



Thermoregulation of male sheep of indigenous or exotic breeds in a tropical environment



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ABSTRACT

Climate change has intensified the frequency of heat waves in the world, thereby exposing farm animals to stressful conditions. For better productive performance it is important to identify the most resilient genotypes. Thus, our objective was to evaluate the thermoregulatory responses of rams of tropical indigenous (Morada Nova and Santa Inês) and exotic breeds (Dorper and Texel), by monitoring the environmental and physiological indicators related to heat tolerance. The experiment was carried out in a tropical climate region (Cwa), in Brazil, for twelve months, which comprised spring, summer, autumn and winter. Thirty-three rams were divided into groups: Morada Nova (MN; n = 8, red-coat), Santa Inês (SI; n = 9, black-coat), Dorper (DO; n = 8, white-coat) and Texel (TX; n = 8, white-coat). The microclimatic variables were monitored, and the THI and BGHI comfort indices were calculated. Coat thickness and body surface temperatures were measured monthly, and serum triiodothyronine-T3 measurements and complete blood tests were performed. The physiological variables were evaluated every fifteen days and skin micro-biopsies were performed in the summer and winter for histological evaluation. During the warmer seasons, the THI and BGHI reached values that indicated thermal discomfort. TX showed higher coat thickness throughout the year, increased physiological variables related to thermolysis, and reduced T3 ($P < 0.05$). The internal temperature was permanently lower in the MN, SI and DO ($P < 0.05$). The body surface temperatures were affected by the coat characteristics and wool length. Hematological parameters varied in the seasons with the highest thermal conditions ($P < 0.05$). The MN showed larger sweat glands, while the area occupied by the sweat glands was higher in the SI. The DO showed higher hair density in the summer and winter ($P < 0.05$). The results indicated that the MN, SI and DO breeds overcome the thermal challenge more easily throughout the seasons due to specific adaptive morphological and physiological characteristics.

1. Introduction

The consolidation of sheep production has contributed to the economic growth of countries located in the tropical areas of the planet, where wool breeds and hair breeds of different origins are used (Cruz Júnior et al., 2015). In tropical climates, sheep breeds can be

categorized as exotic or indigenous (Kim et al., 2016). Exotic animals were gradually introduced in the tropical region to increase the productivity of local herds (Ribeiro and González-García, 2016). On the other hand, the animals of tropical indigenous breeds belong to autochthonous genotypes developed in megathermal climate regions and exhibit specific phenotypic characteristics related to adaptation and

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rusticity (Lv et al., 2014).

The productive potential of sheep in tropical regions is mainly influenced by exposure to high temperatures (Sejian et al., 2017). The current scenario of climate change has intensified the frequency of heat waves in different regions of the world, thereby exposing farm animals to stressful environmental conditions (Sejian et al., 2013). In a heat stress situation, sheep activate their thermoregulatory system in order to dissipate the excess of accumulated thermal energy. However, if the thermolytic capacity is compromised, the internal temperature increases, which reduces food intake, rumination and reproductive efficiency (Das et al., 2016; De et al., 2017). Under these conditions sheep productivity is seriously impaired, which results in significant losses in the production chain (Rana et al., 2014). In general, sheep breeds that originated in cold climates tend to regulate their internal body temperature less effectively than breeds that originated in warmer climate regions (Romero et al., 2013).

In order to provide animal welfare and reduce economic losses due to heat stress, the animals must be monitored, promoting management strategies to keep them in thermoneutral conditions (Macías-Cruz et al., 2016). This can be done by monitoring the physiological variables (Maia et al., 2014), body surface temperatures (Martello et al., 2016; Piccione et al., 2013) and the hormonal (Koluman and Daskiran, 2011) and hematological profiles (Silva et al., 2011). All these physiological elements can undergo changes, individually or in combination, especially in the animals that have lower heat-adaption mechanisms (Beatty et al., 2006).

Identifying the most resilient genotypes that are capable of overcoming the environmental challenges imposed in different production systems is also important to raise productivity levels in tropical regions (Garcia, 2013; Mirkena et al., 2010). Comparative studies on the thermoregulatory capacity of wool breeds and hair breeds are important for the selection of genotypes and potentially more productive individuals when raised in less favorable thermal environments (Titto et al., 2016). Therefore, this study aims at evaluating and comparing the thermoregulatory responses of tropical indigenous (Morada Nova and Santa Inês) and exotic sheep breeds (Dorper and Texel) by monitoring the environmental and physiological indicators related to heat tolerance.

2. Material and methods

2.1. Location and climate

The experiment was conducted at Embrapa Southeast Livestock, São Carlos, SP, Brazil (21°58'30"S, 47°50'58"W, 911 m altitude). The climatic subtype of the region is the Cwa, tropical altitude, according to Köppen climate classification, characterized by dry winters, and hot and rainy summers, with temperatures reaching levels above 37.6 °C in the hottest months of the year. The annual average temperature is 21.5 °C and the mean annual air humidity is 74.3% (Embrapa, 2016). The experimental period was from August 2015 to July 2016 and comprised four climatic seasons: spring (September to December), summer (December to March), autumn (March to June) and winter (June to July). The month of August of 2015 was considered as the adaptation period to the management procedures.

2.2. Bioethics

The experiment was reported according to The Animals in Research: Reporting in Vivo Experiments Guidelines - ARRIVE (Kilkenny et al., 2010) and performed in accordance with Brazilian laws. All experimental procedures were previously approved by the Ethics Committee on the Use of Experimental Animals (CEUA-CPPSE Declaration 06_2013), considering the legal and ethical aspects of the interventions performed.

2.3. Animals and facilities

Thirty-three rams (live weight: 67.6 ± 6.9 kg, age: 20.1 ± 3.1 months) from four different breeds were used. The animals were divided into four experimental groups, according to their genotype: Group Morada Nova (MN; n = 8, red coat), Group Santa Inês (SI; n = 9, black coat), Group Dorper (DO; n = 8, white coat) and Group Texel (TX; n = 8, white coat). The MN and SI breeds developed in Brazil are indigenous, they are hair breeds with short and silky hair coat (Ribeiro and González-García, 2016). The DO breed is exotic in Brazil, from South Africa, and is characterized as animals with short hair coat in the belly and limbs, and small amount of wool in the upper body (DORPERSA, 2017; Milne, 2000). The TX breed is an exotic wool breed in Brazil, originally from the Netherlands, with wool covering the neck and body, and no wool only in the head and lower parts of the limbs (ARCO, 2017; Maia et al., 2011).

Prior to the start of the experiment, the TX animals were sheared for homogenization, with no additional shearing performed during the experimental period. The animals were kept in a single batch under confinement in a shed measuring 570 m², with waterproof concrete floor. The shed was partly covered by a 196 m² galvanized roof and with 4.0-meter-high ceiling. The covered area had a bed of sand and provided shading of 6.0 m²/animal, throughout the day. The daily diet was composed of at will roughage (corn silage) and concentrate (64.45% corn grains, 34.19% soy bran, 1.16% calcitic lime and 0.19% % of mineral salt for sheep), in the amount of 0.7% live weight. The animals were fed once a day (09h00) and had ad libitum access to the feed trough and the automatic drinking fountain.

