Journal of Thermal Biology 84 (2019) 103–110

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Coat and skin morphology of hair sheep breeds in an equatorial semi-arid environment

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ARTICLE INFO

Keywords:
Cutaneous surface
Thermal insulation
Tropical climate
Phenotypic acclimatization

ABSTRACT

This study aims to address if are there annual changes in the hair coat traits and skin morphology of hair sheep breeds raised in an equatorial semi-arid region? Coat and skin samples were taken from thirty Morada Nova (4 ± 2 years old; red coat; ± SD) and twenty Santa Inês multiparous ewes (5 ± 2 years old; brown and black coat; ± SD) every 3 months over a year. Hair coat traits included thickness (mm), density (number of hairs cm^{-2}), length (mm), and diameter (mm), plus epidermal and dermal thickness (μm), sweat glands and blood capillaries area (μm cm^{-2}) were determined. Means of solar irradiance and ambient air temperature were higher between September and December. Annual changes ($P < 0.05$) in hair density, diameter, length and thickness, as well as the skin blood capillaries and sweat gland area differed between breeds. The modifications on hair coat traits resulted in minor changes on the effective thermal conductivity of the hair coat surface both for Morada Nova and Santa Ines sheep. Nevertheless, it was clearly evident that the overall cutaneous thermal insulation for Morada Nova sheep was lowest in September that was coupled with lower hair density, coat thickness, and higher sweat gland and blood capillary area ($P < 0.05$). In conclusion, even in an equatorial region, phenotypic acclimatization on morphological traits of cutaneous surface and skin traits can modify the overall thermal insulation of sheep breeds.

1. Introduction

The hair sheep breeds were introduced into Brazil by successive waves of early exploration and colonization. These sheep survived, reproduced, and adapted to their new environment by developing unique adaptive characteristics to the environment and specific production systems (Mariante and Egito, 2002; Egito et al., 2002). The Morada Nova and Santa Inês sheep provide a source of protein to traditional populations of the Brazilian Northeast, particularly in the semiarid region (McManus et al., 2011; Ribeiro and Gozalez-Garcia, 2016). Typically, this hot environment is characterized by high levels of solar radiation and ambient air temperature, plus low levels of precipitation (Silva et al., 2015).

The ambient air temperature and solar radiation are the two main environmental factors that affect the animal thermal exchanges (Maia et al., 2015; Silva et al., 2010). Sheep, as homoeothermic, continuously adjust their physiology and behavior to maintain a balance between heat produced by metabolism, heat gained from the environment through sensible routes (i.e., radiation, convection, and conduction), and heat dissipation through sensible and insensible avenues (i.e., moisture evaporation from skin and/or upper respiratory tract; Fonseca et al., 2017; Maia et al., 2016; Silva and Maia, 2013). Such regulation is influenced by physical properties of the cutaneous surface traits as hair coat thickness, density, length, and diameter (Cena and Monteith, 1975; Maia et al., 2003, 2005a, 2005b), as well as, skin traits including dermal thickness, capillary bed, sweat gland area and density (Jian et al., 2013; Costa et al., 2014), to determine the overall body thermal insulation.

Seasonal changes or acclimatization in thermal conductance of the cutaneous surface have long been recognized under seasonal climatic

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https://doi.org/10.1016/j.jtherbio.2019.06.007
Received 18 April 2018; Received in revised form 27 April 2019; Accepted 3 June 2019
Available online 24 June 2019
0306-4565/

fluctuations, especially in mammal's species living in temperate zones (Jacobsen, 1980; Walsberg, 1991; Jofré and Caviedes-Vidal, 2003; Lovegrove, 2005; Boyles and Bakken, 2007). Preliminary investigation performed by Berman and Volcani (1960) detected significant thermoperiodic and photoperiodic effects on the annual cycle in the coat of dairy cattle raised under latitude near to 30° N. Nevertheless, to date, we are unaware of any studies that have specifically investigated the annual changes in the skin and coat surface in sheep breeds raised in equatorial semi-arid conditions. As for cattle, the only reports are those that of Maia et al. (2003) and Façanha et al. (2010), who verified seasonal modifications in the hair coat surface traits of Holstein cows raised in latitude near to 20° and 5° S. Therefore, the present study was designed to answer the following questions: (1) are there annual changes in the hair coat traits and skin morphology of hair sheep breeds raised in an equatorial semi-arid region? If yes, are such alterations similar for the Morada Nova and Santa Inês sheep? These data will add valuable information about adaptive traits and heat tolerance of hair-coated sheep breeds raised in equatorial semi-arid conditions.

