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Thermoregulatory responses and reproductive traits in composite beef bulls raised in a tropical climate

Narian Romanello[1] · José de Brito Lourenço Junior[1] · Waldomiro Barioni Junior[2] · Felipe Zandonadi Brandão[3] · Cintia Righetti Marcondes[2] · José Ricardo Macedo Pezzopane[2] · Messy Hannear de Andrade Pantoja[1] · Daniela Botta[1] · Alessandro Giro[1] · Ana Beatriz Bossois Moura[3] · Andréa do Nascimento Barreto[1] · Alexandre Rossetto Garcia[2]

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Abstract
It is believed that increased livestock production is limited by tropical climate. Thermal imbalance in bulls can lead to hyperthermia and alter testicular metabolism, causing subfertility or infertility. Therefore, the thermoregulation of composite Canchim bulls (5/8 Charolais × 3/8 Zebu) raised in tropical climate as well as their consequences in the physiological, hematological, hormonal, and andrological parameters were evaluated monthly. The bulls ($n = 18$; 30.0 ± 1.5 months; 503.8 ± 23.0 kg) were kept on pasture, in a single group, from August 2015 to March 2016, comprising the winter, spring, and summer seasons. Biometeorological variables were continuously monitored, and the Temperature and Humidity Index (THI) was calculated. A greater thermal challenge occurred in spring and summer (THI ≥ 72.0). Nevertheless, the bulls exhibited normothermia (38.6 to 38.9 °C) in these seasons. The cortisol did not vary between seasons (7.0 vs. 8.7 vs. 6.8 ng/mL; $P > 0.05$) and remained within the physiological patterns. Independent of the seasons, stress leukogram was also not observed, refuting the incidence of acute or chronic thermal stress. It is noteworthy that T3 and testosterone increased ($P < 0.0001$, $P < 0.05$) in spring and summer, the time that coincides with the breeding season, when there is increased metabolic requirement from the bulls. The progressive thermal challenge increase did not affect the scrotal thermoregulatory capacity, and in general, scrotal temperature remained at 5.2 °C below the internal body temperature. In summer, there was a 5% reduction in the minor sperm defects ($P < 0.05$) and DNA fragmentation in 2.4% of spermatozoa, a compatible value for high fertility bulls. The results show that the studied composite bulls can be considered as climatically adapted and constitute

✉ Alexandre Rossetto Garcia
alexandre.garcia@embrapa.br

Narian Romanello
narian.r_@hotmail.com

José de Brito Lourenço Junior
joselourencojr@yahoo.com.br

Waldomiro Barioni Junior
waldomiro.barioni@embrapa.br

Felipe Zandonadi Brandão
fzbrandao@id.uff.br

Cintia Righetti Marcondes
cintia.marcondes@embrapa.br

José Ricardo Macedo Pezzopane
jose.pezzopane@embrapa.br

Messy Hannear de Andrade Pantoja
messy.andrade@yahoo.com.br

Daniela Botta
dani_botta@hotmail.com

Alessandro Giro
giro.aless@gmail.com

Ana Beatriz Bossois Moura
anabeatriz.bossois@gmail.com

Andréa do Nascimento Barreto
andreadnb91@gmail.com

[1] Federal University of Pará, Av. dos Universitários, s/n, Castanhal 68746-360, Brazil

[2] Laboratory of Biotechnology and Animal Reproduction, Brazilian Agricultural Research Corporation, Embrapa Livestock Southeast, Rod. Washington Luiz, km 234, São Carlos 13560-970, Brazil

[3] Fluminense Federal University, Rua Vital Brazil, 64, Niterói 24230-340, Brazil

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a viable alternative to be used in production systems in a tropical climate, even if the breeding seasons occur during the most critical thermal condition periods of the year.

Keywords Tropical livestock · Thermoregulation · Animal welfare · Homeothermy · Infrared thermography · Semen quality

Introduction

The urban population expansion associated with the progressive increase of household income will likely increase the world demand for food in the coming years, especially in developing regions (Godfray et al. 2010; FAO 2015). Recent projections indicate that over the next decade, the world consumption of meat will grow by 1.9% per year and that the international beef trade should increase by 2.8% per year (USDA 2014). This scenario requires immediate action by food-producing countries aiming for greater efficiency and productivity in the livestock farming.

Among the largest exporters of beef, some countries with a large part of their territories located in the tropical region of the planet stand out, such as Brazil, India, Australia, and New Zealand (USDA 2014). However, the tropical weather can be a factor considered as limiting for the increased livestock productivity, since it affects the homeostasis of animals (Strong et al. 2015), especially the breeds originating from temperate climate (Nichi et al. 2006; Srikandakumar and Johnson 2004). When subjected to intense solar radiation and high ambient temperatures, animals become more susceptible to circulatory, respiratory, and endocrine changes in an attempt to lose body heat and remain in thermal equilibrium (Kahwage et al. 2017; McManus et al. 2009), which reduces the energy supply for the productive processes.

The thermal discomfort also negatively interferes in animal welfare and production, besides being detrimental to the reproductive processes, with consequent economic losses (Curtis et al. 2017). In bulls, thermal stress can cause gonadal changes with varying degrees of severity (Garcia 2017). In situations of hyperthermia, when scrotal thermoregulation does not occur properly, testicular metabolism increases and there is a reduction in local blood supply. This leads to cellular hypoxia and, consequently, to the establishment of a pathological condition of testicular degeneration (Fernandes et al. 2008), which causes reduced seminal quality and may lead to subfertility or infertility (Lucio et al. 2016).

In this context, the selection of breeders best suited for use in beef cattle production systems in a tropical environment should be among the very important selection criteria. Therefore, the objective of the present study was to evaluate the thermoregulatory response of composite breed bulls raised in a tropical climate, from winter to summer, and its consequences on physiological, hematological, hormonal, and andrological parameters.