2.4. Meteorological variables and comfort indices

During the experimental period, the air temperature (AT, °C), relative air humidity (RH, %) and black globe temperature (BGT, °C) were monitored by an automatic weather station located at the animal accommodation facility. The station had psychrometric sets protected in the micrometeorological shelter. The sensors were coupled to an automatic data acquisition system (CR1000 Datalogger®, Campbell Scientific, Logan, UT, USA), programmed to take readings every five seconds and generate averages every 15 min. As thermal comfort evaluation criterion, the Temperature and Humidity Index (THI) specific for sheep was calculated, as proposed by Marai et al. (2007), using the following equation: $THI = AT - \{(0.31 - 0.31 \cdot RH) \cdot (AT - 14.4)\}$, where: AT is the air temperature (°C) and RH is the relative air humidity (%). The THI values obtained indicate: < 22.2 = absence of thermal stress; 22.2 to < 23.3 = moderate thermal stress; 23.3 to < 25.6 = severe thermal stress; and ≥ 25.6 = extreme severe thermal stress.

The Black Globe Temperature and Humidity Index (BGHI) was calculated as proposed by Buffington et al. (1981), using the equation: $BGHI = BGT + 0.36 (DPT) + 41.5$, where: BGT is the black globe temperature (°C) and DPT is the dew point temperature (°C). The values obtained indicate: < 74 = thermal comfort situation; 74–78 = warning; 79–84 = danger; and > 84 = emergency (Baêta and Souza, 2010). The dew point temperature was calculated from the Tetens (1930), as follows: $DPT = 237.3 \cdot \log [e_a / 0.6108] / 7.5 - \log [e_a / 0.6108]$, where: e_a is the partial steam pressure. Fig. 1 shows the distribution of the meteorological variables and the thermal comfort indices in the experimental period.

2.5. Coat thickness

The coat thickness was measured monthly with a metal caliper graduated in millimeters (mm), positioned perpendicular to the surface of the animal until it touched the skin, with the cursor positioned at the outer surface of the coat (Kahwage, 2015). The result was given in mm.

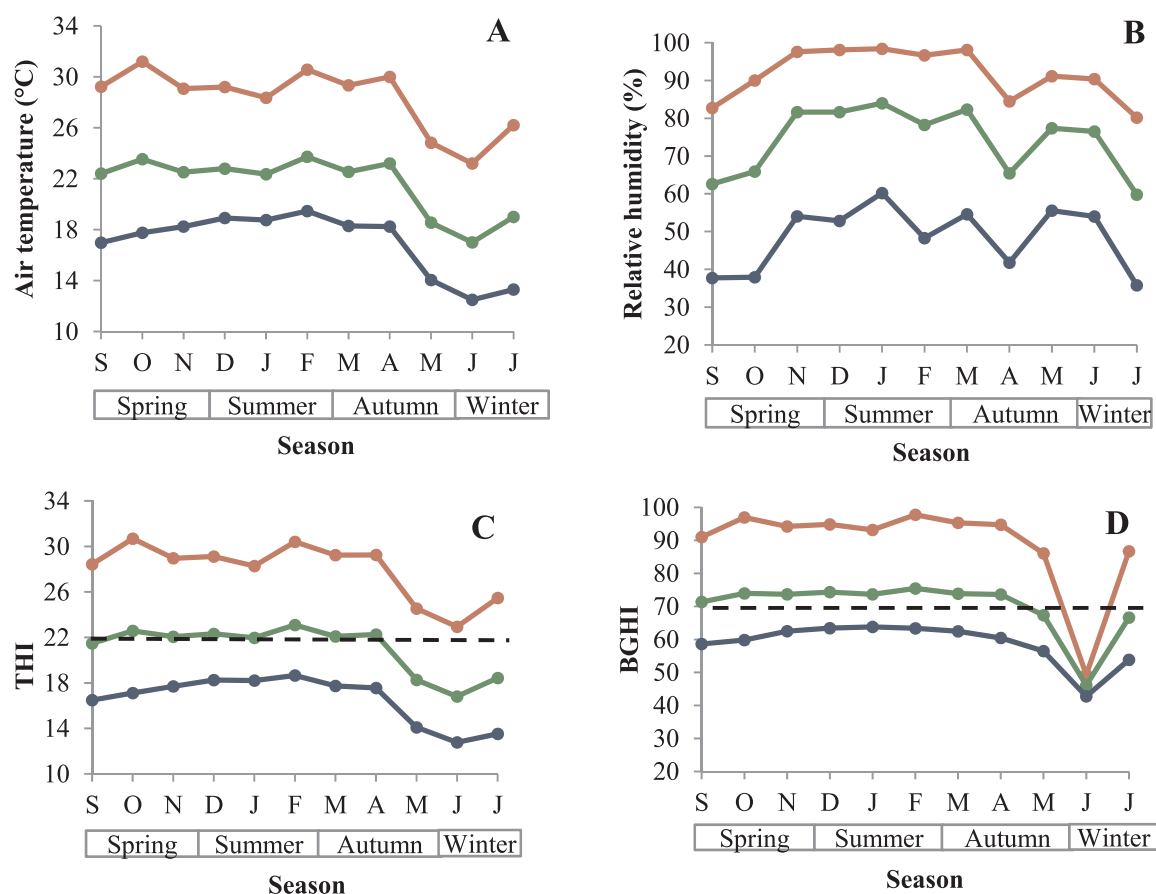


Fig. 1. Maximum (—), mean (—) and minimum (—) values of air temperature (A), air relative humidity (B), Temperature and Humidity Index-THI (C) and Black Globe Temperature and Humidity Index-BGHI (D), recorded monthly at the experimental site throughout the year and characteristic of a tropical climate region. The dotted lines indicate reference values of ITU and BGHI, and above these values the animals exhibit thermal discomfort (Marai et al., 2007; Baêta and Souza, 2010).

