobb Slow™ broilers had the highest final live weight at the end of the growth period.

CONCLUSIONS

Cyclic heat stress for as long as 3 h a day from days 14 to 42 of age does not affect the histology of the thyroid gland or the serum levels of T_3 and T_4 of Cobb Slow™ and Hubbard Flex™ broilers. Also, the broilers conserved metabolism rates and did not change feed consumption; therefore, performance was not reduced.

Under the conditions adopted in the present study, Cobb Slow™ broilers had better live weight at slaughter, compared with the Hubbard Flex™ strain. Also, T_3 levels decreased and T_4 levels increased with age in both strains.

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Ethical approval.- All procedures in this study were carried out in accordance with the guidelines issued by the Ethics Committee in Research with Animals of the Federal University of Uberlândia, state of Minas Gerais (MG), Brazil (CEUA/UFU 065/14).

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CHAPTER 3

(Wrote in accordance with the rules of Arquivo Brasileiro de Medicina Veterinária e Zootecnia)

EFFECT OF CYCLIC HEAT STRESS ON BROILERS AND LITTER TEMPERATURE, FEATHERING, AND INCIDENCE OF PODODERMATITIS

EFEITO DO ESTRESSE CÍCLICO POR CALOR SOBRE A TEMPERATURA DOS FRANGOS E DA CAMA, EMPENAMENTO E INCIDÊNCIA DE PODODERMATITE

BUENO, J. P. R.[1]; NASCIMENTO, M. R. B. M.[2]; GOTARDO, L. R. M.[3]; LITZ, F. H.[1], OLIVIERI, O. C. L.[4]; ALVES, R. L. O. R.[5]; ANTUNES, M. M.[6]; SILVEIRA, A. C. P.[7]

[1]Doutorando (a), Programa de Pós-Graduação em Ciências Veterinárias, UFU
[2]Professora Doutora, Programa de Pós-Graduação em Ciências Veterinárias, UFU
[3]Doutoranda, Programa de Pós-Graduação em Engenharia de Alimentos, USP
[4]Médico Veterinário
[5]Mestrando, Programa de Pós-Graduação em Ciência Animal e Pastagens, ESALQ-USP
[6]Mestre, Médica Veterinária
[7]Professora Doutora, IFTM - Campus Uberaba

ABSTRACT: This study assessed the effect of exposure to cyclic heat on temperatures (of broilers and litter), feathering, and incidence of pododermatitis in broilers. 1120 male broilers (560 Cobb Slow™ and 560 Hubbard Flex™) were lodged in a barn under four thermal settings. Each environment was formed by eight boxes, four for each strain, each containing 35 birds. In the control environment (0), broilers were farmed following the thermal comfort recommendations for each strain. In environments 1, 2, and 3, broilers were exposed to high temperatures for 1 h, 2 h, and 3 h a day, respectively, starting at 11:00 a.m. Heat exposure was carried out until the day broilers were slaughtered. On days 21 and 41, skin, cloacal, and body temperature of six birds of each strain in each environment were measured. Litter temperature was also assessed. On day 42 broilers were slaughtered and feathering and incidence of pododermatitis was evaluated. Heat exposure did not affect feathering or promote pododermatitis, but litter and broilers temperature increased. The temperatures of broilers and of the litter in the middle of the box varied with age. The cloacal temperature of Cobb Slow™ broilers was higher than that of Hubbard Flex™.

KEYWORDS: Animal welfare, cloacal temperature, poultry, skin temperature, thermal stress.

RESUMO: Avaliou-se o efeito da exposição cíclica ao calor sobre as temperaturas (da ave e de cama), o empenamento e incidência de pododermatites em frangos de corte. Foram alojadas 1120 aves, machos (560 Cobb e 560 Hubbard). O galpão foi separado obtendo-se quatro ambientes térmicos, cada um contendo oito boxes, quatro para cada linhagem, com 35 aves por boxe. No ambiente controle (0) as aves foram criadas conforme recomendação térmica para linhagem. Nos ambientes (1, 2 e 3), as aves foram expostas a altas temperaturas, respectivamente, por 1h, 2h e 3h diárias (com início às 11:00h) até o abate. No 21º e 41º dia de idade as temperaturas superficial, cloacal e corporal de seis aves de cada ambiente, dentro de cada linhagem foram mensuradas além da temperatura da cama dos boxes. No abate ao 42º dia, foram avaliados o empenamento e incidência de pododermatites. A exposição ao calor não alterou o empenamento e nem favoreceu a incidência de pododermatites, somente as temperaturas das aves e da cama aumentaram. As temperaturas dos frangos e centro da cama também se alteraram com a idade e a linhagem Cobb apresentou maior temperatura cloacal que a Hubbard.