Material and methods

Location and climatic characterization

The experiment was carried out between August 2015 and March 2016, at the Brazilian Agricultural Research Corporation in São Carlos, Brazil (21° 56′ 23″ S, 47° 50′ 17″ W, altitude 854 m). The local climatic type is Cwa, tropical altitude, according to the Köppen-Geiger climate classification (Kottek et al. 2006), with four climatic seasons defined as follows: winter (June to September), spring (September to December), summer (December to March), and autumn (March to June). During the year, the maximum air temperature ranges from 29.2 to 36.6 °C with peaks of up to 38.0 °C. The mean relative air humidity ranges from 55.3 to 90.5%. The annual rainfall is 1361 mm, and the average solar radiation in summer is 241.3 W/m^2/day (Embrapa 2016).

Biometeorological variables and Temperature and Humidity Index

The air temperature (AT, °C), relative air humidity (RH, %), accumulated rainfall (Rainfall, mm), and solar radiation (SR, W/m^2) were continuously monitored in an automatic weather station installed at the paddock. Subsequently, the Temperature and Humidity Index (THI) was calculated as follows: THI = [(0.8AT) + RH(AT − 14.3) + 46.3] (Thom 1959). Its interpretation was based on the scale used to categorize the levels of thermal stress in cattle: 70.0 ≤ THI < 74.0, animals potentially subject to thermal stress; 74.0 ≤ THI < 79.0, animals on alert; 79.0 ≤ THI < 84.0, animals in distress; and THI ≥ 84.0, animals in a state of emergency (LCI 1970).

Experimental animals and management

Eighteen Canchim bulls (5/8 *Bos taurus* × 3/8 *Bos indicus*) were used, a composite breed characterized by the white to yellow coat color, short hair, and pigmented skin. At the beginning of the experiment, the animals were 30.0 ± 1.5 months of age, had a live weight of 503.8 ± 23.0 kg and a body condition score of 5.5 ± 0.1 (scale from 1 to 9), and were clinically healthy. The bulls were previously selected from a herd of 74 contemporary males, with similar clinical reproductive condition and body condition score, to guarantee the homogeneity of the individuals. The selected animals were allocated in a single plot, under rotational grazing in a *Brachiaria decumbens* area, with natural shading offered by remaining trees distributed in

the paddocks (36.2 m^2/animal). The animals were kept under the same conditions of sanitary and nutritional management, with ad libitum access to automatic drinking fountains and mineral salt in covered troughs. At the end of the experiment, the bulls presented a live weight of 667.4 ± 22.9 kg and a body condition score of 6.3 ± 0.1. The management adaptation period was 15 days, in the month of July.

Experimental design

The animals were evaluated from August 2015 to March 2016, a period that comprised the winter, spring, and summer seasons. Monthly, the physiological and hematological parameters, body surface temperatures, and hormonal concentrations were evaluated in the animals. Also monthly, testicular parenchyma integrity and seminal quality assessments were performed. For each variable, measurement campaigns were carried out in the winter (twice), in the spring (four times), and in the summer (four times). The animals were kept in cultivated pastures and on the physiological assessments days they were gently taken to the handling pen early in the morning, adjacent to the pasture area. In the corral, they waited for 15 min in an area devoid of shading before the beginning of the measurements to avoid the influence of management and the facilities on the analyzed variables. Next, each animal was contained in a cattle crush sheltered from direct solar radiation and rain and was immediately evaluated. The reproductive assessments were always carried out 7 days after the others, so as not to interfere in the records of the features that have responses more sensitive to instantaneous variations. The physiological evaluations were performed in the cattle crush by a permanent team of four experienced members, while the evaluations of testicular parenchyma integrity and seminal quality and the interpretation of the thermograms were always performed by the same technician. Physiological evaluations always occurred between 0700 hours and 0930 hours. The data were grouped by climatic season to verify the possible seasonal changes of the variables.

Physiological parameters, hemogram, and hormonal dosages

The respiratory rate (RR, breaths/m) and rectal temperature (RT, °C) were checked in the morning shift (0700 hours to 0930 hours), on typical days of each season and not on rainy days. The RR was measured by observation of the thoracoabdominal region, and the RT was evaluated by digital thermometry. Subsequently, blood was collected by jugular venipuncture in vacuum tubes containing 10% EDTA. The samples were refrigerated at 8 °C and immediately transported to the laboratory for complete blood count. The values were for red blood cells (RBC, 10^6/μL), hematocrit (%), hemoglobin (g/dL), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, %), platelets (10^6/μL), leukocytes (10^6/mm^3), lymphocytes (10^6/mm^3), neutrophils (10^6/mm^3), eosinophils (10^6/mm^3), and monocytes (10^6/mm^3) (Roland et al. 2014).

For determination of serum hormone concentrations, blood was collected in siliconized vacuum tubes. The samples were centrifuged at 1350×*g* for 15 min, fractionated in aliquots, and stored in microtubes at − 20 °C. Cortisol dosages were performed with the ImmuChem™ Cortisol Coated Tube RIA kit (MP Biomedicals, LCC Diagnostics Division, USA). The sensitivity and intra-assay coefficients were 0.17 μg/dL and 11%, respectively. The triiodothyronine (T3) dosages were carried out with T3 Antibody Coated Tubes kit, T3 Tracer [125I], and T3 Standards Set (MP Biomedicals, Diagnostics Division, USA). The sensitivity, the intra-assay coefficient, and the inter-assay coefficient were 6.7 ng/dL, 9%, and 10%, respectively. For the determination of testosterone, the ImmuChem™ Testosterone Double Antibody kit was used (MP Biomedicals, Diagnostics Division, USA). The sensitivity and intra-assay coefficient were 0.03 ng/mL and 12.2%, respectively. All data were within the maximum and minimum points of the curve.

