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ZOOTECNIA

TIBIA BONE INTEGRITY IN BROILERS SUBJECTED TO CYCLIC HEAT STRESS

INTEGRIDADE ÓSSEA DE TÍBIAS DE FRANGOS DE CORTE SUBMETIDOS AO ESTRESSE CÍCLICO DE CALOR

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Abstract
Macroscopic and microscopic changes in the epiphyseal region of the tibia were recorded in the 42nd day of life of broilers subjected to one-hour heat stress in different rearing phases. The treatments comprised both broilers reared under room temperature and humidity conditions from the 1st to the 42nd day of life (control) and broilers subjected to heat stress from the 16th to the 21st, from the 22nd to the 42nd, and from the 16th to the 42nd day of life. The adopted design was completely randomized with six replicates; 35 broilers were used in each experimental unit. Fragments from the epiphyseal region were extracted and sectioned lengthwise for macro and microscopic analyses. Data on tibia lesion scores were analyzed by Kruskal Wallis test at 5%. The one-hour cyclic heat stress did not change the morphologic integrity in the epiphyseal region of the tibia in the different treatments. Broilers subjected to one-hour cyclic heat stress did not develop lesions that suggested tibial dyschondroplasia, regardless of the rearing phase.
Keywords: bone development; broilers; leg issues; thermal stress; tibial dyschondroplasia.

Resumo
Foram estudadas alterações macroscópicas e microscópicas na região epifisária da tíbia no 42º dia de frangos de corte submetidos ao estresse cíclico de calor por uma hora em diferentes fases de criação. Os tratamentos compreenderam aves criadas em condições naturais de temperatura e umidade relativa do primeiro ao 42º dia de idade (controle), ou estressadas por calor do 16º ao 21º dia, do 22º ao 42º dia e do 16º ao 42º dia. O delineamento utilizado foi o inteiramente casualizado com seis repetições sendo 35 aves por unidade experimental. Para análises macro e microscópicas da tíbia, fragmentos da região epifisária foram extraídos e seccionados longitudinalmente. Os dados de escores de lesões tibiais foram analisados pelo teste de Kruskall Wallis a 5%. O estresse cíclico por calor por uma hora não alterou a integridade morfológica da região epifisária da tíbia nos diferentes tratamentos. Frangos de corte submetidos por uma hora diária ao estresse de calor não desenvolvem lesões sugestivas de discondroplasia tibial independente da fase de criação.

Palavras-chave: ave; desenvolvimento ósseo; discondroplasia tibial; estresse térmico; problemas de pernas.

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Introduction

High room temperature is an important environmental factor for poultry production, mainly in the inter-tropical region[1]. When broilers are under heat stress they undergo behavioral changes and their organisms respond with hormonal, physiological, and chemical changes in order to keep homeostasis[2]. This fact that characterizes a stress condition[3].

The housing environmental conditions broilers are subjected to may also trigger locomotion issues due to changes in the acid-base balance[4]. Chlorine ion (Cl^-) levels in the plasma increase under such condition, which leads to H^+ ions excretion reduction and HCO_3^- reabsorption by the kidneys. These changes contribute to blood acidification in response to the respiratory alkalosis caused by heat stress[5]. Therefore, electrolytic balance changes generated by heat-caused organic stress may affect bone development and increase the incidence of leg issues in broilers[4,6].

Tibial dyschondroplasia is among the most frequent diseases associated with leg issues in broilers. It often occurs between the second and the fifth week of life[7] in fast growth broilers, mainly in males[8]. The disease is characterized by lesions that are most commonly found in both pelvic members[9].

Despite the great number of studies on the subject, dyschondroplasia still lacks total clarification. This disease may be caused by multiple factors, but fast growth[10] and electrolytic imbalance (cation/anion) are its most likely causes[11,12]. These factors may impair calcification and result in the cartilaginous mass accumulation in the epiphyseal region of the tibia and the femur. These lesions are typical of tibial dyschondroplasia and they could represent another adaptive response triggered by stressful conditions, such as heat stress, over the animal's organism[13].