PALAVRAS-CHAVE: avicultura, bem estar animal, estresse térmico, temperatura superficial, temperatura cloacal.

INTRODUCTION

Of the animal species used for food production, broilers present the fastest metabolic rate due to genetic and nutritional improvements in addition to better farming and environment conservation practices currently adopted. With such marked metabolism and late maturation of their thermoregulation system, the ability of broilers to control temperature is affected, mainly when they are exposed to the high temperatures and humidity values frequently observed in tropical countries (Boschini et al., 2011). It is known that the environment conditions broilers are exposed to are cyclic, not constant, since temperatures are higher in late morning and the afternoon, posing greater challenges to broilers, and lower at night and early morning.

As a response to the cyclic heat exposure, farming of broilers may be affected in different ways. Cloacal temperature represents the internal temperature of the animal and may be used as reference value for comfort condition or stress of broilers (Brown-Brandl et al., 2003). In turn, mean skin temperature represents the outer surfaces of the bird, which respond more quickly to the changes in the thermal environment (Nääs et al., 2010). The mean body temperature (MBT) of broilers is calculated considering the cloacal and mean skin temperatures.

Broilers also exhibit changes in feathering patterns due to the challenges posed by the temperature they are farmed at (Nascimento et al., 2011). Also, the litter used in broiler farming barns is an element of either comfort or stress, since the temperature of this material is also affected by the environment (Bueno et al., 2014). Depending on the thermal environment conditions, lesions may merge on feet of broilers. These lesions are called pododermatites and may impair the movement of these broilers (Shepherd and Fairchild, 2010), causing discomfort and pain to the animal, which is contrary to the basic principles of animal welfare.

The market offers several commercial strains of broilers and genetic improvement is unique to each company. Thus, it is important to investigate the differences between strains regarding responses to the hot environment. It is essential to also consider the significant broilers growth speed every week, which makes them more sensitive to heat.

In this scenario, this study assessed the effect of cyclic heat stress on skin, cloacal, body, and litter temperature as well as feathering and incidence of pododermatites in two commercial broilers strains farmed under different heat exposure times.

MATERIALS AND METHODS

This study was approved by the Ethics Committee (CEUA/UFU 065/14) of the Federal University of Uberlândia, state of Minas Gerais (MG), Brazil.

The experiment was conducted in the Poultry Unit, Farm “Glória”, Federal University of Uberlândia. The barn was built in brick and mortar, steel structures, and fiber cement roofing. In addition, the floor is made of concrete, and the walls of the barn are made of wire and double lining. The ceiling is made of plastic lining, and temperature and humidity are controlled using fans and humidification nozzles. Infrared bulbs were used in the first week of broiler farming. Wood shavings were used as litter.

In total, 1120 broilers were used (560 Cobb Slow™ and 560 Hubbard Flex™). All chicks were bred from the same breeding stock and purchased from the same incubation company. Chicks were incubated in the same apparatus under equivalent conditions and distributed according to a randomized block design with blocks subdivided for space and time (to analyze skin, cloacal, body and litter temperature) and blocks subdivided for space (feathering and pododermatitis). Thirty-two boxes measuring 1.90 m x 1.50 m were used, each equipped with tubular feeder and automatic nursery drinker (first week) and hanging poultry drinker (week two onwards).

All broilers were given feed formulation based on Rostagno et al. (2011) for each age (Tab. 1). Bromatological analyses of raw materials were conducted to confirm the composition of feeds. Broilers were given feed and drinking water (3 – 5 mg/mL chlorine) *ad libitum*. The lighting program was 2 h, 4 h, and 2 h dark sessions from days 1 to 7, 8 to 21, and 21 to 42, respectively.