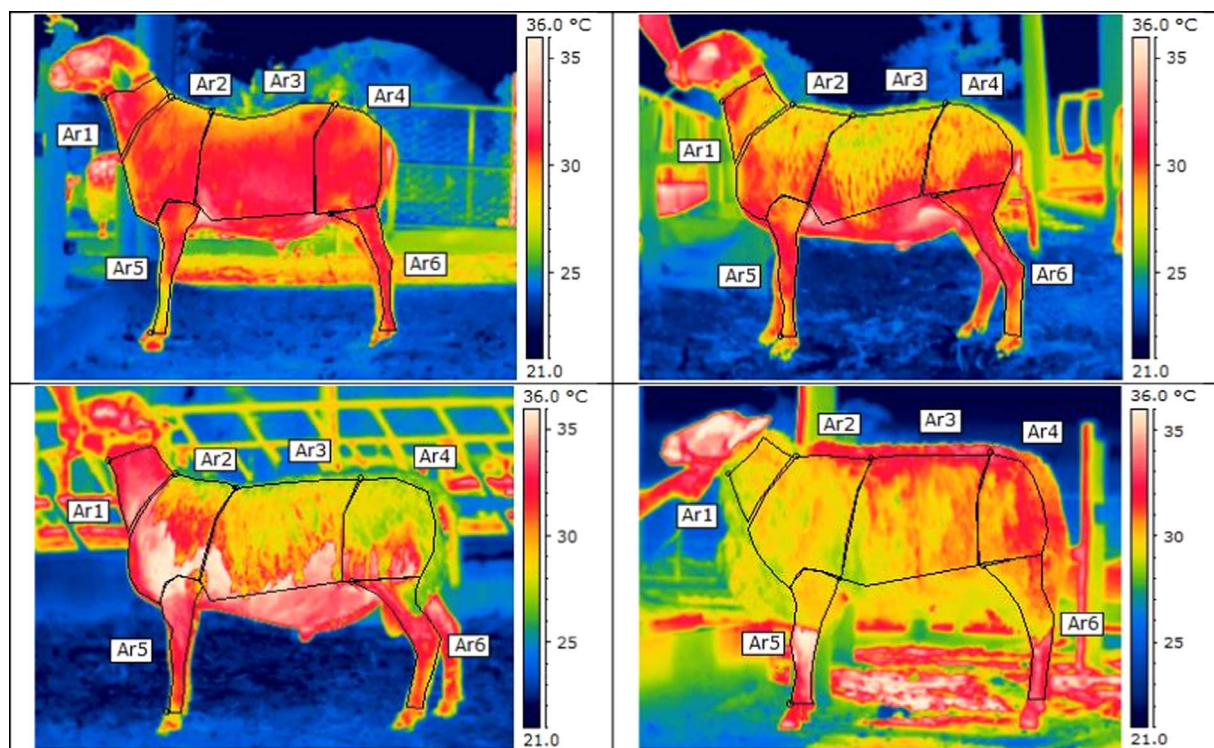


Fig. 2. Thermographic images of the left antimer of different genotypes of sheep breeders: Santa Inês (A), Morada Nova (B), Dorper (C), Texel (D). Analytical regions defined according to Kotrba et al. (2007): neck (Ar1), body forepart (Ar2), barrel (Ar3), body hind part (Ar4), forelimb (Ar5) and rear limb (Ar6).

2.6. Physiological variables and body surface temperatures

Respiratory rate (RR, breaths.m⁻¹) and rectal temperature (RT, °C) were measured every fifteen days in the morning (08h00) and afternoon (14h00), and the results of the measurements are presented as means. The RR was obtained by observing thoracoabdominal movements for one minute. The RT was considered as the core temperature (Schaefer et al., 2007) and was obtained with a digital clinical thermometer (McManus et al., 2015). Body surface temperatures were monthly measured by infrared thermography, with an auto-focus thermographic camera and thermal sensitivity of 0.05 °C (T300, FLIR Systems, Wilsonville, OR, USA). The thermograms generated were later analyzed in the FLIR Tools+ software (Version 5.6, FLIR Systems, Oregon, USA).

Thermographic images were obtained of the left eyeball region, of the testicular region and of the left antimer of the animals. The camera was kept at the level of the anatomical region analyzed at approximately 0.5 m from the eyeball, 0.7 m from the scrotum and 2.0 m from the body of the animal. The emissivity used was 0.98 (Hoffmann et al., 2013). The orbital area temperature (ORB, °C) was analyzed by a circular tracing over the orbital region, including the eyeball and approximately 1 cm of the ocular cavity, to include the lacrimal gland. The maximum ORB (hot spots) values were used in the statistical analysis (Schaefer et al., 2007; Hoffmann et al., 2013).

To study the left antimer, the body of the animal was subdivided into areas, as proposed by Kotrba et al. (2007). Using the software, the following mean temperatures were evaluated: neck temperature (TNeck), body forepart (TBfore), barrel (TBarrel), body hind part (TBhind), forelimb (TFlimb) and rear limb (TRLimb). In the thermographic analysis, polygon areas compatible with the subdivisions used were created (Fig. 2). The temperature of the back (TBack) was determined by a line drawn from the iliac crest to the scapula. The mean testicular temperature (TTest, °C) was determined by polygonal demarcation, which included the region of the right and left testis (Kahwage et al., 2017).

2.7. Hematological parameters and triiodothyronine (T3) dosage

The blood samples from each animal were collected monthly, from the jugular vein in a vacuum tube system containing EDTA 10% (McManus et al., 2011). The samples were refrigerated at 8 °C and transported immediately to the laboratory for analysis. The values determined were erythrocytes (10⁶/μL), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, %), leukocytes (10⁶/mm³), eosinophils (10⁶/mm³), neutrophils (10⁶/mm³), lymphocytes (10⁶/mm³), monocytes (10⁶/mm³) and platelet count (10⁶/μL) (Wojtas et al., 2014). For the triiodothyronine dosage, blood samples were collected by external jugular vein puncture in vacuum tubes, without anticoagulant. The samples were centrifuged at 3600 rpm for 30 min. The determination of T3 concentrations was performed by radioimmunoassay, using commercial T3 Antibody-Coated Tubes kit, T3 Tracer [125I] and T3 Standards Set (MP Diagnostics Division, Orangeburg, NY, USA). The sensitivity and intra- and inter-assay coefficients were 6.7 ng/dL, 9% and 10%, respectively. All data were within the maximum and minimum points on the curve.

2.8. Morphological structure of the skin

Two skin fragments from each animal were collected in winter and summer, taken from the upper region of the right scapula. The collection was performed using a micro-biopsy punch, 8 mm diameter, after the application of local anesthetic without vasoconstrictor (lidocaine hydrochloride 2%). Each fragment was histologically processed, in six longitudinal and six cross-sections of 4 μm thickness, then stained by Masson's trichrome method (Kahwage, 2015). The sections were

evaluated by optical microscopy (Leica DME, Leica Microsystems, Wetzlar, Germany), magnification of 100× and submitted to photo-documentation. Eight to 12 images were scanned per section, later evaluated in the Image J software. In the images of the longitudinal sections, the area occupied by the secretory portion of the sweat glands (ASG, μm²) and the proportion of the glandular area (PGA, %) were measured. The PGA was obtained by the centesimal ratio of the sum of the glandular areas, in relation to the total area of the image evaluated (Bianchini et al., 2006). In the cross-sectional images the identification, quantification and calculation of the density of the primary hair follicles (PF.mm⁻²) and secondary hair follicles (SF.mm⁻²) were performed. The former were differentiated for being associated with the sweat gland, erector muscle and sebaceous gland. The total density of hair follicles (HF.mm⁻²) was obtained by the sum of the primary and secondary follicles, in relation to the total area of the analyzed image (Moore et al., 2015).