Most studies about the effect of heat stress on broilers have been conducted in climatic chambers adjusted to high and constant room temperatures[14-17]. However, room temperature is not constant under natural conditions. There are air diurnal variations that often rapidly increase after 08:00 am, and the highest temperatures peak between 12:00 am and 03:00 pm. After that, temperature decreases and gets stable at 09:00 pm[18].

By considering that there are yet little reports on the duration, the challenging periods, and the effects of heat cyclic stress on bone integrity of broilers' locomotion limbs, it becomes necessary to investigate such aspects.

Therefore, the current study aims to investigate the effect of one-hour daily cyclic heat stress on the tibia bone integrity of *Cobb* Avian48^{TM} male broilers housed in an opened side house with conventional ventilation and nebulization systems and challenged between 16 and 21, 22 and 42, and 16 and 42 days of age.

Material and Methods

The present research was conducted at the broiler house of Gloria Experimental Farm of Universidade Federal de Uberlândia. All the procedures adopted in the current study were performed according to Protocol Registration CEUA/ UFU 024/10, which was approved by the Ethics Committee for the Use of Animals of Universidade Federal de Uberlândia.

Eight hundred and forty one-day old Cobb Avian48^{TM} male broiler chicks were used in the experiment and housed in an opened side house with conventional ventilation and nebulization systems. They were kept under comfortable thermic conditions from the 1st to the 15th day of life, according to Cassuce et al.[19]. Subsequently, the sample was transversally divided by double face plastic canvas into four different environments: the control group and three other groups, each of them kept under heat cycles from the 16th to the 21st day of life; from the 22nd to the 42nd day of life; and from 16th to the 42nd day of life. One hour under temperatures higher than 36 °C, from 12:00 am to 01:00 pm– on a daily basis–, was considered to be the cyclic heat stress period. The experiment was performed in a completely randomized design with four treatments (environments) and six replicates with 35 broilers each.

All animals were supplied with potable water (3-5 mg/mL chlorine) and fed *ad libitum.* Feeds were formulated to supply the nutritional levels recommended by Rostagno et al.[20] and formulated based on sorghum, soybean meal, degummed soybean oil, dicalcium phosphate, limestone, sodium chloride, vitamin and mineral supplement, besides the commercial additives. Feeds also answered the requirements of the description phases: pre-initial (from 1 to 7 days old), initial (from 8 to 21 days old), fattening (from 22 to 33 days old) and slaughter (from 34 to 42 days old). The used lightening program was as follows, according to COBB[21]: from 1 to 7 days, two hours in the dark; from 8 to 21 days, four hours in the dark; from 22 to 42 days, two hours in the dark. As for featuring the thermic environment (Figure 1) in each group of broilers challenged at different ages (16-21, 22-42, and 16-42), the temperature and the relative humidity were monitored daily at 10:00 am and 05:00 pm with the aid of six psychrometers placed 3 meters from each other, 30 cm from the poultry litter. Subsequently, the means and standard variations in the periods of 16-21, 22-28, 29-35, and 36-42 days of life were calculated and were compared to the maximum and minimum temperatures recommended by the standards for the strain[21].

Starting from the 16th day, the environment was pre-heated by infrared brooder heating for 10 minutes, before the one-hour period to reach heat stress temperatures. Throughout the stress period, the lateral curtains were closed and the fans were kept on to ensure aeration in the environment. Water and feed were supplied to all broilers. During this time, temperature and air relative humidity were monitored every 10 minutes and the means and standard deviations (Figure 2) were calculated in the periods from 16 to 21, 22 to 28, 29 to 35, and 36 to 42 days of life in each group. The broilers were challenged at different ages (16-21, 22-42, and 16-42 days of life) and compared at maximum and minimum temperatures according to the thermal comfort recommended by the standard for the strain. By the end of the heat stress period, the curtains were opened in order to reach natural temperature and humidity conditions within 10 minutes. Whenever necessary, fans and nebulizers were turned on to achieve thermal comfort conditions.