Table 1. Composition and nutritional levels of the prestarter (days 1 to 7), starter (days 8 to 21), grower (days 22 to 33), and finisher (days 34 to 42) feeds given to broilers.

Ingredients (%)	Prestarter	Starter	Grower	Finisher
Ground sorghum 8.5	52.87	56.94	58.51	63.35
Soybean meal 46.0	40.69	36.68	34.04	29.07
Soybean oil	2.71	2.85	4.17	4.61
Calcitic limestone 36%	1.36	1.38	1.16	1.45
Dicalcium phosphate 18.5	1.03	0.90	0.82	0.32
Ground table salt	0.50	0.45	0.38	0.36
DL-Methionine 98%	0.08	0.04	0.14	0.09
L-Threonine 98%	0.04	0.03	0.05	0.01
Premix VMA FC	0.70[1]	0.70[1]	0.70[2]	0.70[3]
TOTAL	100	100	100	100
Nutritional levels				
Apparent metabolizable energy (kcal/kg)	3.050	3.099	3.219	3.299
Total protein (%)	23.53	22.00	21.00	19.00
Linoleic acid (%)	2.24	2.32	2.99	3.23

Available calcium (%)	1.03	1.00	0.90	0.90
Available phosphor (%)	0.48	0.45	0.43	0.33
Potassium (%)	0.97	0.90	0.85	0.77
Sodium (%)	0.23	0.21	0.18	0.17
Digestible lysine (%)	1.28	1.18	1.16	1.00
Digestible methionine (%)	0.65	0.59	0.59	0.49
Digestible methionine + cystine (%)	0.96	0.88	0.87	0.75
Digestible threonine (%)	0.81	0.75	0.74	0.64

[1]**Starter (kg/product):** Lysine 110 g, methionine 350g, vitA 1,000,000 IU, vitD3 285,700 IU, vitE 1.571 IU, vitK3 214 mg, vitB1 257 mg, vitB2 714 mg, vitB6 343 mg, vitB12 1,428.50 mcg, niacin 5,000mg, pantothenic acid 1,643 mg, folic acid 114.27 mg, biotin 5.70 mg, choline 42.85 g, manganese 8,570 mg, zinc 7,140 mg, iron 5.714 mg, copper 1,142.86 mg, iodine 114.30 mg, selenium 42.86 mg, phytase 71.429 un., protease 53.571 un., amylase 53.571 un., B-glucanase 44.643 un., xylanase 89.286 un., cellulase 80.357 un., ethoxyquin 9.524 mg, virginamycin 2,358 mg, nicarbazin+maduramicin 6,250 mg. [2]**Grower (kg/product):** Lysin 170 g, methionin 230 g, vitA 785,000 IU, vitD3 171,000 IU, vitE 1,428 IU, vitK3 171 mg, vitB1 171 mg, vitB2 571 mg, vitB6 271 mg, vitB12 1,142 mcg, niacin 4,000 mg, panthotenic acid 1,285 mg, folic acid 85.70 mg, choline 37.19 g, manganese 8,500 mg, zinc 7,100 mg, iron 5,700 mg, copper 1,142 mg, iodine 114 mg, selenium 35,70 mg, ethoxyquin 9,430 mg, phytase 71,429 un., protease 53,571 un., amylase 53,571 un., B-glucanase 44,643 un., xylanase 89,286 un., cellulase 80,357 un., virginamycin 2,357 mg, salinomycin 9,428.57mg. [3]**Finisher (kg/product):** Lysin 114 g, methionin 187 g, vitA 285,714 IU, vitD3 71,429 IU, vitE 785.71 IU, vitK3 78.56 mg, vitB2 285.70 mg, vitB12 714.28 mcg, niacin 2,857 mg, pantothenic acid 928.60 mg, choline 17.10g, manganese 8,570mg, zinc 7,143 mg, iron 5,714mg, copper 1,142.86mg, iodine 114.30mg, selenium 28.57mg, phytase 71,430 un., protease 53,571 un., amylase 53,571 un., B-glucanase 44,643 un., xylanase 89,286 un., cellulase 80,357un., ethoxyquin 9,524mg.