Surface temperatures

The body surface temperature was evaluated by infrared thermography. A portable thermography camera (FLIR T300; FLIR Systems, USA), with thermal sensitivity of 0.05 °C and optical resolution of 320 × 240 pixels, was used. The emissivity adopted was 0.98 (Hoffmann et al. 2013). The images were generated by the same operator, and the following anatomical regions were considered: right orbital area (ORB), right flank (RFL), and scrotum (SCR), always in this sequence (Fig. 1). The distances used between the thermograph and the animal were standardized: 0.5 m for ORB, 2.0 m for RFL, and 1.0 m for SCR (Hoffmann et al. 2013; Menegassi et al. 2015; Barros et al. 2016). The generated thermograms were analyzed in laboratory using FLIR Tools software (version 5.6; FLIR Systems, USA). The maximum values were considered (hot spots) for the ORB temperature, and their analysis was obtained by circular tracing over the orbital region, including the ocular globe (Schaefer et al. 2012; Hoffmann et al. 2013). The RFL temperature was analyzed by rectangular tracing superimposed on the flank region and its value presented as the mean (Montanholi et al. 2008). The SCR temperature was measured by free hand tracing, comprising the entire scrotal region, from the vascular cone to the ventral testicular pole, and its result was expressed as the mean temperature (Vencato et al. 2014).

Biometry, consistency, and testicular ultrasound

The scrotal perimeter was measured with a malleable graduated tape in millimeters (CBRA 2013). To determine the testicular

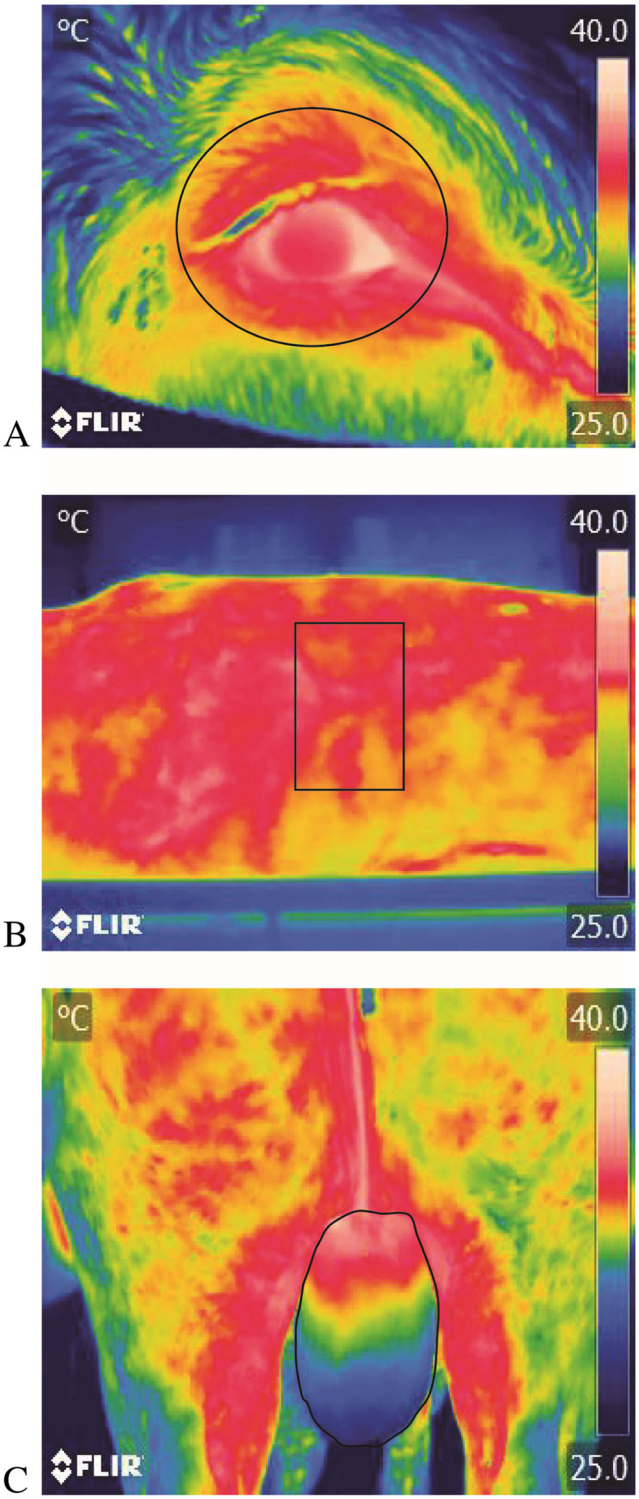

Fig. 1 Illustrative images of thermograms of Canchim bulls. **a** Right eyeball. **b** Right flank. **c** Scrotum. The tracings indicate the areas analyzed. Parameterized for rainbow color palette and thermal scale from 25.0 to 40.0 °C

volume (TV, cm^3), the width and length of the testicles were individually measured using a metal caliper (mm). Then, the testicular volume was calculated by the following equation: $TV = 4 / 3\ (\Pi)(L / 2)(W / 2)^2$, where L is the length of the testicle (cm), W is the width of the testicle (cm), and $\Pi = 3.1415$ (Bailey et al. 1998). The evaluation of the testicular consistency (CONS, 1 to 5) was performed by bilateral palpation and the tonus classified on a scale, where 1 = flaccid and 5 = firm (CBRA 2013). The testicles were then evaluated by ultrasound examination in mode B (DP-3300 Vet; Mindray, China), with a linear probe of 7.5 mHz. The parenchyma of the right and left testicles was evaluated for homogeneity (HOM, 0 to 2) and hyperechogenicity (HYP, 0 to 3), and their classification followed previously established diagnostic criteria (Kahwage et al. 2017). The results of testicular volume, consistency, homogeneity, and hyperechogenicity were presented as the mean of contralateral testicles.

Semen quality

Semen samples from all the bulls were collected by electroejaculation (Nichi et al. 2006). The samples were maintained at 37 °C during the execution of the immediate gross motility (GM, 0 to 5), sperm vigor (VIG, 1 to 5), and progressive sperm motility (PM, %) analyses performed by bright-field optical microscopy (CBRA 2013). The spermatic concentration (CONC, $\times 10^6$ sptz/mL) was evaluated in a Neubauer counting chamber (1:200), and the spermatozoid plasma membrane integrity (PMI, %) was evaluated by the hypoosmotic swelling test (Revell and Mrode 1994). The chromatin fragmentation (CFrag, %) was evaluated by the toluidine blue-stained smear technique (Beletti and Mello 2004). The spermatic morphology analysis was performed under phase contrast microscopy, with a magnification of × 1000. The abnormal cells were classified according to their morphological characteristics, as carriers of minor defects (MiDef, %) or major defects (MaDef, %). The total defects (TDef, %) represent the sum of the MiDef and MaDef in each sample (Bloom 1973).