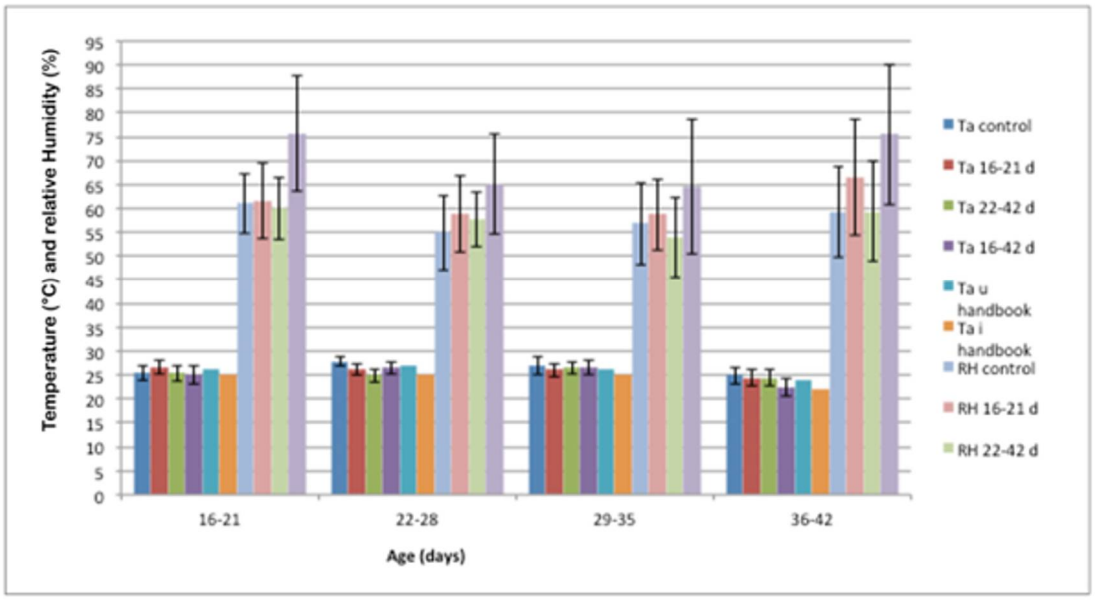

Figure 1. Weekly temperature means and standard deviations of dry bulbs (Ta) (°C) and relative humidity (RH) (%) outside the heat stress period in the following treatments: kept under natural temperature and humidity conditions from the 16th to the 42nd day of age (Ta control) and subjected to cyclic heat stress for one hour from the 16th to the 21st (Ta 16-21 d), from the 22nd to the 42nd (Ta 22-42 d), and from the 16th to the 42nd days (ta 16-21 d) of life, and inferior (Ta i handbook) and upper (ta u handbook) temperatures of thermal comfort suggested by the standard for the strains at each breeding age (days).