From days 1 to 13, broilers were farmed according to the thermal comfort recommendations for each strain. On day 14, the barn was divided into four parallel chambers (5.60 x 10.20 x 2.80 m) using double-face plastic lining. The white side of the lining was exposed in the effort to obtain four thermal environments, each comprising eight boxes, four for each strain. In total, 35 broilers were lodged in each box. The broilers in the control environment (0) were farmed as stipulated for each strain considering thermal comfort throughout the experiment. In the test environments (1, 2, and 3), broilers were exposed to high temperatures for 1 h, 2 h, and 3 h a day starting at 11:00 a.m. (which is the peak hour in heat exposure in the region this study was carried out), from day 14 to slaughter.

Temperature and relative humidity of the environment were measured using specific instruments (HOMIS 404A) placed in three sites in the barn 30 cm away from the litter. The temperature-humidity index (THI) was calculated using the equation

$$THI = 0.8T + [H(T-14.3)/100] + 46.3$$

T: temperature

H: humidity

According to Yakabu et al. (2018), THI values above 79.92 indicate heat stress (Tab 2). Cyclic heat stress was induced using infrared bulbs. Mean temperatures used were 36 ºC from days 14 to 20, 35 ºC from days 21 to 27, 34 ºC from days 28-34, and 33 ºC from days 35 to 42. Fans were kept working throughout the periods bulbs were on as a means to make the environment temperature uniform in boxes. Also, CO_2 levels did not exceed the recommended value of 3000 ppm (Equipment: Li-cor^{TM} LI-8100).

Table 2. THI and mean temperature and humidity values (with standard deviation) broilers were exposed to in Uberlândia, MG, Brazil

	Control			
	14-20 days	21-27 days	28-34 days	35-41days
Temperature (°C)	26.6±1.9	25.9±1.7	24.8±2.2	27.0±1.6
Humidity (%)	63.9±5.5	63.4±2.1	67.9±6.3	60.0±2.8
THI	75.43	74.37	73.26	75.5
	Stress (1 h, 2 h, and 3 h)			
	14-20 days	21-27 days	28-34 days	35-41days
Temperature (°C)	35.1±1.1	34.2±0.9	33.2±0.9	32.3±0.8
Humidity (%)	65.2±2.2	55.3±1.5	58.7±1.1	60.3±2.0
THI	87.9	84.66	83.95	82.99

From the beginning (11h00) to the end (14h00) of artificial heating, measurements were conducted every 10 min in the four environment to prevent temperature variation.

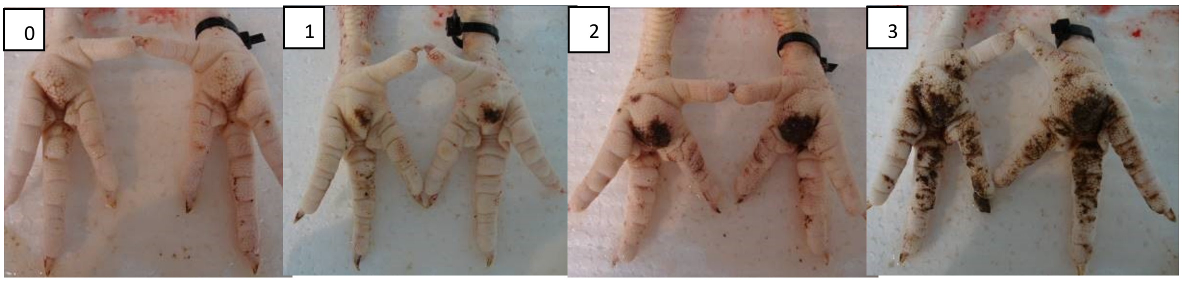
On days 21 and 41, six broilers from each environment and strain (48 animals in total) were selected always at the same time during heat exposure. Wing, head, back, and foot temperatures were measured. The temperature of the wattle was measured only on day 41. All measurements were conducted using a digital infrared thermometer (Instrutemp DT8530). Cloacal temperature was assessed using a mercury thermometer (Incoterm L185/06). These data were employed to calculate mean skin temperature (MST) with the equation proposed by Nascimento (2010) on day 21 ($MST = 0.23T_{wing} + 0.13\ T_{head} + 0.6T_{back}$) and on day 41 ($MST = 0.27T_{wing} + 0.1T_{head} + 0.05T_{foot} + 0.5T_{back} + 0.1T_{wattle}$). MBT was obtained according to Richards (1971) ($MBT = 0.3MST + 0.7T_{cloaca}$).