2.9. Statistical analysis

Data analysis was performed using the MIXED procedure of the SAS software (SAS Institute Inc., Cary, NC, USA), considering repeated measures. For all variables, the normality check was performed previously. The effects of breed (DO, MN, SI and TX) and season (spring, summer, autumn and winter) and their interactions were evaluated following the model: $Y = \text{mean} + \text{age} + \text{THI} + \text{breed} + \text{season} + \text{breed} \times \text{season} + \text{error}$. In the coat data and hematological parameters the effects of breed (DO, MN, SI and TX) and season (spring, summer, autumn and winter) and their interactions were considered, following the model: $Y = \text{age} + \text{breed} + \text{season} + \text{breed} \times \text{season} + \text{error}$. In the sweat glands data the effects of breed (DO, MN, SI and TX) and season (summer and winter) and their interactions were considered following the model: $Y = \text{age} + \text{season} + \text{breed} + \text{breed} \times \text{season} + \text{error}$. The other fixed effects were considered as classificatory in the model. In order to meet the assumptions of the linear models, the variables of leukocytes, lymphocytes, neutrophils, platelets, hair density, primary follicles, secondary follicles, sweat gland area and proportion of glandular area were used on a scale transformed to log¹⁰. The erythrocyte, MCHC, eosinophil, monocyte and hematocrit variables were used on a transformed scale to **2. The MCH and MCV variables were used on a scale transformed to 1/MCH and 1/MCV. To present the results, the data was returned to the original condition. The surface temperature charts were assembled with the average data of each collection, to highlight the profile of each variable studied. Tukey's test was used to compare the treatment averages. The level of significance previously used for all analyses was 5%.

3. Results

The mean AT fluctuated to around 22.0 °C in the hottest periods of the year, peaking at 31.2 °C in the spring, while the mean RH ranged from 60% to 80% over the seasons. The mean THI showed values close to 22.0, for most of the year, with maximum THI values that surpassed 30.0, indicating the possibility of severe stress. Similarly, BGHI showed values close to 74.0 from spring to autumn (Fig. 1).

The animals of the TX breed exhibited greater coat thickness than the other breeds, which did not differ throughout the seasons (Fig. 3). In addition, the coat thickness of the TX animals increased throughout the year, showing an increase over the months as described by equation $y = 24.135\ln(x) + 49.624$ ($R^2 = 0.945$). For these animals, in the first measurement of spring the coat thickness observed was 47.3 mm, and at the end of winter it was 104.1 mm.

In all seasons, the TX animals had higher RR averages (Table 1). During the warmer seasons, the lowest RR values were observed in the MN and SI animals. Similarly, the RT exhibited by the TX animals was significantly higher, regardless of the climatic seasons, ranging between 39.1 and 39.3 °C. The other breeds showed RT variation ranging

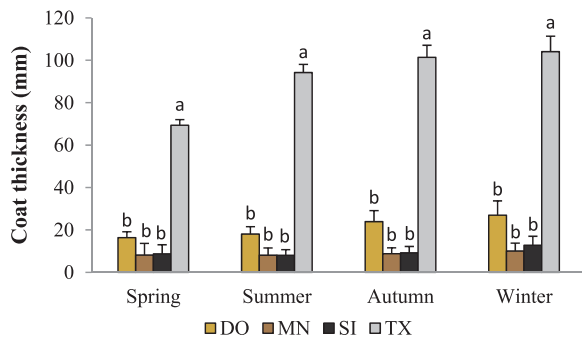


Fig. 3. Mean values of hair coat thickness of Dorper (DO; $n = 8$), Morada Nova (MN; $n = 8$), Santa Inês (SI; $n = 9$) and Texel (TX; $n = 8$) rams recorded monthly in a tropical climate region. Different lowercase letters (a, b) indicate significant difference within the climatic season by the Tukey test ($P < 0.05$).

Table 1

Mean and standard error values of physiological variables (RR and RT) of Dorper (DO; $n = 8$), Morada Nova (MN; $n = 8$), Santa Inês (SI; $n = 9$) and Texel (TX; $n = 8$) rams measured throughout the year in a tropical climate region.

| Breed | Spring | Summer | Autumn | Winter |
|--|--------------------------|---------------------------|---------------------------|---------------------------|
| Respiratory Frequency (RR, breaths.m ⁻¹) | | | | |
| DO | 66.7 ± 4.0 ^{bA} | 66.4 ± 4.7 ^{bA} | 59.8 ± 6.9 ^{bAB} | 72.0 ± 8.7 ^{Ba} |
| MN | 48.5 ± 7.5 ^{cA} | 31.5 ± 5.0 ^{cB} | 44.6 ± 4.3 ^{bca} | 42.6 ± 4.9 ^{CaB} |
| SI | 50.7 ± 5.9 ^{cA} | 41.3 ± 3.9 ^{cCB} | 43.2 ± 4.3 ^{cAB} | 45.7 ± 5.5 ^{cAB} |
| TX | 97.9 ± 3.9 ^a | 98.4 ± 5.2 ^a | 99.0 ± 7.6 ^a | 95.4 ± 9.4 ^a |
| Rectal Temperature (RT, °C) | | | | |
| DO | 38.7 ± 0.1 ^b | 38.7 ± 0.1 ^b | 38.8 ± 0.1 ^b | 38.8 ± 0.1 ^b |
| MN | 38.8 ± 0.1 ^{bA} | 38.4 ± 0.1 ^{cB} | 38.4 ± 0.1 ^{cB} | 38.7 ± 0.1 ^{bca} |
| SI | 38.7 ± 0.1 ^{bA} | 38.4 ± 0.1 ^{cB} | 38.5 ± 0.1 ^{cAB} | 38.6 ± 0.1 ^{CaB} |
| TX | 39.1 ± 0.1 ^a | 39.1 ± 0.1 ^a | 39.3 ± 0.1 ^a | 39.2 ± 0.1 ^a |

A,B different capital letters indicate significant difference in the rows ($P < 0.05$).

a,b different lowercase letters indicate significant difference in columns ($P < 0.05$).

Table 2

Mean and standard error values of triiodothyronine (T3) of Dorper (DO; $n = 8$), Morada Nova (MN; $n = 8$), Santa Inês (SI; $n = 9$) and Texel (TX; $n = 8$) rams measured throughout the year in a tropical climate region.

| Breed | Spring | Summer | Autumn | Winter |
|-------|----------------------------|----------------------------|-------------------------|---------------------------|
| DO | 102.9 ± 7.7 ^{bcA} | 91.5 ± 8.4 ^{abAB} | 49.4 ± 8.4 ^C | 54.8 ± 12.6 ^{BC} |
| MN | 125.0 ± 7.7 ^{abA} | 121.7 ± 8.7 ^{aAB} | 88.9 ± 9.4 ^B | 86.9 ± 14.4 ^B |
| SI | 114.7 ± 7.3 ^{abA} | 97.9 ± 7.9 ^{abAB} | 80.5 ± 7.9 ^B | 63.9 ± 11.9 ^B |
| TX | 73.1 ± 7.7 ^c | 74.2 ± 8.7 ^b | 51.5 ± 8.8 | 83.3 ± 13.4 |

A,B different capital letters indicate significant difference in the rows ($P < 0.05$).

a,b different lowercase letters indicate significant difference in the columns ($P < 0.05$).

between 38.4 and 38.8 °C. During spring and summer, the TX animals also exhibited lower T3 means (Table 2).

The ORB did not differ between genetic groups, nor was it influenced by climatic seasons ($P > 0.05$) (Fig. 4).