2. Material and methods

2.1. Study site, animals, and experimental design

This research was reviewed and approved by the Federal University of Paraíba Animal Care and Use Committee before the study started (process number: 081/2015). Coat and skin samples were assessed from thirty Morada Nova (4 ± 2 years old; red coat) and twenty Santa Inês multiparous ewes (5 ± 2 years old; brown and black coat) on September 21, 2014, December 21, 2014, March 21, 2015, and June 21, 2015. The study was carried out using a fully randomized split-plot design, with breed as the main plot and period of the year as the subplots.

Data on Morada Nova and Santa Ines subjects were collected from the Experimental farm of the Federal University of Paraíba (07° 23′ 27″ S, 458 m altitude), and from a commercial farm (06° 52′ 06″ S, 555 m altitude), respectively. A semi-intensive system was in place on both farms that animals grazed on native pastures (*Caatinga*) and were fed a mineral supplement with additional dietary supplementation during the dry season. Body score condition for both herds (Santa Ines and Morada Nova) ranged from 3.0 to 3.5. The meteorological data including ambient air temperature (°C), solar irradiance (W m^{-2}), precipitation (mm), and mean day length were obtained between 2014 and 2015 from a weather station of the National Meteorological Institute (07° 29 ‘20′ S, 436 m altitude; Fig. 1).

2.2. Hair coat traits and effective thermal conductivity

In vivo measurements and coat samples were collected between the 12th and 13th vertebrae, approximately 10 cm below the vertebral spine, always on the right side of each animal to determine the hair coat thickness (T; mm), density (N; number of hairs cm^{-2}), length (L; mm), and diameter (D; mm). Firstly, the coat thickness was measured *in vivo* using a digital millimeter caliper, according to the method described by Silva (2000). Then, coat samples were collected from an area of 0.18 cm^2 using specially adapted clippers, according to the procedure described by Silva (2000). After sampling, the coat samples were stored in paper bags. Density was calculated by counting the number of hairs per sample area and then converting the number of hairs counted into the number of hairs per cm^2 of surface area. The length and diameter of the ten longest hairs within each sample was measured with a digital caliper and micrometer (Model IP40, Digimess, Brazil. Accuracy ± 0.1 µm), according to the method described by Udo (1978).

Using the approach of Davis and Bikerbak (1974), taking into consideration the conduction in the absence of free convection and radiation process, the effective thermal conductivity (k_{eff}) was determined as follow:

$$k_{eff} = k_{hair}\left(\frac{0.25\pi D^2 LN}{T}\right) + k_{air}\left[1 - \left(\frac{0,25\pi D^2 LN}{T}\right)\right] \quad [1]$$

where k_{hair} is the hair thermal conductivity = 0.27 W m^{-1} K^{-1}; D is hair diameter (cm); L is hair length (cm); N is hair density (n/cm^2); T is hair coat thickness (cm); k_{air} is the air thermal conductivity = 0.026 W m^{-1} K^{-1}.

2.3. Skin and sweat gland traits

Skin samples were taken with a skin biopsy punch (diameter = 0.5 cm) from six animals of each breed, from three body regions, namely the upper central region of the shoulder; the side between the 12th and 13th ribs, 10 cm below the vertebral spine; and the central region of the leg, 10 cm below the ilium (Fig. 2). The procedure was performed 10 min after the administration subcutaneous of a local anesthetic (2 mL of lidocaine hydrochloride; Lidovet - Bravet). The thickness (µm) of the epidermis and dermis and the sweat glands and blood capillaries (µm cm^{-2}) area were determined.

Skin samples were fixed by immersion in 10% formaldehyde to preserve the samples until processing. The tissue fragments were sent for histological analysis and embedded in paraffin according to standard histological processing (Heleno et al., 2011). Longitudinal 5-µm-thick sections were prepared using a microtome and stained with hematoxylin and eosin. Photomicrographs of the histological slides were digitized using the software Motic Images Plus 2.0 and an Olympus BX-60 microscope with a Motic digital camera attached to the computer. Subsequent measurements were made using the same software. The 40× and 5× objectives were used to measure the thickness of the epidermis and dermis, respectively. Two photomicrographs per side-region fragment were digitized, and four measurements were performed per photomicrograph, totaling 48 measurements (6 animals x 2 photomicrographs x 4 measurements) in each breed, per period of the year.