The temperature of litter was measured during heat exposure cycles in each box using a digital infrared thermometer (Instrutemp DT8530). Seven measuring sites were used (the four corners, the center of the litter, near the feeder, and near the drinker). Mean litter temperature (MTT) was calculated as the mean corner temperature (MCT) and central litter temperature (CLT) as described by Bueno et al. (2014).

On day 42, six broilers (of each environment and each strain, totaling 48 birds) were slaughtered following current standards and procedures in Brazil (Brasil, 1998). Broilers were manually plucked by the same person. The weights before (WB) and after plucking (WP) were recorded, and the weight of feathers (WF) was obtained as the difference between values (WF = WB – WP).

The plantar surface of the feet of the slaughtered broilers was inspected to determine the incidence of pododermatitis, which was scored from 0 to 3. Score 0 indicates no lesion and scores 1, 2, and 3 highlight lesions in less than 50%, between 50% and 100%, and 100% of the plantar surface, in that order (Hashimoto et al., 2011) (Fig 1).

Figure 1. Score of pododermatitis lesions in broilers on day 42 of age.



Source: the authors

All data were tested for normality (Shapiro-Wilk test, number of blocks smaller than 50) and homogeneity (Levene test). An analysis of variance was carried out using the SISVAR software and means of quantitative data were compared using the Tukey test (5% probability). The qualitative data describing the incidence of pododermatitis were analyzed using the Kruskal-Wallis test (5% probability).

RESULTS AND DISCUSSION

Broilers exposed to heat, independently of exposure times (1 h, 2 h, or 3 h), presented mean skin temperature (MST), cloacal temperature (CT), and mean body temperature (MBT) higher than control broilers (Tab. 3).

Table 3. Mean skin temperature (MST), cloacal temperature (CT), and mean body temperature (MBT) of Cobb Slow™ and Hubbard Flex™ broilers farmed under different heat exposure regimens.

		MST(ºC)	CT (ºC)	MBT (ºC)
Environment	0	35.1b	41.3b	39.4b
	1	37.7a	41.7a	40.5a
	2	38.1a	41.8a	40.7a
	3	37.6a	41.8a	40.6a
CV%				
Strain	Cobb Slow™	37.1	41.8a	40.4
	Hubbard Flex™	37.2	41.5b	40.2
CV%				
Age	21	36.4b	41.5b	40.5a
	41	37.9a	41.8a	40.1b
CV%				
P-value	E x S x A	0.2299	0.0943	0.5381
	E x S	0.9502	0.6000	0.7227
	E x S	0.6719	1.0000	0.8674
	L x S	0.8881	0.0664	0.2839
	Environment	0.0000*	0.0000*	0.0000*
	Strain	0.7979	0.0084*	0.1265
	Age	0.0000*	0.0072*	0.0000*

Means followed by different letters in the same column differ in the Tukey test (5%).

*Significance: $P < 0.05$

0 = Control group (environmental temperature and humidity conditions); 1, 2, and 3 = broilers exposed to 1-h, 2-h, and 3-h heat cycles, respectively, starting at 11:00 a.m.

E= Environment, S= Strain and A= Age

The rise in environment temperature kept for as long as 3 h induced the increase in skin temperature of broilers. According to Giloh et al. (2012), this is caused by peripheral vasodilation as a characteristic physiological response of broilers in the effort to improve heat loss. The authors also discovered that cloacal temperature increases with the rise of skin temperature, as observed in the present study.

Nääs et al. (2010) and Nascimento et al. (2014) found that the correlation is more robust between featherless areas of a broiler's body such as wattle and feet and environment temperature, compared with areas covered with feathers (head, back, and wings). This is explained in view of the higher blood flow in feathered regions. Nääs et al. (2010) also observed a difference of as much as 6 ºC between feathered and featherless areas.