Independently of climatic seasons, the TX breed showed significantly lower TNeck, TBfore, TBarrel, TBhind, TFlimb, TRlimb and TBack than the other breeds (Fig. 5). The DO rams exhibited the same TX variation for TBhind and TBack throughout the year. The TTest ranged between 31 and 32 °C, in spring and summer, with no difference between genotypes and reduced in the autumn and winter, with lower values for the DO and MN breeds ($P > 0.05$).

The TX animals had lower erythrocyte values in spring and winter (Table 3). There were no differences between the genotypes for hemoglobin and hematocrit variables. The DO animals exhibited the lowest MCV values in the spring. The DO and TX animals exhibited extreme MCH values in the spring. The MN animals had lower MCHC values, when compared to the SI breed, in the summer ($P < 0.05$).

There was no significant difference in the concentration of

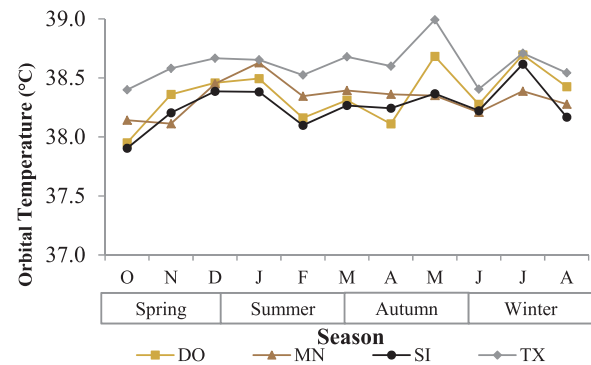


Fig. 4. Mean values of orbital area temperature (ORB) in Dorper (DO; $n = 8$), Morada Nova (MN; $n = 8$), Santa Inês (SI; $n = 9$) and Texel (TX; $n = 8$) rams recorded monthly throughout the year in a tropical climate region. Different lowercase letters (a, b) indicate significant difference within the climatic season by the Tukey test ($P < 0.05$).

leukocytes, eosinophils and lymphocytes among the genotypes, independent of the climatic seasons (Table 4). The DO and TX animals exhibited the highest and lowest neutrophil values in the autumn, respectively. The DO breed showed higher monocyte values in the winter. The mean platelet values for the SI breed were higher in the winter ($P < 0.05$).

There was no significant difference in SGA between breeds, independent of whether the measurements were taken in the summer or winter (Table 5). There was a difference in PGA between genotypes only in winter, with lower values for the MN animals and higher values for SI animals ($P < 0.05$). In the winter, the DO animals showed significantly higher primary follicle density. The density of secondary follicles and the total density of hair follicles of the DO animals were higher than those of the other breeds, both in summer and winter.

4. Discussion

Average temperatures and air relative humidity observed throughout the year were typical of the Cwa climate subtype, tropical altitude, temperature peaks reached 31.2 °C. During the spring and summer, the average THI reached values that indicate the possibility of moderate stress to sheep, according to the scale proposed by Marai et al. (2007). Similarly, the mean BGHI indicated a biometeorological heat warning situation (Baêta and Souza, 2010). Therefore, the animals had to activate their thermoregulatory system to maintain body temperature, especially in the spring and summer seasons.

The coat thickness of the TX animals increased throughout the year. In the spring, the beginning of the hair growth probably is related to the preparation for the subsequent cold season (Sumner and Bigham, 1993). The difference in coat thickness between the genotypes was probably because the TX breed originates from the crossing of English breeds, among them the Lincoln breed, which has long wool, and is the most influential in the formation of the Texel breed (ARCO, 2017). This genotype originates from a temperate region, where animals need to protect their body against the cold, to prevent excessive heat loss and maintain their thermal equilibrium. However, in tropical climate regions, especially in the hotter seasons of the year, the higher coat thickness and the presence of wool act as physical barrier, impairing the heat transfer to the external part. Thus, the greater the coat thickness, the lower the heat exchange capacity, making the animals less adapted to tropical conditions (Maia et al., 2009).

The respiratory route is considered to be the most efficient way to heat dissipation in sheep (Marai et al., 2007). The MN and SI breeds showed RR of 40–60 breaths m⁻¹, indicating a reduced need for sensible heat loss through the respiratory route. The DO breed showed 61–80 breaths m⁻¹, suggesting that these animals were in an intermediate thermal discomfort situation. However, the TX animals showed RR of 81–120 breaths m⁻¹, indicating substantial use of panting to