The 5× objective was used to measure the sweat gland area digitizing two photomicrographs for each fragment, totaling 12 samples (6 animals x 2 photomicrographs) per body region (side, shoulder, and leg) per period of the year. In each of these 12 photomicrographs, the total area of the sweat glands found in the dermis was measured and subsequently converted to the value per cm^2 of the sample area. Finally, the 40× objective was used to measure the blood capillary area, digitizing seven photomicrographs per fragment, totaling 42 samples (6 animals x 7 photomicrographs), for each body region (side, shoulder, and leg), per period of the year. The total blood capillary area found in the dermis was measured in each of the 42 photomicrographs and subsequently converted to the value per centimeter squared of the sample area.

2.4. Statistical analyses

Data were analyzed by the least square method using the mixed linear models procedure of the statistical analysis system (SAS). Means were compared by the Tukey tests. The following models were employed:

Model 1 (Coat surface traits)

$$Y_{ijk} = \mu + R_i + e_{ik} + E_j + RE_{ij} + e_{ijk}$$

where Y_{ijkl} is the l-th observation of coat surface traits; µ is the overall mean; R_i is the fixed effect of i-th breed (i = Morada Nova and Santa Inês sheep); e_{ik} residual due to effect of the interaction of the random animal effect within each breed; E_k is the fixed effect of the period of the year (k = September 21, 2014; December 21, 2014; March 21, 2015; June 21, 2015); RE_{ik} represents the interaction effects of i-th breed and the k-th period of the year; e_{ijk} is the residual term due to effect of the interaction between breed and period of the year.

Model 2 (Skin morphology traits)

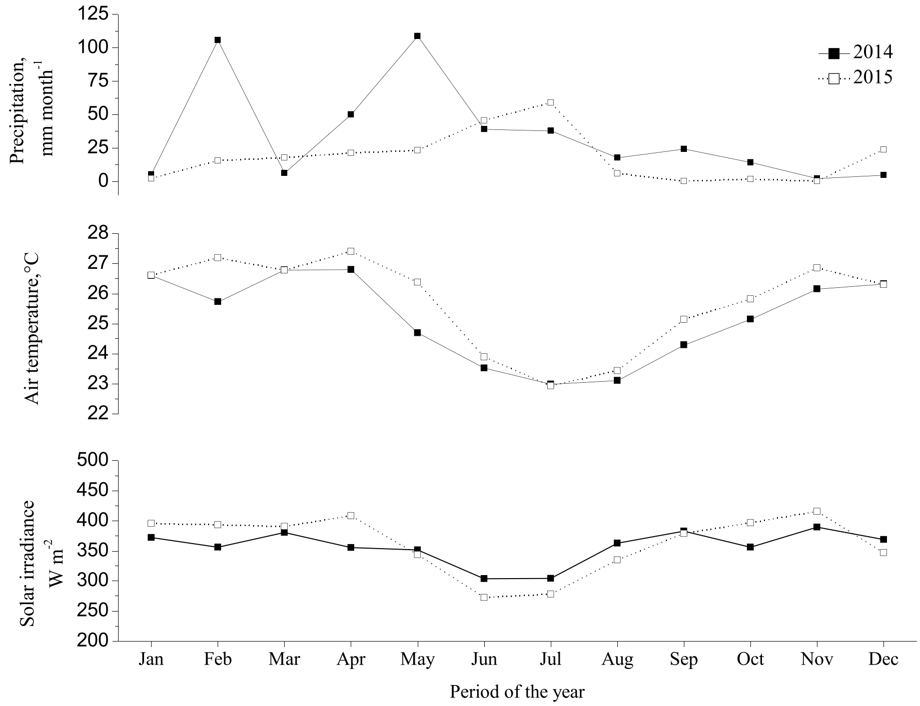

Fig. 1. Monthly pattern for meteorological variables during the study.