In a study with Cobb Slow™ broilers, Nascimento et al. (2015) observed that MST on day 41 is approximately 1ºC higher when broilers are exposed to 1-h heat cycles and roughly 3 ºC higher when these broilers are exposed to 2-h and 3-h heat cycles. Similar results were recorded in the present study, where an increase of 2 ºC to 3 ºC was noticed after a 1-h heat cycle. Nascimento et al. (2015) also reported that cloacal temperature increased by approximately 0.5 ºC in broilers exposed to heat for 1 h.

It is known that cloacal temperature represents the internal temperature of the bird and that, in a thermally comfortable environment, the value should be approximately 41 ºC (Bueno et al., 2014). High cloacal temperatures indicate heat stress. Marchini et al. (2007) found that, after 1 h under heat conditions, broilers already demonstrate thermal discomfort, increasing internal temperature to approximately 42.7 ºC.

Under thermal comfort conditions, MST vary considerably in broilers farmed in conventional barns. The values of MST depend on broiler's body area used to collect data, season of the year, and thermal control system. MST values can vary between 30 ºC and 35 ºC (Bueno et al., 2014; Martins et al., 2015), while the cloacal temperature is essentially constant (between 41 ºC and 41.5 ºC) in broilers nearing slaughter age, since this parameter responds less quickly to environment temperatures variations.

The mean body temperature recorded in the present study under thermal comfort settings are similar to the values reported by Martins et al. (2015) and Welker et al. (2008). Using different kinds of orientation and thermal control in conventional barns, Welker et al. (2008) discovered that MBT may vary between 38 ºC and 39.8 ºC. Higher values indicate heat stress to broilers, since, concomitantly with increase in MST, cloacal temperature also rises, increasing MBT.

Cobb Slow™ broilers had higher cloacal temperatures compared with Hubbard Flex™ broilers (Tab. 3). High cloacal temperatures may indicate a more considerable challenge faced by Cobb Slow™ when losing heat under the temperature settings used in the present study. Besides, the metabolism of Cobb Slow™ is faster in the early production stages (Api et al., 2017), when temperatures are higher.

In the comparison of values for age of broilers, cloacal temperature and MST were higher on day 41 than on day 21, while MBT was higher on day 21, against day 41 (Tab. 3). Cangar et al. (2008) and Bueno et al. (2014) observed a drop in skin temperature with growing age, contrasting with the results obtained in the present study.

By comparison, Marchini et al. (2007) reported that cloacal temperature rises from 40 ºC on day 1 to 41.2 ºC on day 42, when broilers are farmed in a thermally comfortable environment. In turn, Martins et al. (2015) also observed an increase in cloacal temperature with age accompanied by a rise in MBT, while Bueno et al. (2014) reported a drop in MBT.

Broilers keep constant their core body temperature within the limit of their species. Therefore, its production expenditure is maximum. Under cyclic heat stress, broilers manage to recover from this energy loss during the cooler parts of the day, increasing the heat dissipation to the environment at that time and not harming its performance which does not happen under constantly high temperatures.

Broilers feathering on day 41 did not vary among environments and strains (Table 4). The feather cover, according to Nascimento et al. (2011), represents an adaptive response of a broiler to high environment temperatures in the effort to improve heat loss, when heat levels are critical to the bird.

Fukuyama et al. (2005) explained that broilers tend to decrease feathering depending on the level of stress in the environment they are farmed, in the effort to lose excess heat and, therefore, improve performance. This was not observed in the present study, probably because thermal comfort was ensured in the cooler parts of the day, inducing broilers not to resort to this kind of adaptation mechanism.

During heat exposure, litter temperature (mean, central, and corners) were higher than the control group. CLT was lower on day 41 compared with day 21, and no variation was observed in the parameter across the strains used (Tab. 4).

The increase in litter temperature with environment temperature independently of the location values were measured in the box and heat exposure cycles agrees with the findings published by Carvalho et al. (2011). The authors observed that litter temperature is directly proportional to the air temperature of the barn. Mendes et al. (2010) added that, as long as litter temperature does not exceed 36ºC, broilers are exposed to no harmful conditions.