Statistical analysis

The data for all variables were submitted to analysis of variance using the GLM procedure of SAS (SAS 2011). For each variable in the model, the effect of the climatic seasons was considered (winter, spring, and summer; Y = season + error), with the animal as the experimental unit ($n = 18$). The multiple comparisons between means were performed by the LSMEANS option, using the Tukey test. $P < 0.05$ was considered to indicate a statistically significant result.

Results

The variation of climatic elements that occurred along the seasons is shown in Fig. 2. High temperatures and relative humidity were recorded in spring and summer. As a

consequence, the average Temperature and Humidity Index of these seasons presented values of 72, reaching a maximum value of 86 in the summer and 87 in the spring.

Respiratory rate and rectal temperature were significantly higher in winter and spring (Table 1).

The corpuscular hemoglobin values had a significant difference between winter and summer (Table 2). Hematocrit and mean corpuscular volume were lower in winter, while hemoglobin was higher in summer. The mean corpuscular hemoglobin concentration was also higher in winter and summer.

The platelet value presented a significant difference between winter and summer (Table 3). Leukocyte and neutrophil values were lower in winter, while lymphocyte values did not differ. There was a difference for eosinophils between winter and spring, while the highest value for monocytes was recorded in the spring.

Cortisol did not differ between seasons, whereas triiodothyronine had lower values during winter. In turn, testosterone showed a higher concentration in summer when compared to winter (Fig. 3).

Ocular globe and right flank temperatures showed no significant difference between seasons (Fig. 4). However, scrotal temperature was lower in summer when compared to winter.

The scrotal perimeter showed an increase throughout the seasons, with a higher value observed in the summer (Table 4). There was no difference in testicular consistency in any of the seasons. The testicular volume presented higher values in spring and summer. The homogeneity of the

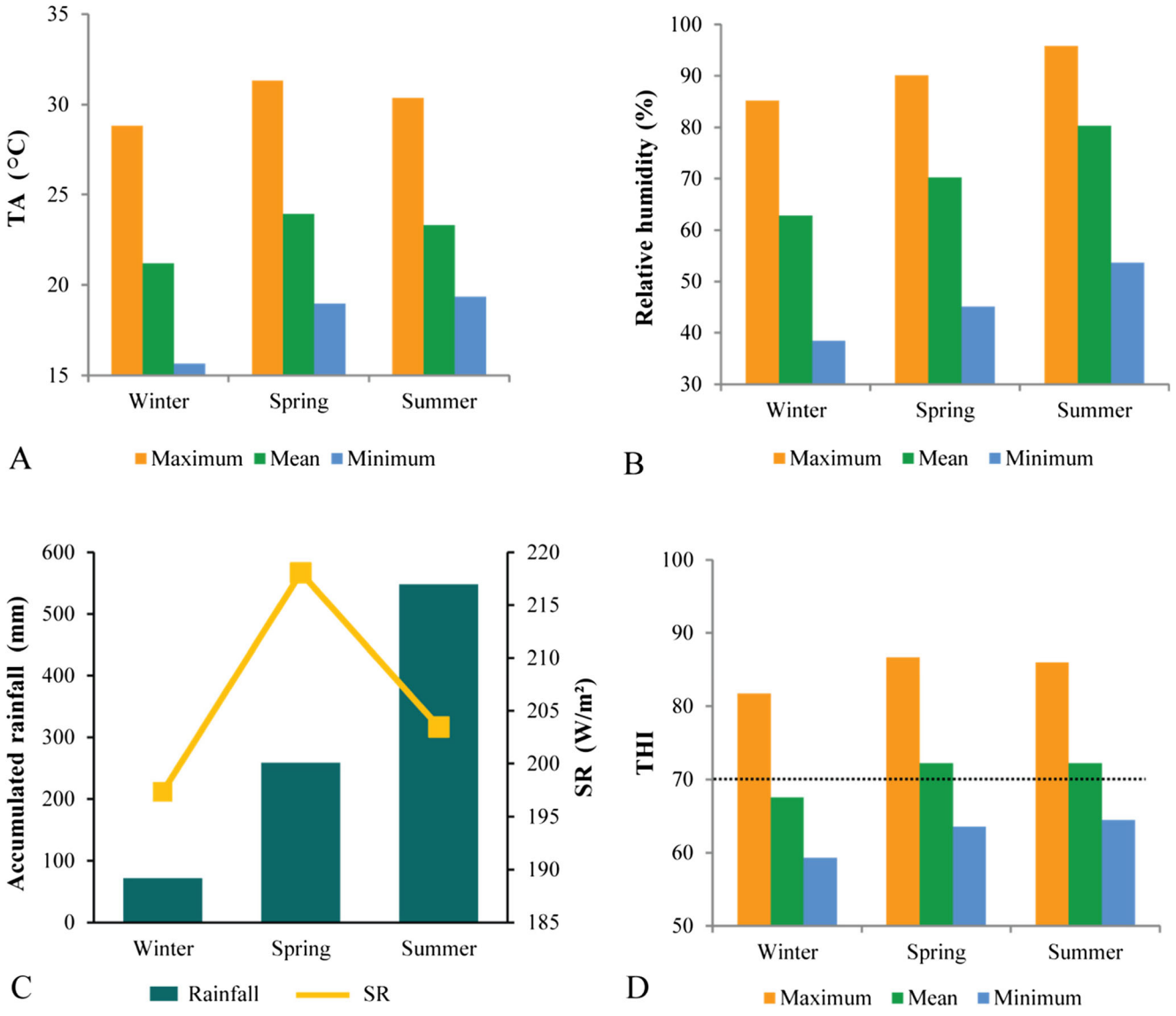

Fig. 2 Values of **a** mean air temperature (AT), **b** mean relative air humidity (RH), **c** accumulated rainfall (Rainfall) and average solar radiation per day (SR), and **d** Temperature and Humidity Index (THI) per climatic season. The dotted line indicates THI reference value (70.0), above which cattle are potentially vulnerable to thermal stress (LCI 1970)

Table 1 Mean values (± standard error) of respiratory rate (RR) and rectal temperature (RT) of cattle bulls (n = 18) in a tropical environment

Variable	Season		
	Winter	Spring	Summer
RR (breaths/m)	40.8 ± 1.20[a]	38.7 ± 0.90[a]	33.2 ± 1.10[b]
RT (°C)	39.0 ± 0.06[a]	38.9 ± 0.04[a]	38.6 ± 0.04[b]

Averages followed by different lowercase letters on the same line differ significantly ($P < 0.05$)

testicular parenchyma did not differ between seasons, with higher hyperechogenicity in the summer.