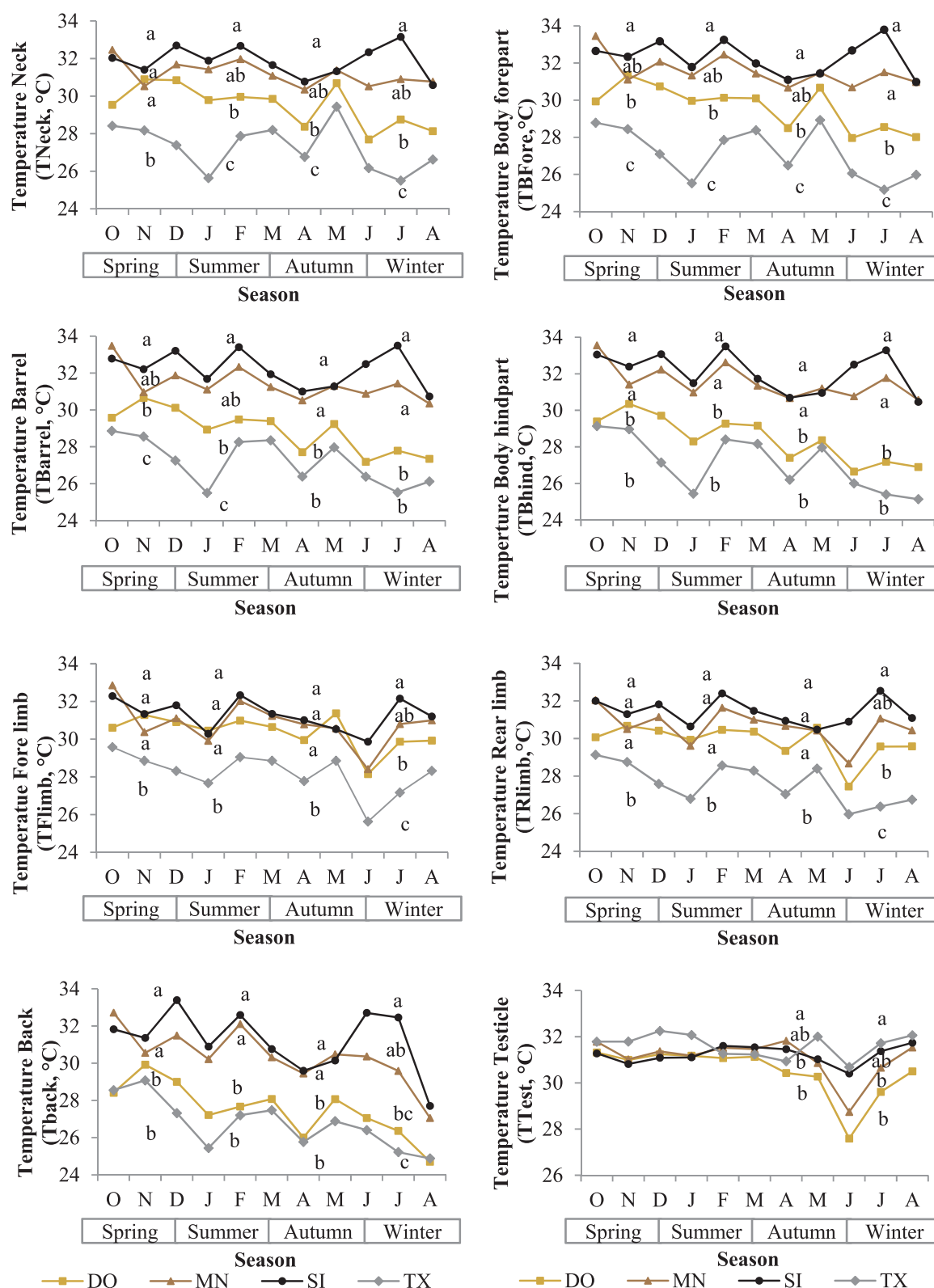


Fig. 5. Temperatures of body surface of Dorper (DO; $n = 8$), Morada Nova (MN; $n = 8$), Santa Inês (SI; $n = 9$) and Texel (TX; $n = 8$) rams recorded monthly by infrared thermography throughout the year, in a tropical climate region. Different lowercase letters (a, b) indicate significant difference within the climatic season by the Tukey test ($P < 0.05$).

alleviate a situation likely to progress to heat stress (McManus et al., 2015). The RR of TX animals was always higher, demonstrating their need to activate their thermoregulatory system more intensely. As the coat thickness was higher in this genotype, there was difficulty in the sensible heat loss, forcing the animals to use other means to dissipate

thermal energy (Moore et al., 2015). In this case, the woolled animals activated a mechanism of latent heat loss, increased their respiratory rate to increase body heat dissipation by evaporative thermolysis (Carvalho et al., 2014). Even so, throughout the year the TX animals expressed higher RT than the other genotypes.

Table 3

Mean and standard error values of erythrocyte constituents of Dorper (DO; n = 8), Morada Nova (MN; n = 8), Santa Inês (SI; n = 9) and Texel (TX; n = 8) rams measured throughout the year, in a tropical climate region.

| Breed | Spring | Summer | Autumn | Winter |
|---|---------------------------|----------------------------|----------------------------|--------------------------|
| Erythrocytes (10 ⁶ /μL) | | | | |
| DO | 10.0 ± 0.3 ^a | 10.5 ± 0.5 | 9.97 ± 0.7 | 11.9 ± 0.8 ^a |
| MN | 9.9 ± 0.6 ^{ab} | 10.2 ± 0.5 | 10.4 ± 0.5 | 10.4 ± 0.5 ^{ab} |
| SI | 9.7 ± 0.5 ^{ab} | 10.0 ± 0.4 | 10.0 ± 0.4 | 9.5 ± 0.6 ^{ab} |
| TX | 7.9 ± 0.3 ^b | 8.7 ± 0.5 | 8.5 ± 0.7 | 9.0 ± 0.9 ^b |
| Hemoglobin (g/dL) | | | | |
| DO | 11.0 ± 0.4 ^C | 11.9 ± 0.6 ^C | 12.7 ± 0.8 ^{BC} | 14.7 ± 1.1 ^{AB} |
| MN | 12.1 ± 0.8 ^C | 13.5 ± 0.6 ^C | 14.3 ± 0.6 ^{CB} | 13.9 ± 0.7 ^{AB} |
| SI | 11.8 ± 0.6 | 12.6 ± 0.5 | 13.4 ± 0.6 | 12.4 ± 0.7 |
| TX | 9.6 ± 0.4 | 10.7 ± 0.6 | 10.8 ± 0.9 | 11.9 ± 1.2 |
| Hematocrit (%) | | | | |
| DO | 31.2 ± 1.2 | 33.0 ± 1.6 | 33.3 ± 2.2 | 39.5 ± 2.9 |
| MN | 35.3 ± 2.2 | 37.3 ± 1.6 | 39.5 ± 1.6 | 38.5 ± 1.7 |
| SI | 33.3 ± 1.7 | 33.6 ± 1.2 | 35.9 ± 1.4 | 33.7 ± 1.9 |
| TX | 28.5 ± 1.2 | 30.0 ± 1.7 | 31.3 ± 2.4 | 32.7 ± 3.1 |
| Mean Corpuscular Volume (MCV, fL) | | | | |
| DO | 31.5 ± 0.8 ^c | 31.5 ± 1.1 | 33.4 ± 1.5 | 33.1 ± 2.1 |
| MN | 35.8 ± 1.6 ^{cb} | 37.0 ± 1.1 | 38.1 ± 1.0 | 37.1 ± 1.1 |
| SI | 34.2 ± 1.2 ^{cb} | 33.8 ± 0.8 | 35.9 ± 0.9 | 35.4 ± 1.3 |
| TX | 36.8 ± 0.8 ^{ab} | 34.7 ± 1.2 | 37.4 ± 1.7 | 36.7 ± 2.2 |
| Mean Corpuscular Hemoglobin (MCH, pg) | | | | |
| DO | 10.9 ± 0.24 ^b | 11.3 ± 0.4 ^c | 11.7 ± 0.5 ^c | 12.4 ± 0.6 |
| MN | 12.2 ± 0.5 ^{abC} | 13.3 ± 0.3 ^{abB} | 13.8 ± 0.3 ^{abAB} | 13.5 ± 0.3 ^{BC} |
| SI | 12.0 ± 0.4 ^{ab} | 12.6 ± 0.3 ^{bc} | 13.4 ± 0.3 ^{ab} | 13.1 ± 0.4 |
| TX | 12.4 ± 0.2 ^a | 12.4 ± 0.4 ^{bc} | 12.9 ± 0.5 ^{bc} | 13.4 ± 0.7 |
| Mean Corpuscular Hemoglobin Concentration (MCHC, %) | | | | |
| DO | 35.0 ± 0.2 ^C | 36.0 ± 0.3 ^{abBC} | 35.0 ± 0.5 ^{Cc} | 37.5 ± 0.6 ^{AB} |
| MN | 34.3 ± 0.4 ^B | 35.9 ± 0.3 ^{bb} | 36.2 ± 0.4 ^{bca} | 36.2 ± 0.4 ^A |
| SI | 35.3 ± 0.3 ^C | 37.4 ± 0.3 ^{aA} | 37.3 ± 0.3 ^{abB} | 36.9 ± 0.4 ^{BC} |
| TX | 33.8 ± 0.2 ^C | 35.8 ± 0.4 ^{abB} | 34.6 ± 0.5 ^{cBC} | 36.4 ± 0.7 ^{AB} |

A,B different capital letters indicate significant difference in the rows (P < 0.05).

a,b different lowercase letters indicate significant difference in the columns (P < 0.05).