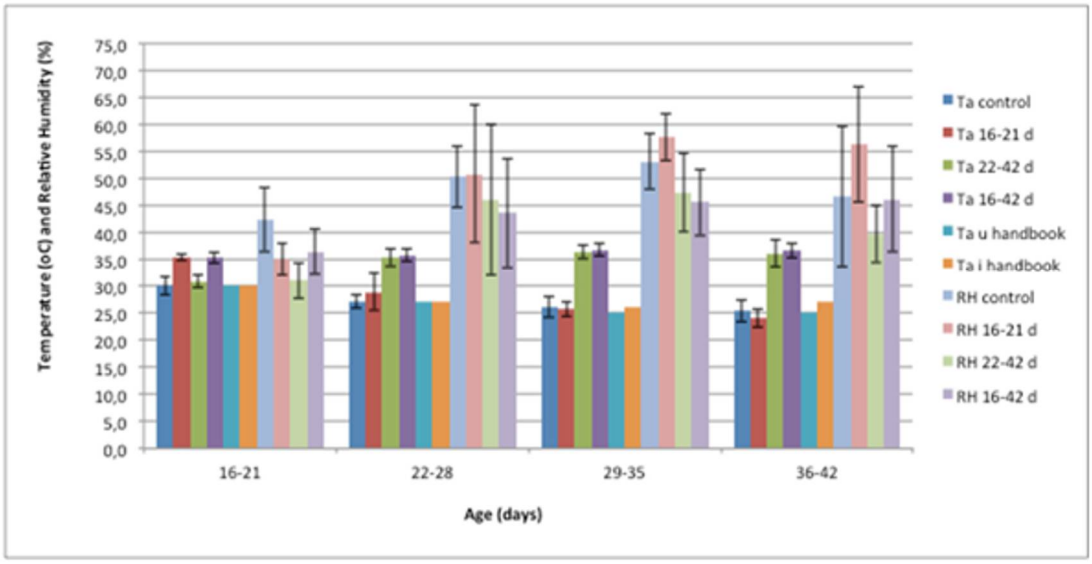

Figure 2. Temperature means and standard deviation of a dry bulb (Ta) (°C) and relative humidity (RH) (%) throughout the heat stress period within the following environments: natural temperature and humidity conditions from the 16th to the 42nd day of life (Ta control), heat stress for an hour from the 16th to the 21st (Ta 16-21d), from the 22nd to the 42nd (Ta 22-42 d), and from the 16th to the 42nd (Ta 16-42 d) days of life, and inferior (Ta i manual) and upper (Ta u manual) temperatures of thermal comfort recommended by the strains' management handbook at each breeding age (days).

Six broilers, at 42 days of age, from each thermic environment representing the mean weight from each experimental portion, were chosen and subjected to euthanasia–by means of cervical

dislocation–for the microscopic analysis of tibia bone integrity in the epiphyseal region. Three-centimeters-long fragments were extracted from the epiphyseal region of the tibia and sectioned lengthways. Scores were set to the bone integrity and they varied from 0 to 2 (Figures 3A, B, and C), according to the methodology described by Almeida Paz[22], who set the following scores: score 0 = normal bone, without epiphyseal growth plane thickening; score 1 = lesion in which the growth plate presents thickening varying from 1 to 3 mm; score 2 = lesion in which the growth plate presents thickening greater than 6 mm (Figures 3A, B, and C).

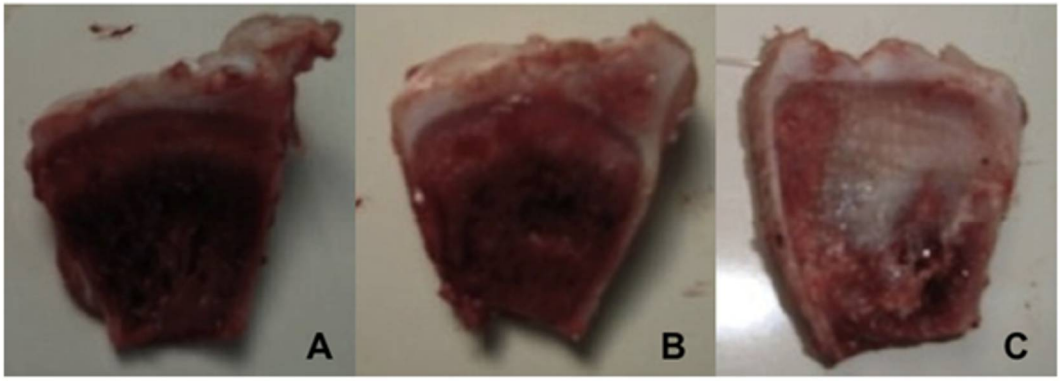

Figure 3. Lengthwise cuts from the epiphyseal region of the tibia presenting macroscopic lesion with score 0 – normal bone presenting pineal gland without growth plate thickening (A); score 1 – growth plate with thickening lesion varying from 1 mm to 3 mm (B); and score 2 – growth plate with thickening lesion greater than 6 mm (C) found in *Cobb*Avian48™ male broilers from different breeding environments: kept under natural temperature and humidity conditions (control) and subjected to heat cyclic stress for one hour at ages from 16 to 21, from 22 to 42, and from 16 to 42 days.