Table 4. Feathering and litter temperature (mean litter temperature, MLT; litter corner temperature, LCT; and central litter temperature, CLT) in boxes Cobb Slow™ and Hubbard Flex™ broilers were farmed under different heat exposure regimens and ages

		Feathering (kg)	MLT (ºC)	LCT (ºC)	CLT (ºC)
Environment	0	0.20	30.6b	31.3c	29.1b
	1	0.18	32.1a	32.3b	31.7a
	2	0.18	32.2a	32.3b	32.0a
	3	0.19	33.1a	33.4a	32.6a
CV%		22.07	3.49	4.23	5.32
Strain	Cobb Slow™	0.18	32.1	32.5	31.3
	Hubbard Flex™	0.19	31.9	32.2	31.4
CV%		18.99	2.45	3.82	4.84
Age	21	-	32.1	32.2	31.9a
	41	-	31.9	32.4	30.8b
CV%		-	2.98	4.41	4.18
P-value	E x S x A	-	0.8275	0.7736	0.4744
	E x S	0.6013	0.4550	0.7860	0.7521
	E x S	-	0.0610	0.0601	0.1115
	L x S	-	0.7172	0.9931	0.3786
	Environment	0.4219	0.0009*	0.0144*	0.0012*
	Strain	0.3287	0.3572	0.2679	0.6644
	Age	-	0.2607	0.6588	0.0017*

Means followed by different letters in the same column differ in the Tukey test (5%).

*Significance: P < 0.05

0 = Control group (environmental temperature and humidity conditions); 1, 2, and 3 = broilers exposed to 1 h, 2 h, and 3 h heat cycles, respectively, starting at 11:00 a.m.

E= Environment, S= Strain and A= Age

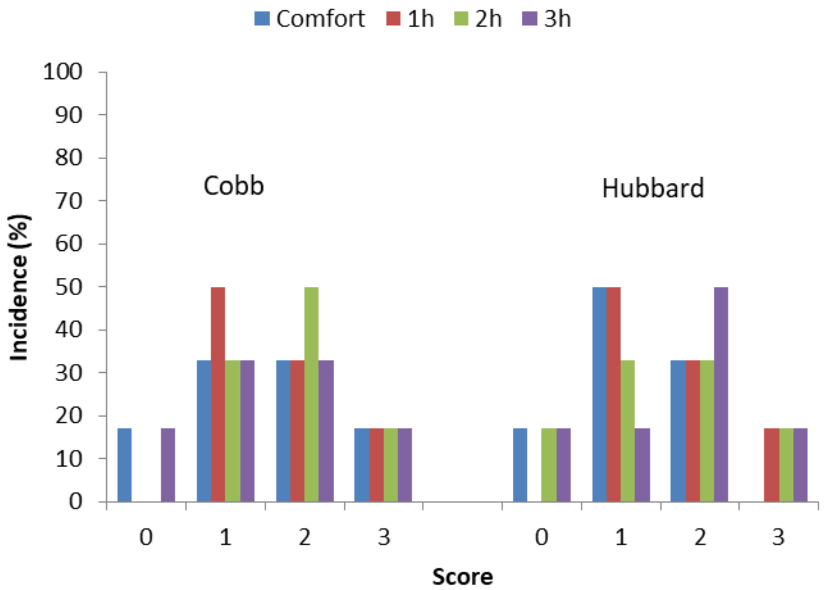
The lower CLT recorded on day 41 compared with the value observed on day 21 (Tab. 4) is different from the findings published by Bueno et al. (2014). The authors observed that litter temperature increases with age of broilers due to the increase in size of these broilers, while the actual physical space they are farmed in remains the same. However, it is possible to assume that the environment temperature required by broilers is lower on day 41 than on day 21. For this reason, CLT was lower for broilers on day 21.

The similarity between litter temperatures of the two strains can be explained by being directly related to the air temperature (Carvalho et al., 2011) and by the structure of the broiler (Bueno et al., 2014) because their growth rate is similar to the end of the production cycle (Api et al., 2017).