The animals of the DO, MN and SI breeds presented coat characteristics that facilitate convective and evaporative thermolysis on the skin surface. This may have contributed to greater resistance to higher ambient temperature and therefore showed lower RT averages than the TX animals. On the other hand, the TX breed has a longer coat, which prevents air renewal inside the fleece and results in greater difficulty to eliminate heat (Gerken, 2010; Titto et al., 2016). By associating these characteristics with the effects of high air temperature and relative humidity, it can be assumed that there were reduced evaporative changes in the TX animals, causing them to accumulate more thermal energy, which explains their higher internal temperature.

However, although the TX animals had the highest RT, they were able to keep it below the maximum limit for the species, which is of 39.9 °C (Liu et al., 2012), possibly due to the lower production of T3, mainly in the spring and summer. Since T3 is a thyroid hormone with anabolic and catabolic actions, the secretion reduction can be seen as an effort by the animals to decrease the production and accumulation of body heat (Garcia, 2013; Koluman and Daskiran, 2011), and to maintain the thermal equilibrium. The temperature of the orbital area is a characteristic considered as sensitive to thermoregulatory changes in the body (Schaefer et al., 2007). Therefore, the maximum ORB temperature is the measure that shows high correlation with internal body temperature (Barros et al., 2016). However, unlike that observed for RT, there was no significant ORB difference between the genotypes in the present study.

On the other hand, the TNeck, TBfore, TBarrel, TBhind, TFlimb, TRLimb TBack may have been influenced by the color of the coat. The lower surface temperatures found in the TX animals are justified by the physical characteristics of the wool, which acts as a thermal insulator, re-irradiating heat and exhibiting higher thermal resistance (Castanheira et al., 2010), besides the greatest wool length and color, which is white. In fact, animals with lighter colored coats have higher

Table 4

Mean and standard error values of the constituents of white blood cell count of Dorper (DO; n = 8), Morada Nova (MN; n = 8), Santa Inês (SI; n = 9) and Texel (TX; n = 8) rams measured throughout the year in a tropical climate region.

| Breed | Spring | Summer | Autumn | Winter |
|---|-------------------------|-------------------------|--------------------------|--------------------------|
| Leukocytes (10 ⁶ /mm ³) | | | | |
| DO | 9.1 ± 1.3 ^B | 9.8 ± 1.9 ^B | 18.6 ± 2.7 ^A | 17.8 ± 3.3 ^A |
| MN | 8.5 ± 2.3 | 9.6 ± 1.9 | 8.1 ± 2.3 | 8.9 ± 2.5 |
| SI | 11.2 ± 1.8 | 12.2 ± 1.5 | 13.5 ± 2.0 | 16.0 ± 2.4 |
| TX | 8.6 ± 1.3 | 10.1 ± 2.0 | 9.8 ± 3.0 | 13.8 ± 3.6 |
| Eosinophils (10 ⁶ /mm ³) | | | | |
| DO | 1.0 ± 0.2 | 0.9 ± 0.4 | 0.7 ± 0.5 | 0.9 ± 0.6 |
| MN | 1.4 ± 0.4 | 1.2 ± 0.4 | 0.5 ± 0.5 | 0.5 ± 0.5 |
| SI | 1.2 ± 0.3 | 1.2 ± 0.3 | 1.3 ± 0.4 | 1.5 ± 0.5 |
| TX | 0.9 ± 0.2 | 1.1 ± 0.4 | 1.1 ± 0.6 | 1.7 ± 0.7 |
| Neutrophils (10 ⁶ /mm ³) | | | | |
| DO | 4.6 ± 0.7 ^{AB} | 4.6 ± 1.0 ^B | 10.7 ± 1.4 ^{AA} | 10.2 ± 1.7 ^{AC} |
| MN | 3.7 ± 1.2 | 4.6 ± 1.0 | 4.3 ± 1.3 ^{ab} | 4.1 ± 1.4 |
| SI | 5.7 ± 0.9 | 6.1 ± 0.8 | 5.4 ± 1.1 ^{ab} | 7.1 ± 1.3 |
| TX | 3.6 ± 0.7 | 4.0 ± 1.1 | 4.2 ± 1.6 ^b | 5.1 ± 1.9 |
| Lymphocytes (10 ⁶ /mm ³) | | | | |
| DO | 3.1 ± 0.5 | 3.9 ± 0.7 | 6.3 ± 1.1 | 5.5 ± 1.3 |
| MN | 3.2 ± 1.0 | 4.6 ± 0.7 | 3.4 ± 0.8 | 4.2 ± 0.9 |
| SI | 4.0 ± 0.7 | 4.5 ± 0.6 | 5.5 ± 0.7 | 6.8 ± 0.9 |
| TX | 3.9 ± 0.5 | 4.5 ± 0.8 | 4.0 ± 1.2 | 6.4 ± 1.4 |
| Monocytes (10 ⁶ /mm ³) | | | | |
| DO | 0.4 ± 0.7 ^B | 0.3 ± 0.1 ^B | 0.7 ± 0.1 ^{BC} | 1.0 ± 0.2 ^{aAC} |
| MN | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 ^b |
| SI | 0.4 ± 0.9 | 0.5 ± 0.8 | 0.5 ± 0.1 | 0.4 ± 0.1 ^b |
| TX | 0.3 ± 0.7 | 0.4 ± 0.1 | 0.3 ± 0.2 | 0.4 ± 0.2 ^b |
| Platelets (10 ⁶ /μL) | | | | |
| DO | 0.5 ± 0.6 | 0.3 ± 0.8 | 0.2 ± 0.1 ^c | 0.2 ± 0.1 |
| MN | 0.8 ± 0.1 | 0.6 ± 0.1 | 0.5 ± 0.1 ^{bc} | 0.5 ± 0.1 |
| SI | 0.6 ± 0.1 ^A | 0.4 ± 0.1 ^{AB} | 0.8 ± 0.1 ^{abA} | 0.1 ± 0.1 ^B |
| TX | 0.5 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.1 ^c | 0.2 ± 0.2 |

A,B different capital letters indicate significant difference in the rows (P < 0.05).

a,b different lowercase letters indicate significant difference in the columns (P < 0.05).