Sperm concentration increased in spring and summer (Table 5), while the gross motility showed a decrease between these seasons. Sperm vigor and progressive motility were higher in winter and spring. There was no seasonal variation for spermatozoid plasma membrane integrity, chromatin fragmentation, major defects, and total defects. However, there was a significant decrease in the percentage of minor defects from winter to summer.

Discussion

The air temperature, relative air humidity, rainfall, and solar radiation showed a typical behavior of tropical high-altitude climate (Peel et al. 2007), with higher values observed in spring and summer. As a result, the THI values were also higher in these seasons, reaching maximum values indicative of a conductive thermal environment for the animals to exhibit alert, danger, and even emergency conditions at certain times of the day (Mader et al. 2006). As expected, spring and summer were the most challenging seasons for animals. Thus, spring can be considered as a transitional period, when there was a progressive increase in air temperature, accumulated rainfall, and relative humidity, as well as an increase in solar radiation. The highest amount of rainfall observed in summer can have a thermolytic effect on cattle. However, this effect is instantaneous and does not determine a lasting condition of greater thermal comfort, since the increase of relative air humidity, associated with the consequent higher vapor pressure, hinders the thermolysis of cattle in hot environments (Collier and Gebremedhin 2015).

Respiratory rate and rectal temperature are consistent physiological indicators for determining the thermoregulatory condition of animals (Silanikove 2000). In adult bovines, the basal respiratory rate can range from 24 to 36 breaths per min (Ferreira et al. 2006), while the normal rectal temperature range is 38.1 to 39.1 °C for beef cattle (Robinson 2014). The respiratory rate was highest in spring and winter but did not achieve values considered indicative of heat stress. It is assumed that in winter, this was due to the experimental adaptation period, considered relatively short, when the animals were still more reactive to management. Meanwhile, in spring, increased respiratory rate can be attributed to the bulls' real need to dissipate heat to maintain homeothermia. In this season, the ambient conditions had high solar radiation, high temperature, and high relative air humidity, a combination considered to be responsible for thermal stress in cattle (Kamal et al. 2016).

Regardless of the climatic season, the rectal temperature remained within the range considered normal for cattle (Robinson 2014). The normothermia observed in this season indicates that the animals efficiently performed sensitive thermal changes, which may have been accompanied by sweating, a characteristic that was not monitored in the present study. The behavior of these variables throughout the seasons demonstrates that the animals had efficient homeothermia, keeping their physiological parameters within normal ranges, even in adverse climatic conditions.

In a situation of thermal discomfort by heat, the animal turns on mechanisms to dissipate thermal energy, which involves perceptible physiological responses, as well as in the circulatory system (Barros et al. 2016; McManus et al. 2009). Although the erythrogram and leukogram constituents showed higher values in times of greater thermal challenge,

Table 2 Mean values (± standard error) of erythrogram constituents: red blood cells (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) of cattle bulls (n = 18) in a tropical environment

Variable	Season			Reference value
	Winter	Spring	Summer	
RBC (10^6/μL)	7.8 ± 0.23[b]	8.1 ± 0.13[ab]	8.4 ± 0.14[a]	5–10*
Ht (%)	33.7 ± 1.05[b]	37.5 ± 0.61[a]	39.8 ± 0.62[a]	28–38*
Hb (g/dL)	11.6 ± 0.35[b]	12.3 ± 0.21[b]	13.5 ± 0.21[a]	9–14*
MCV (fL)	43.5 ± 1.26[b]	46.9 ± 0.73[a]	47.7 ± 0.75[a]	46–65*
MCH (pg)	14.9 ± 0.36[b]	15.3 ± 0.21[ab]	15.9 ± 0.22[a]	11–17**
MCHC (%)	34.3 ± 0.53[a]	32.0 ± 0.31[b]	33.4 ± 0.31[a]	30–36**

Means followed by different lowercase letters on the same line differ significantly ($P < 0.05$)

*Values based on Kraft and Durr (2005)

**Values based on Radostits et al. (2007)

Table 3 Mean values (± standard error) of the constituents of platelets (Pt) and leukogram: total absolute leukocytes (Lc), lymphocytes (Lf), neutrophils (Nt), eosinophils (Eos), and monocytes (Mon) of cattle bulls in a tropical environment

Variable	Season			Reference value
	Winter	Spring	Summer	
Pt (10^3/μL)	476.1 ± 34.34^{a}	422.5 ± 20.01^{ab}	367.1 ± 20.60^{b}	300–800*
Lc (/μL)	9361.1 ± 638.65^{b}	12,641.5 ± 372.19^{a}	12,154.9 ± 379.41^{a}	4000–12,000**
Lf (/μL)	5492.1 ± 415.06^{a}	6207.5 ± 241.89^{a}	6331.8 ± 246.58^{a}	2500–7500**
Nt (/μL)	2590.4 ± 380.56^{b}	4320.0 ± 221.78^{a}	4305.3 ± 226.08^{a}	600–4000**
Eos (/μL)	773.7 ± 218.67^{b}	1405.7 ± 127.44^{a}	1070.3 ± 129.91^{ab}	0–2400**
Mon (/μL)	504.9 ± 79.46^{b}	714.6 ± 46.31^{a}	451.1 ± 47.21^{b}	25–840**

Means followed by different lowercase letters on the same line differ significantly ($P < 0.05$)

*Values based on Kraft and Durr (2005)

**Values based on Radostits et al. (2007)

especially in the summer, they remained within the reference values for adult bovines (Kraft and Durr 2005; Radostits et al. 2007). The erythrogram increase can be associated with the rising ambient temperature, because in the attempt to dissipate heat, the animal loses body fluids by skin evaporation or by respiratory evaporation, which contributes to reduce the plasma volume and a higher concentration of the blood constituents (Srikandakumar and Johnson 2004). However, the condition known as stress leukogram occurs in situations of chronic stress and is mediated by the release of glucocorticoids. It is characterized by leukocytosis due to neutrophilia, lymphopenia, and eosinopenia (Van Engen et al. 2014), which were not observed, independent of the climatic season.