Another explanation for this result would be the similar feathering between lineages in this study, which could increase the heat loss by conduction if there were broilers with less amount of feathers.

The incidence of pododermatitis did not differ between values recorded based on environments (P = 0.747) and strains (P = 0.423) (Fig. 2). The level of humidity in litter may be the main factor behind the emergence of callosities on the feet of broilers (Sheperd and Fairchild, 2010). However, in this study, the highest humidity value was 67.9%, which didn't exceed that recommended by Abreu and Abreu (2011), that is 70%.

Figure 2. Incidence of pododermatitis in broilers of two strains on day 42 of age farmed under different heat cycle regimens.



Source: the authors

Another cause of pododermatitis is the type of litter. Mendes and Komiyama (2011) cite that this is more prevalent when wood shavings are used as litter by comparison with rice husk. This explains the pododermatites incidence of the condition in all farming environments. The authors also claim that the incidence of pododermatitis increases in the rainy season.

Sheperd and Fairchild (2010) add that the genetic constitution is another predisposition factor for pododermatitis, which was not observed in the present study, since the incidence of the condition did not vary between the strains used.

Even though the commercial use of feet of broilers was not investigated in the present study, the incidence of pododermatitis should not be neglected. Depending on the score of lesions, broilers may find difficulty to move (for discomfort and pain) in order to feed or drink water, which affects performance and is contrary to the basic principles of animal welfare.

CONCLUSION

Broilers adjust to high temperature cycles for as long as 3 h, from 14 days of age, since feathering is not affected and there is no increase in incidence of pododermatitis. However, litter and broilers temperatures are directly correlated with environment temperatures. Cobb Slow™ strain presents higher cloacal temperature than Hubbard Flex™.

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CHAPTER 4 – FINAL CONSIDERATIONS

Broilers adjust to high temperature cycles for as long as 3 h a day from days 14 to 42 of age, since the histology of the thyroid gland, the serum levels of T_3 and T_4 and feathering of Cobb Slow™ and Hubbard Flex™ broilers is not affected. Also, the broilers conserved metabolism rates and did not change feed consumption; therefore, performance was not reduced. There is no increase in incidence of pododermatites, ensuring the welfare of the broilers.

However, litter and broilers temperatures are directly correlated with environment temperatures.

Under the conditions adopted in the present study, Cobb Slow™ broilers had better live weight at slaughter, compared with the Hubbard Flex™ strain and Cobb Slow™ presents higher cloacal temperature than Hubbard Flex™. Cobb Slow™ is more adapted to the climatic conditions of the Brazilian tropical regions.

Also, T3 levels decreased and T4 levels increased with age in both strains.

ATTACHMENT A – Certificado CEUA/UFU

Universidade Federal de Uberlândia

– Comissão de Ética na Utilização de Animais –

Comissão de Ética na Utilização de Animais CEUA

CERTIFICADO

Certificamos que o protocolo para uso de animais em experimentação nº 065/14, sobre o projeto de pesquisa intitulado "Hormônios tireoidianos e desempenho de duas linhagens de frangos de corte mantidos em estresse cíclico de calor e o rendimento, características, qualidade e composição da carcaça", sob a responsabilidade da **Profª. Drª. Mara Regina Bueno de Mattos Nascimento** está de acordo com os princípios éticos na experimentação animal conforme regulamentações do Conselho Nacional de Controle e Experimentação Animal (CONCEA) e foi **APROVADO** pela Comissão de Ética na Utilização de Animais (CEUA) – UFU em reunião de **29 de agosto de 2014.**

(We certify that the protocol nº 065/14, about "Hormônios tireoidianos e desempenho de duas linhagens de frangos de corte mantidos em estresse cíclico de calor e o rendimento, características, qualidade e composição da carcaça", agrees with the ETHICAL PRINCIPLES ON ANIMAL RESEARCH as regulations of National Advice of Control and Animal Experimentation (CONCEA) and approved by Ethics Commission on Use of Animals (CEUA) – Federal University of Uberlândia in 29/08/2014).

Uberlândia, 01 de setembro de 2014.

Prof. Dr. César Augusto Garcia
Coordenador da CEUA/UFU