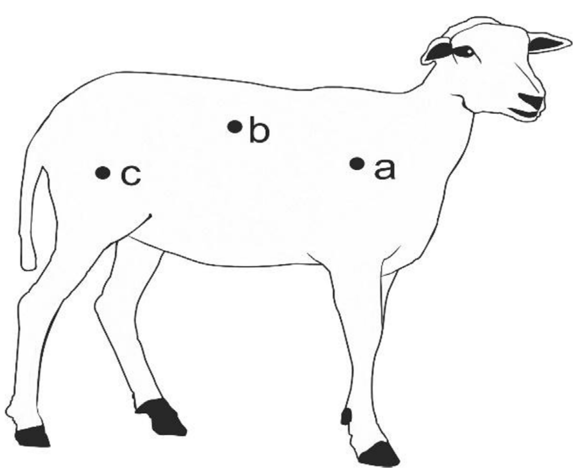

Fig. 2. Body regions for skin sampling (a = shoulder; b = side; c = leg).

$$Y_{ijkl} = \mu + R_i + C_j + RC_{ij} + e_{ijl} + E_k + RE_{ik} + EC_{kj} + RCE_{ijk} + e_{ijkl}$$

where Y_{ijkl} is the m-th observation of the skin morphology traits; μ is the overall mean; R_i is the fixed effect of breed (i = Morada Nova and Santa Inês); C_j is the fixed effect of j-th part of the body (j = shoulder, between the 12th and 13th ribs, and the central region of the leg); RC_{ij} is the i-th breed versus j-th body region interaction effect; e_{ijl} is residual due to effect of the interaction between breed and body region; E_k is the k-th fixed effect of period of the year (k = September 21, 2014; December 21, 2014; March 21, 2015; June 21, 2015); RE_{ik} represent the interaction between i-th breed and the k-th period of the year; CE_{jk} represent the interaction between j-th part of the body and the k-th period of the year; RCE_{ijk} represent the interaction between i-th breed, j-th part of the body and the k-th season; and e_{ijkl} residual due to effect of the interaction between interaction between i-th breed, j-th part of the body and the k-th period of the year.

3. Results

3.1. Meteorological variables

The overall mean for meteorological variables between 2014 and 2015 are depicted in Fig. 1. Lowest means for air temperature and solar irradiance occurred between June and August, both for 2014 and 2015. These periods were coupled with the highest levels of precipitation and shortest day length. In September (both 2014 and 2015), means for air temperature, solar irradiance and day length began to increase. Please, see the supplementary document for more details about meteorological variables.

3.2. Hair coat surface traits

The annual pattern for hair coat traits and effective thermal conductivity of Santa Ines and Morada Nova sheep are depicted in Fig. 3 and 4. Except for hair coat density and length, fixed effects (breeds vs period of the year) showed significant interaction ($P < 0.05$) on hair coat diameter, coat thickness and effective thermal conductivity. Hair coat of Morada Nova were denser and longer ($P < 0.05$) than Santa Ines, with both breeds increasing the density and length of their hair coats in June. The hair diameter of Morada Nova was significantly wider ($P < 0.05$) in all periods of the year when compared with Santa Inês (Fig. 3). Moreover, wider diameters ($P < 0.05$) were observed in September for Morada Nova and June for Santa Ines sheep.

The Santa Inês had greater ($P < 0.05$) coat thickness when compared with Morada Nova sheep in September, December, and June (Fig. 3). The coat thickness of Morada Nova was lower ($P < 0.05$) in September and December, whereas coat thickness remained statistically unchanged ($P > 0.05$) across the periods for Santa Ines. The effective thermal conductivity of hair coat of Morada Nova was significantly higher ($P < 0.05$) than that of Santa Inês for all periods of the year. Moreover, the effective thermal conductivity for Morada Nova sheep was slightly higher ($P < 0.05$) in September, while was statistically indistinguishable ($P > 0.05$) across year for Santa Ines (Fig. 4).