Table 5

Mean and standard error values of morphological characteristics of Dorper (DO; n = 8), Morada Nova (MN; n = 8), Santa Inês (SI; n = 9) and Texel (TX; n = 8) rams measured throughout the year in a tropical climate region.

| Breed | Summer | Winter |
|--|-------------------------|--------------------------|
| Area of Sweat Glands (ASG, × 10 ³ μm ²) | | |
| DO | 13.8 ± 5.4 | 10.0 ± 3.2 |
| MN | 29.0 ± 3.4 ^A | 7.0 ± 5.8 ^B |
| SI | 21.0 ± 3.2 | 14.8 ± 4.4 |
| TX | 15.3 ± 5.8 | 5.3 ± 3.0 |
| Proportion of Glandular Area (PGA, %) | | |
| DO | 7.5 ± 1.2 ^A | 4.5 ± 0.7 ^{abB} |
| MN | 8.5 ± 0.7 | 1.5 ± 1.2 ^b |
| SI | 7.2 ± 0.7 ^A | 5.0 ± 1.0 ^{ab} |
| TX | 6.8 ± 1.3 | 2.6 ± 0.7 ^{ab} |
| Primary Follicle Density (PF.mm ⁻²) | | |
| DO | 3.0 ± 0.2 | 3.5 ± 0.2 ^a |
| MN | 2.6 ± 0.2 | 2.5 ± 0.2 ^b |
| SI | 2.4 ± 0.1 | 2.5 ± 0.2 ^b |
| TX | 2.4 ± 0.2 | 2.3 ± 0.2 ^b |
| Secondary Follicle Density (SF.mm ⁻²) | | |
| DO | 20.7 ± 1.5 ^a | 26.4 ± 1.4 ^a |
| MN | 10.1 ± 1.5 ^b | 11.8 ± 1.5 ^b |
| SI | 9.4 ± 1.2 ^b | 10.9 ± 1.2 ^b |
| TX | 14.3 ± 1.5 ^b | 14.0 ± 1.7 ^b |
| Density of Hair Follicles (HF.mm ⁻²) | | |
| DO | 23.5 ± 1.6 ^a | 29.9 ± 1.4 ^a |
| MN | 12.7 ± 1.5 ^b | 14.3 ± 1.5 ^b |
| SI | 11.8 ± 1.2 ^b | 13.5 ± 1.3 ^b |
| TX | 16.7 ± 1.5 ^b | 16.3 ± 1.8 ^b |

A,B different capital letters indicate significant difference in the rows (P < 0.05).

a,b different lowercase letters indicate significant difference in the columns (P < 0.05).

reflectivity and absorb between 40% and 50% less radiation than those with dark colored coats (McManus et al., 2011). The lower absorption of solar shortwave radiation reduces the thermal energy storage, which contributed to maintaining the temperature of the light colored coats at lower levels. Therefore, the animals of the SI breed, which have short hair and black fur, always presented higher body surface temperatures, regardless of the anatomical region evaluated. It is noteworthy that during the summer the back temperature of SI animals reached values of 5.5 °C higher than the woolled animals. In contrast, the woolled regions of the TX animals always presented lower surface temperature in comparison to the genotypes without any wool coats, throughout the experimental period, reinforcing the role wool has in minimizing the absorption of direct solar radiation.

Independently of the genotype, the animals were able to establish similar testicular thermoregulation pattern in the more challenging thermal seasons, which resulted in similar *T*-Test. The countercurrent heat exchange in the pampiniform plexus may have contributed to the testicular thermoregulation efficiency between the hottest arterial blood and cooler venous blood, the action of the cremaster muscle, which approaches or moves the testis away from the body, and the activation of scrotal sweat glands which favor testicular evaporative cooling (Kastelic et al., 1997; Kastelic, 2014). However, genotypic differences were observed in autumn and winter, regarding scrotal thermoregulation. The smallest *T*-Test found in the DO and MN animals contrasted with the values observed in the TX animals. However, the difference between the RT and *T*Test was always close to or above 6.0 °C, the thermal gradient recommended for normal spermatogenesis for animal species bearing extra-cavitary testes (Waite, 1970). Interestingly, the *T*-Test was the variable that showed the behavior with a higher degree of parallelism in the BGHI records, possibly for being a thinner skin area, relatively hairless (Kastelic, 2014), probably making it more responsive to combined variations of air temperature, relative humidity and direct sunlight.

The evaluation of hematological parameters can be an important tool to check the animal welfare, since the blood is an important response indicator to heat stress (Okourwa, 2015). Despite the higher erythrocyte means found for the DO breed in the spring, the values for this constituent were within the range considered as reference value for the species (Madureira et al., 2013). The lack of significant differences in hemoglobin and hematocrit variables were due to compensatory physiological changes, which helped the body overcome the thermal challenge. As the animals were able to efficiently dissipate excess heat, the thermal challenge effects may have been mitigated on these blood parameters throughout the experiment. The MCV and MCH reduction in the spring can be attributed to the hemodilution effect caused by a possible water consumption increase, which is transported in the circulatory system for evaporative cooling (El-Nouty et al., 1990). The MCHC increased significantly in the summer, which can be explained by a decrease in plasma volume (Hashem, 2014). However, the averages of the leukogram constituents show that despite the increased number of neutrophils and monocytes in the DO animals in autumn and winter, the leukogram constituents found in this study were always within the reference values for sheep (Radostits et al., 2002).

The role of the sweat glands is to produce sweat and its evaporation on the skin causes heat loss (Gebremedhin et al., 2008) to maintain the heat balance. In this study, due to high temperatures and humidity, the THI value reached 23.1, which indicates the possibility of moderate heat stress in sheep (Marai et al., 2007) and consequently an increased need for heat dissipation. The main functional requirement of sweat glands leads to increased sweat production, and as a consequence to increase the area of the secretory glands (Kahwage, 2015). The main activation of sweat glands contributes to greater heat loss through the skin and consequently, lower need for panting, which reduces RR. The increased secretory portion of sweat glands in the MN breed demonstrates its greater heat dissipation capacity by sweating and may indicate the animal's tropical climate adaptation (Bianchini et al., 2006).

The tissue area occupied by the secretory portion of sweat glands was lower for the MN breed in the winter, since this season represents a lower thermal challenge and therefore determines a lower need for heat dissipation by sweating. In winter, the sweat glands are also located in deeper layers of the dermis, indicative of lower functional activity (Ferreira et al., 2009), and therefore, the PGA was lower in the MN breed during this season.

Finally, the physical properties of the coat may affect the efficiency of cooling the skin surface. Hair density, for example, can impede heat transfer, mainly in tropical conditions (Maia et al., 2009). The primary follicles produce the thick hairs, which provide mechanical protection, while the secondary follicles are more numerous and produce fine fibers, providing thermal protection (Ansari-Renani et al., 2011). The lesser hair coat thickness of the DO breed could be an unfavorable feature because it would facilitate the incidence of direct sunlight (Silva et al., 2001). However, in the DO animals, this effect may have been mitigated by the greater hair density observed in the summer, which is a protective barrier that minimizes the direct solar radiation incidence on the skin (Maia et al., 2003), which is less pigmented in these animals. In contrast, woolless animals from hot climate regions have shorter, darker and less abundant hair coats (McManus et al., 2009), as observed in the MN and SI breeds.

5. Conclusions

Throughout the year the tropical climate presented challenging thermal conditions for the animals, with the exception of the milder winter. Despite climatic variations and the warm climate, the genotypes Morada Nova, Santa Ines and Dorper showed a lower need to activate the respiratory evaporative route and maintained lower internal temperatures. The Texel animals showed lower body surface temperatures due to the physical characteristics of their light-colored and woolled coat. However, its wool impaired sensible heat loss, forcing them to increase their respiratory rate and reduce their metabolism to maintain thermal equilibrium. Therefore, the animals Morada Nova, Santa Ines, considered tropical indigenous breeds, as well as the Dorper animals, were able to better overcome the thermal challenges imposed, showing superior thermolytic performance due to specific adaptive morphological and physiological characteristics, and were more heat-tolerant.

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