The samples collected for macroscopic analyses were fixed in 4% formaldehyde solution, buffered at pH 7.4 for 24 hours, decalcified in 80% alcohol solution and nitric acid, embedded in paraffin, cut 4 μm thick, stained in hematoxylin and eosin. The blades were assembled according to Carvalho et al.[23]. These same fragments were used for the microscopic analyses of cellular structure and arrangement in the epiphyseal region of the tibia. Lesion scores were given to the fragments based on the structure, arrangement and condition evaluation of the bone cells in the growth plate of the tibia, according to the methodology described by Almeida Paz[22], who have set the following scores: score 0, normal growth plate presenting proliferative zone regions, well distinct pre-hypertrophic zone, and hypertrophic zone; score 1, presenting growth plate with large edge and distinct from pre-hypertrophic chondrocytes; score 2, presenting growth plate with degenerative changes in the cells (Figures 4, 5, and 6).

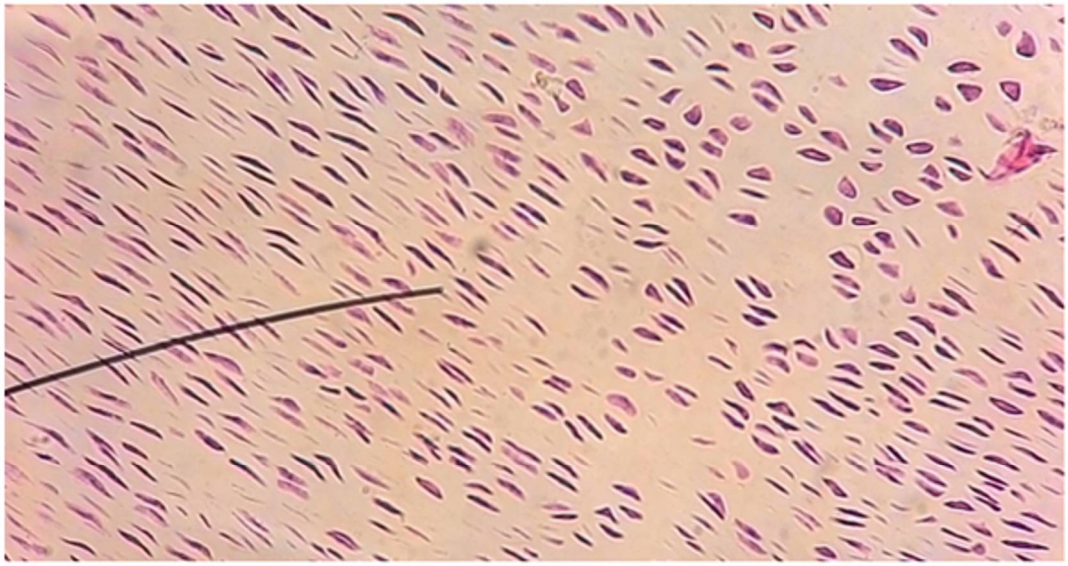

Figure 4. Histological section in the epiphyseal region of the tibia from broilers at the 42nd day of life presenting score 0, i.e., normal growth plate with proliferative zone regions, well distinct pre-hypertrophic zone and hypertrophic zone (arrow). H. E., magnification 40X.