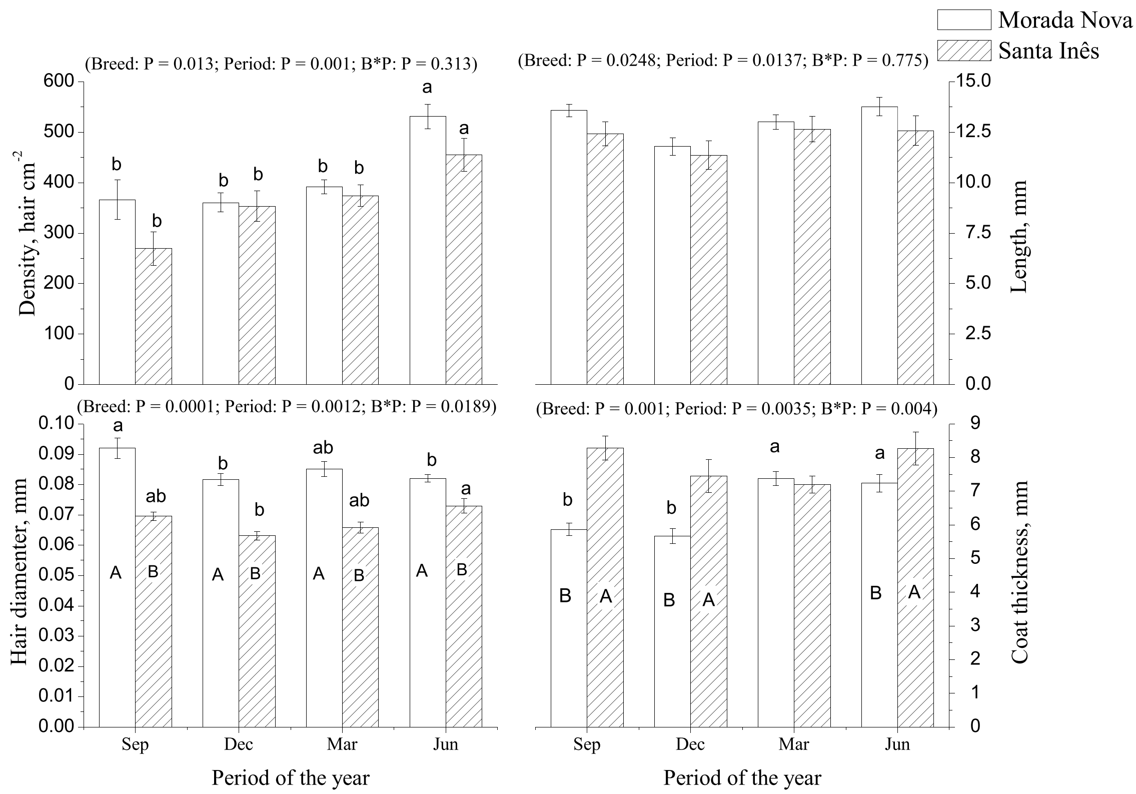

Fig. 3. Annual pattern of hair coat traits of Morada Nova and Santa Inês sheep raised in an equatorial semi-arid region. Uppercase letters correspond to the effect of period of the year, and lowercase letters correspond to the breed effect. Sep = September 2014; Dec = December 2014; Mar = March 2015; Jun = June 2015; B*P = Interaction between the fixed factors (breed and period of the year). Data are presented with mean ± SEM.

3.3. *Skin morphological traits*

The annual pattern of dermal and epidermal thickness, sweat gland and blood capillary area of Morada Nova and Santa Ines sheep are presented in Figs. 5 and 6. The interaction between fixed effects (breed vs period of year) on all skin traits was statistically significant ($P < 0.05$). The dermal thickness of Morada Nova was greater ($P < 0.05$) than that of Santa Ines only in September. At this time, the Morada Nova presented higher ($P < 0.05$) dermal thickness, while for Santa Ines was greater in June. Except for June, the epidermal thickness did not differ ($P > 0.05$) between breeds (Fig. 5). Additionally, both Morada Nova and Santa Ines sheep had thicker epidermis in December.

The Morada Nova showed higher ($P < 0.05$) sweat gland area than that of Santa Ines only in September, while the opposite was observed in March (Fig. 5). In fact, the largest sweat gland area for Morada Nova across the year was recorded in September, and the blood capillary