Activation of the hypothalamic-pituitary-adrenal axis and the consequent increase of plasma cortisol concentration are considered to be the most remarkable responses to stress conditions (Curley et al. 2008). The increase of cortisol induces physiological adjustments, so that the animal is able to tolerate the stress caused by several factors, such as environmental heat (Gifford et al. 2015). When bovines are under thermal stress, the plasma cortisol concentration rises (Maibam et al. 2014). Although the serum concentration of cortisol did not differ between seasons ($P > 0.05$) and was consistent with normal values for adult bovines (Lockwood et al. 2017), the value observed in the spring was approximately 2.0 ng/mL higher than that in the summer. This indicates that the bulls did not exhibit chronic stress. However, it may suggest that the behavior of cortisol in the transitional period could be the object of further studies, mainly associated with the effect of air temperature, in an intra-seasonal circadian approach.

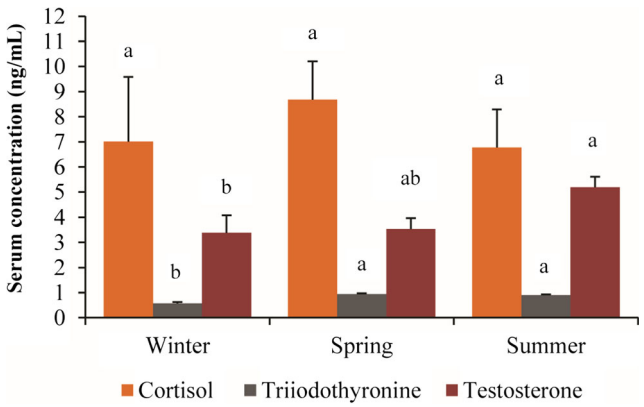

Fig. 3 Mean serum concentrations of cortisol, triiodothyronine (T3), and testosterone of cattle bulls ($n = 18$) in a tropical environment. Different lowercase letters among seasons indicate significant difference ($P < 0.05$)

Triiodothyronine is a hormone produced by the thyroid gland and is responsible for regulating metabolism (Villanueva et al. 2013). In the case of thermal stress, its secretion decreases for a lower production of metabolic heat (Pereira et al. 2008). The increase in triiodothyronine concentration in spring and summer indicated that the temporary reduction of the metabolic rate was unnecessary (Christopherson et al. 1979). The breeding season in beef cattle production systems coincides with the hottest periods of the year in tropical climate regions, when bulls are most demanded due to constant deambulation and sexual activity (Berry et al. 2011). The triiodothyronine increase in this period of higher energy demand may be an extremely favorable characteristic for bulls with more efficient thermoregulation. This emphasizes the importance of selecting genotypes and individuals that are able to more sensibly regulate their metabolic rate, helping in the maintenance of homeothermia.

Leydig cells are responsible for the production of testosterone, a key hormone for the maintenance of spermatogenesis and the expression of male secondary sexual characteristics (Gulia et al. 2010). High concentrations of testosterone can positively influence sperm production and motility, libido, and bull fertility (Andersson 1992). When submitted to thermal stress, bulls show a significant reduction in the serum concentration of testosterone (Minton et al. 1981). Given that the serum testosterone profile was not adversely affected by climatic changes, and even an increase from winter to summer, it is assumed that steroidogenesis was not impaired by the environmental heat, which could be related to efficient body and scrotal thermoregulation and could favor seminal production, the quality of the ejaculates, and the libido.

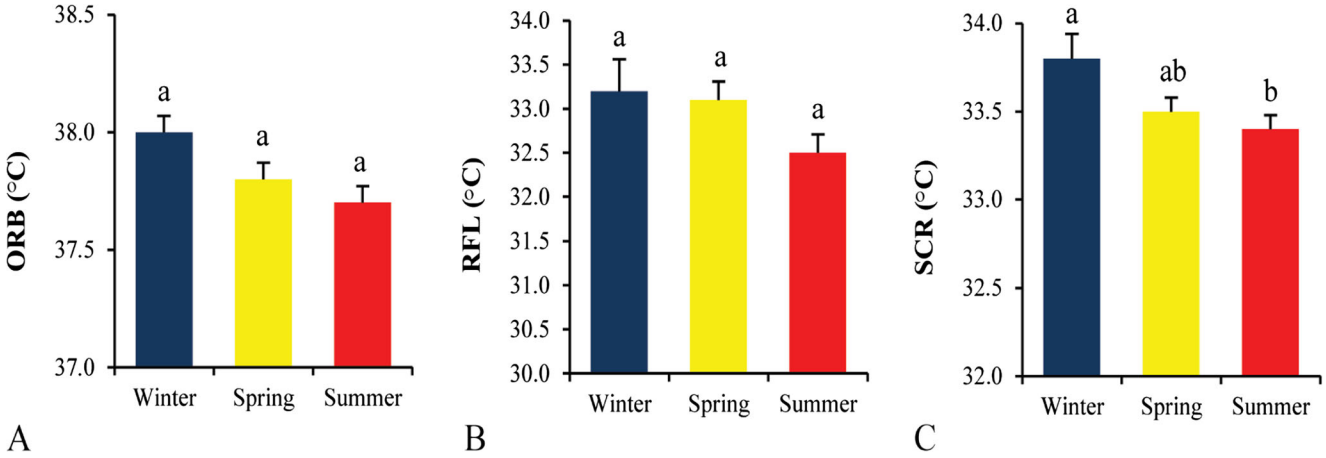

Fig. 4 Average temperatures of **a** ocular globe (ORB), **b** right flank (RFL), and **c** scrotum (SCR) of cattle bulls ($n = 18$) in a tropical environment. Means followed by different lowercase letters among seasons differ significantly ($P < 0.05$)