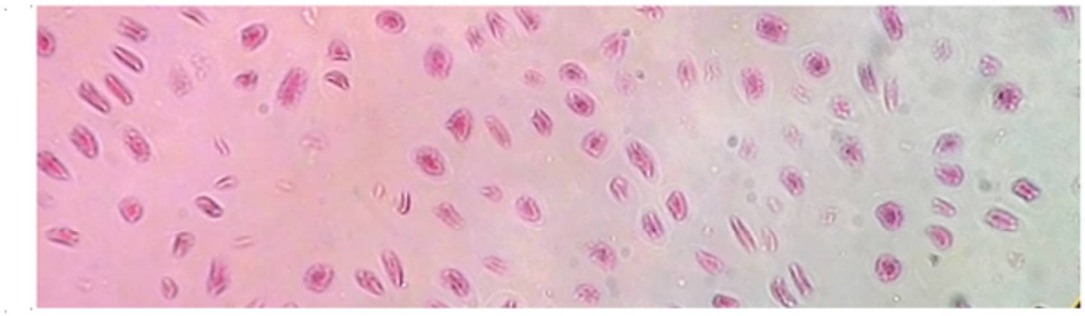

Figure 5. Histoligical section in the epiphyseal region of the tibia from broilers at the 42nd day of life presenting score 1, i.e., growth plate with large edge and distinct pre-hypertrophic chondrocytes. H.E., magnification 40X.

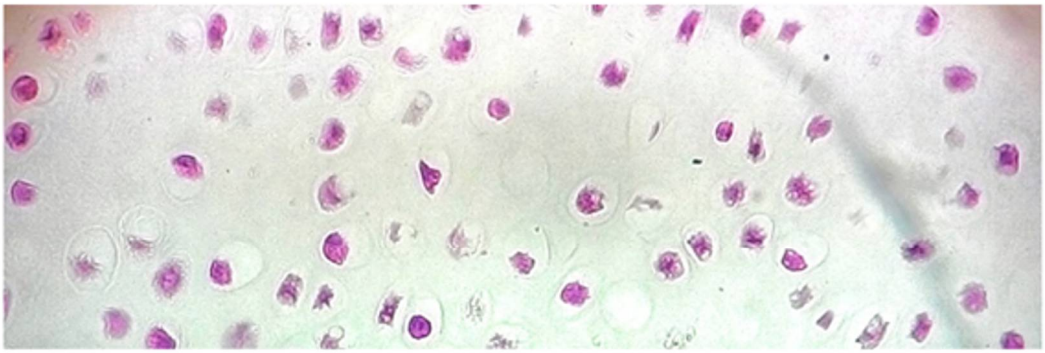

Figure 6. Histological section in the epiphyseal region of the tibia from broilers at the 42nd day of life presenting score 3, i.e., growth plate with degenerative changes in the cells. H.E., magnification 40X. Data of macroscopic and microscopic tibia lesion scores were analyzed by means of Kruskal Wallis test at 5%, using the Biostat software.

Results and Discussion

*Cobb*Avian48™ male broilers exposed to room temperature varying from 36 °C to 36.8 °C for one hour, from 16th-21st, 22nd-42nd, and 16th-42nd day of life, did not present changes in macroscopic (p=0.03) and microscopic features in the epiphyseal region (p=0.06) of the tibia (Table 1).

Table 1. Macroscopic and microscopic lesion scores of tibia's epiphyseal region from *Cobb*Avian48™ male broilers at the 42nd day of life, kept under room temperature and humidity conditions (control) and subjected to cyclic heat stress for an hour from 16th-21st, 22nd-42nd, and 16th-42nd day of life

Repetition	Control	Cyclic heat stress		
		16th to 21st day	22nd to 42nd day	16th to 42nd day
	Macroscopic lesions[1]			
1	0	0	2	1
2	0	1	0	0
3	0	0	1	2
4	0	0	1	0
5	0	2	2	1
6	1	0	0	1
Microscopic lesions[2]				
1	0	0	2	1
2	0	1	0	2
3	1	1	1	2
4	0	0	2	1
5	0	2	2	1
6	1	0	1	1

Score values analyzed by means of Kruskal Wallis test at 5%

[1]Macroscopic lesion scores: score 0 = normal bone presenting pineal gland without growth plate thickening; score 1 = growth plate presenting thickening lesion varying from 1mm to 3mm (initial lesion); score 2 = growth plate presenting thickening lesion greater than 6mm.