In order to maintain body temperature, endothermic animals dissipate thermal energy by sensible heat transfer and the direction of energy flow is always from the higher to lower temperature region (Collier and Gebremedhin 2015), with a thermal gradient between the animal and the environment. When the temperature of the animal exceeds the temperature of the environment, the heat exchange occurs through conduction, convection, and radiation. Therefore, in a tropical environment, it is desirable that the temperature of the coat is always lower than the temperature of the skin surface, in order to favor the establishment of a gradient for heat loss. Because of this, coat color can decisively influence heat dissipation of cattle by the dermal route. Animals with dark-colored hairs exhibit higher absorbance of solar radiation (Hillman et al. 2001), which raises the surface temperature of their fur. In quadrupedal animals, the dorsum and flank are anatomical regions that receive direct solar radiation incidence. Although the radiation was substantially higher during spring and summer, the absence of changes in flank temperature throughout the seasons may be associated with the greater reflectivity of the light-colored coat. This is a desirable phenotypic characteristic in bulls raised in tropical climate and is beneficial to their thermal equilibrium (Maia et al. 2005), because the higher reflectance reduces the accumulation of thermal energy on the hair surface. On the other hand, there were no differences between seasons regarding ocular globe temperature, with values similar to those previously reported for cattle (Church et al. 2014).

From the reproductive point of view, the temperature increase of the environment can reduce the efficiency of bull spermatogenesis as a consequence of the temperature increase in the scrotal surface (Berry et al. 2011). Therefore, bulls with an abnormal scrotal thermogram usually also exhibit low seminal quality (Kastelic and Brito 2012). However, the skin of the cattle scrotal region is thin, flexible, and relatively glabrous and has a large number of sweat glands, characteristics that help in the heat loss (Kastelic et al. 1997). The lower scrotal temperature observed in the summer indicated the maintenance of the testicular temperature within normal levels, since the bovine testis should operate at a temperature up to 6.0 °C lower than body core temperature (Garcia 2004). Although the biometeorological indicators revealed a growing thermal challenge throughout the studied seasons, the difference between the rectal and scrotal surface temperatures remained at 5.2 °C in winter as well as in summer. The bulls exhibited scrotal thermograms with a pattern of three distinct and homogeneous thermal bands, denoting a decreasing temperature of the testicular vascular cone to the epididymis, and confirmed their thermoregulatory scrotal efficiency (Silva et al. 2017), similar to the findings for bulls raised in subtropical climate (Menegassi et al. 2015).

Table 4 Mean values (± standard error) of the scrotal circumference (SC), testicular consistency (TES), testicular volume (TV), testicular homogeneity (HOM), and testicular hyperechogenicity (HYP) of cattle bulls ($n = 18$) in a tropical environment

Variable	Season		
	Winter	Spring	Summer
SC (cm)	35.3 ± 0.28^{c}	37.7 ± 0.23^{b}	39.0 ± 0.24^{a}
CONS (1 to 5)	3.2 ± 0.05^{a}	3.3 ± 0.04^{a}	3.3 ± 0.04^{a}
TV (cm^3)	192.9 ± 10.57^{b}	273.9 ± 6.16^{a}	257.7 ± 6.28^{a}
HOM (0 to 2)	0.1 ± 0.12^{a}	0.2 ± 0.07^{a}	0.3 ± 0.07^{a}
HYP (0 to 3)	0.4 ± 0.22^{b}	0.5 ± 0.13^{b}	1.4 ± 0.13^{a}

Means followed by different lowercase letters on the same line differ significantly ($P < 0.05$)

HOM (0 to 2): 0 = homogeneous parenchyma (no pathological anechoic points), 1 = little heterogeneous (few pathological anechoic points), and 2 = very heterogeneous (many pathological anechoic points)

HYP (0 to 3): 0 = no hyperechoic points, 1 = up to five hyperechoic points, 2 = over five hyperechoic points close to the mediastinum, and 3 = over five diffuse hyperechoic points in the testicular parenchyma (Kahwage et al. 2017)

Table 5 Mean values (± standard error) of spermatic concentration (CONC), gross motility (GM), sperm vigor (VIG), progressive sperm motility (PM), integrity of sperm plasma membrane (PMI), chromatin fragmentation (CFrag), minor defects (MiDef), major defects (MaDef), and total defects (TDef) of ejaculates of cattle bulls ($n = 18$) in a tropical environment

Variable	Season		
	Winter	Spring	Summer
CONC ($\times 10^6$ sptz/mL)	1239 ± 230.25^{b}	2092 ± 191.58^{a}	2099 ± 193.45^{a}
GM (0–5)	2.7 ± 0.17^{ab}	3.1 ± 0.14^{a}	2.4 ± 0.14^{b}
VIG (0–5)	2.9 ± 0.13^{a}	2.6 ± 0.11^{a}	2.2 ± 0.11^{b}
PM (%)	69.7 ± 2.41^{a}	66.3 ± 2.00^{a}	56.1 ± 2.03^{b}
PM I (%)	66.2 ± 3.81^{a}	66.7 ± 2.96^{a}	67.9 ± 3.05^{a}
CFrag (%)	3.2 ± 0.48^{a}	2.0 ± 0.39^{a}	2.4 ± 0.40^{a}
MiDef (%)	15.1 ± 1.31^{a}	12.0 ± 1.08^{ab}	9.9 ± 1.10^{b}
MaDef (%)	6.2 ± 0.95^{a}	5.7 ± 0.78^{a}	8.0 ± 0.80^{a}
TDef (%)	21.3 ± 1.81^{a}	17.7 ± 1.49^{a}	17.9 ± 1.52^{a}

Means followed by different lowercase letters on the same line differ significantly ($P < 0.05$)

Testicular hyperthermia can lead to testicular degeneration (Ohashi et al. 1988; Rahman et al. 2011), which consists of a set of changes in the testicular parenchyma that lead to structural variations in the cells of the germ line, a reduction in the functionality of the gonad, and fertility of the animal (Garcia 2017). Animals with testicular degeneration have reduced testicular consistency and volume, and if the causative agent is not removed, there is progression and chronification of the condition. This leads to a decrease in the space occupied by the tubular epithelium, which can progress to calcification, fibrosis, and atrophy (Van Camp 1997). Throughout the climatic seasons, the testicles of the bulls presented fibroelastic consistency, with firm tone, a normal and desirable characteristic in cattle (CBRA 2013). The progressive increase observed in the scrotal perimeter can be attributed to the increase in testicular volume, since these are interdependent and related characteristics, among other factors, to the size and body condition of the animals (Bailey et al. 1998). Considering that the bulls evaluated were young animals, the evolution of their weights and the maintenance of testicular tone help to explain the increase of the scrotal perimeter. Since the scrotal circumference has a high and positive genetic correlation with age at puberty and with the pregnancy rates of females of the progeny (Toelle and Robison 1985), the preservation of the integrity of the testicular parenchyma is fundamental to the accurate scrotal biometry and possible discrimination of bulls capable of increasing the reproductive potential of the herd.

Testicular ultrasonography can be used as an auxiliary tool in the selection of bulls for breeding (Ahmad et al. 2011), as it is a non-invasive and efficient method for the identification of macroscopic testicular and epididymal lesions (Kastelic and Brito 2012). In the present study, the homogeneity of the testicular parenchyma did not change and this was a positive indication of tissue integrity during the seasons. Associated with the tissue homogeneity data, the presence of calcification in the testicular parenchyma may indicate the degree of macroscopic integrity of the gonads (Jensen et al. 2008), and these characteristics are directly related to sperm production and seminal quality (Kahwage et al. 2017; Kastelic and Brito 2012). Although a greater number of hyperechoic spots were observed in the testicular parenchyma during the summer, this variation was not enough to impair the testicular echotexture, nor did it negatively affect the seminal quality.

The increase in testicular temperature raises the oxidative metabolism of sperm cells. This leads to higher consumption of energy reserves and greater generation of reactive oxygen species, causing mitochondrial disturbances and irreversible cellular damage (Nichi et al. 2006; Rhoads et al. 2013). In these cases, there may be reduced sperm motility and viability, associated with increased morphological abnormalities and increased incidence of sperm chromatin fragmentation (Rahman et al. 2011). However, the increase in the biometeorological variables and in THI over time was not sufficient to alter the capacity of scrotal thermoregulation and cause a significant reduction in seminal quality. Although a slight decrease was observed in gross motility, sperm vigor, and progressive motility during the summer, the efficient thermoregulation preserved spermatogenesis, so that environmental factors did not reduce the percentage of cells with intact plasma membrane. The thermal environment also did not negatively impact the percentage of morphologically normal spermatozoa, and there was also a reduction in the incidence of minor defects, which involved curved tails and distal cytoplasmic droplets. In the hottest seasons of the year, in regions of subtropical or tropical climate, this finding is only recorded in bulls with greater scrotal thermal dissipation capacity (Menegassi et al. 2015; Nichi et al. 2006).

Heat testicular stress is one of the major factors responsible for the induction of sperm DNA fragmentation, with peaks of changes in the integrity of the chromatin structure detectable approximately 20 days after the thermal challenge was installed (Garcia 2004). However, toluidine blue is a sensitive external agent that is incorporated into damaged chromatin and becomes metachromatic when bound to DNA (Beletti and Mello 2004).

The absence of difference between seasons in the percentage of spermatozoa with chromatin fragmentation indicates that the process of DNA protamination during hypercondensation of chromatin in the spermiogenesis phase occurred normally. This is a relevant finding because DNA integrity is directly related to the sperm fertilization capacity (Karoui et al. 2012). The integrity of the chromatin structure is also directly related to normal sperm morphology (Fortes et al. 2012), as observed in the present study. The chromatin fragmentation values registered in spring and summer, close to 2.0%, were lower than those reported for bulls kept in an artificial insemination center (Bochenek et al. 2001) and equivalent to the values reported for high fertility bulls (Dogan et al. 2015).

Sperm concentration in spring and summer was almost twice to that observed in winter, the milder weather season. If the bulls had undergone systemic or testicular thermal stress, their sperm concentration would be reduced (Kastelic et al. 2001; Menegassi et al. 2015). The highest number of cells per unit volume in an ejaculate may not only favor its yield in the production of inseminating doses but also favor the fertility of bulls that may have compensable spermatic morphological defects (Saacke 2008). The increase in sperm production during spring and summer, coupled with the other qualitative characteristics of the ejaculate, indicates that the bulls studied could be used successfully in the hottest seasons of the year, whether for use in natural mounts or for sperm cryopreservation in semen processing centers.

Conclusions

The altitude tropical climate was more challenging in the spring and summer seasons. Even when there was an increase of the biometeorological elements capable of leading to heat stress, the bulls had an effective body and scrotal thermoregulatory response, maintained the condition of normothermia, demonstrating adaptability and resilience to the thermal environment. Although under greater thermal challenge, the animals did not enter acute or chronic stress, a situation evidenced by the monitoring of the physiological, hematological, and hormonal indicators. In the hottest times of the year, the bulls exhibited a quantitative increase in seminal production and qualitative characteristics related to fertility. Therefore, they can be considered as climatically adapted and constitute a viable alternative for use in production systems in tropical regions, even if the breeding seasons are set in the most critical thermal condition periods of the year.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The experiment complies with the Brazilian current laws, and all procedures performed were approved by the Committee on Experimental Animal Use and Ethics (Protocol CEUA-CPPSE Declaration 12_2014). Procedures were related according The ARRIVE Guidelines (Kilkenny et al. 2010)

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