[2]Microscopic lesion scores: score 0, normal growth plate presenting proliferative zone regions, well distinct pre-hypertrophic zone and hypertrophic zone; score 1, presenting growth plate with large and distinct edge of pre-hypertrophic chondrocytes; score 2, presenting growth plate with degenerative changes in the cells.

According to Borges et al.[5,24], heat stress leads to important changes in broilers' electrolytic balance since they increase respiratory frequency in order to diminish body temperature[2,25]. Panting results in acid-base unbalance and in respiratory alkalosis due to both the severe CO_2 elimination and pCO_2 reduction[25] and the reduction in plasma calcium levels, blood pCO_2 and HCO^-_3 in broilers subjected to 34 °C after 6, 12, and 18 hours exposure, respectively, during panting[26].

The reduction in the carbonic anhydrase enzyme activity results in bicarbonate and carbonate reduction[27], as well as in the reduction of ionized free calcium available for bone deposition[28,29]. As the ionized free calcium reduction takes place, the calcification process can be impaired. It favors hypertrophic zone area increase due to the calcification deficiency in the chondrocytes that have remained pre-hypertrophic. Such process results in the accumulation of cartilaginous mass, which is not calcified in the area, characterizing thus tibia dyschondroplasia[30].

Therefore, the calcification process may be impaired and an increase in the hypertrophic zone area may be favored due to the non-calcification of chondrocytes that would remain pre-hypertrophic. It would then result in the accumulation of the cartilaginous mass that does not calcify in this area, which characterizes tibial dyschondroplasia. Due to the great diversity of causes and to the absence

of common factors linking all causing agents, it is possible that one-hour cyclic heat stress might have generated just a stress state that helped to change some physiological mechanism able to result in pre-hypertrophic chondrocytes and in cartilaginous mass formation: cellular changes that characterize tibial dyschondroplasia.

The results presented herein contrast those reported by Sahin et al.[29], who have shown that the exposition of Japanese quail (*Coturnix coturnux japonica*) to cyclic heat stress (34 °C, from 09:00 am to 05:00 pm, from the 10th to the 42nd day of life) reduced calcium, phosphorus, magnesium and manganese concentration in the tibia due to the higher excretion of these minerals; a fact that has impaired bone mineralization. Likely, the heat exposure time has influenced such response, namely: in the current study, broilers were exposed to heat for one hour a day rather than for eight hours.

As for the present experiment, broilers recovered their metabolic balance during the thermo-neutral phase of 23 daily hours when the room temperature was within the comfort limit set by the standards for the strain for different development phases (Figure 1). Therefore, the stress was not intense and long enough to trigger severe and long metabolic unbalance able to impair calcium mobilization and deposition in the bone, and consequently, to impair chondrocyte maturation.

There are other factors to be considered and they may partially explain the herein results. Broilers subjected to cyclic heat stress present a reduction in thyroid hormone level[31]. In this case, the frequency of bone remodeling activation is reduced and the remodeling phase is prolonged, as it was observed by Sahin et al.[29], who showed that cyclic heat stress led to impairment of bone mineralization due to an increased excretion of tibial calcium, magnesium, manganese, and phosphorus concentrations. As thyroid hormone levels were not assessed in the current study, future investigations about this particular aspect should be performed.

Conclusion

The one-hour cyclic heat stress did not change the morphologic integrity in the epiphyseal region of the tibia in the different treatments. Broilers subjected to one-hour cyclic heat stress did not develop lesions that suggested tibial dyschondroplasia, regardless of the rearing phase.

Acknowledgments

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