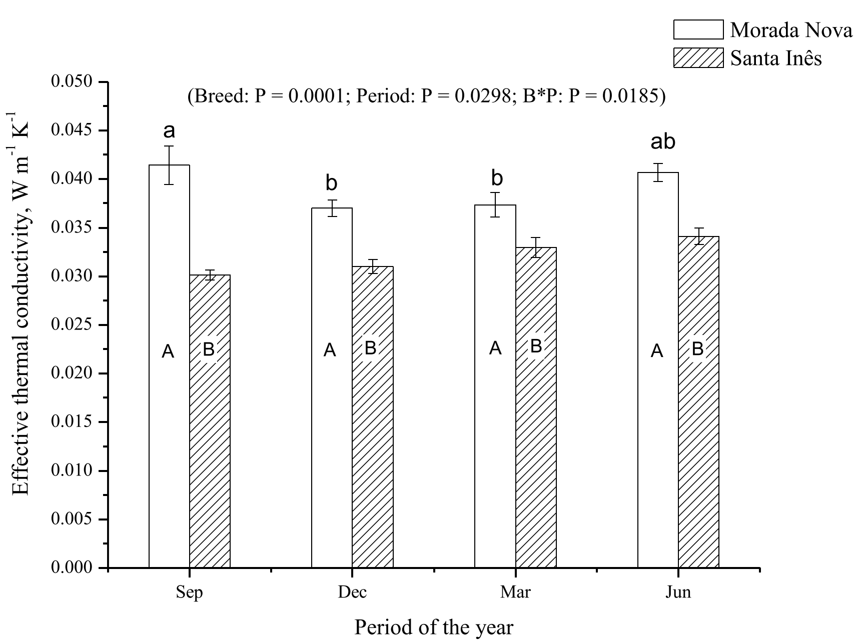

Fig. 4. Annual pattern in effective thermal conductivity of Morada Nova and Santa Inês sheep raised in an equatorial semi-arid region. Uppercase letters correspond to the effect of period of the year, and lowercase letters correspond to the breed effect. Sep = September 2014; Dec = December 2014; Mar = March 2015; Jun = June 2015; B*P = Interaction between the fixed factors (breed and period of the year). Data are presented with mean ± SEM.

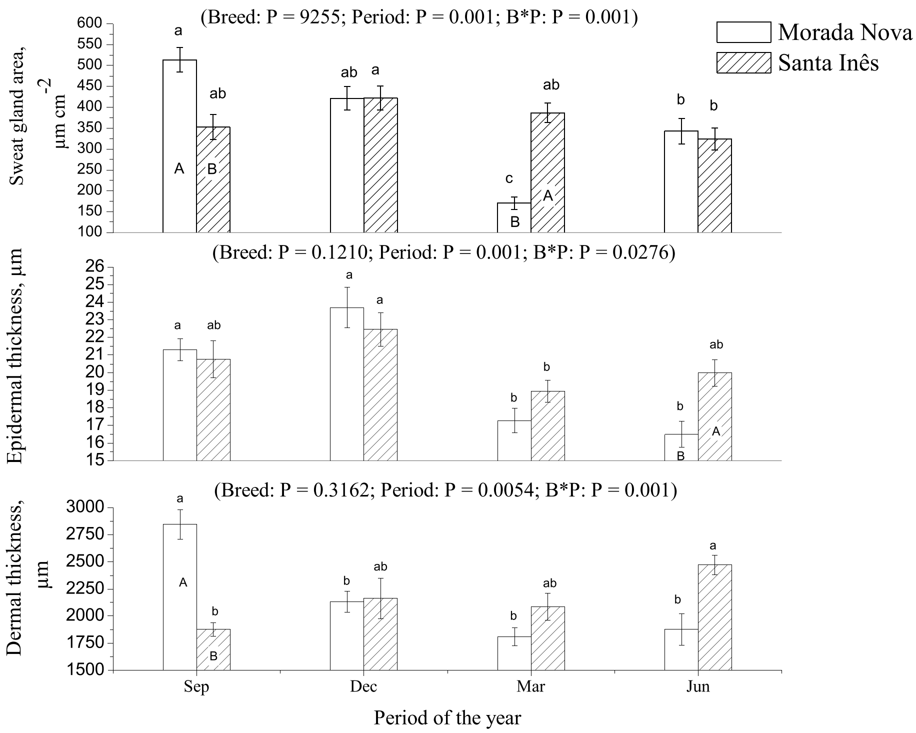

Fig. 5. Annual pattern for sweat gland area, epidermal and dermal thickness of Morada Nova and Santa Inês sheep raised in an equatorial semi-arid region. Uppercase letters correspond to the effect of period of the year, and lowercase letters correspond to the breed effect. Sep = September 2014; Dec = December 2014; Mar = March 2015; Jun = June 2015; B*P = Interaction between the fixed factors (breed and period of the year). Data are presented with mean ± SEM.

area, as well (Fig. 6). For both breeds, sweat gland area was statistically indistinguishable (P > 0.05) between shoulder, side, and leg. Otherwise, blood capillary area was higher on leg for Morada Nova and on side region for Santa Ines (Fig. 6).

4. Discussion

The condition of any semi-arid tropical region can be described as a dry and extremely hot condition with marked fluctuations of water and food resources (Leroy et al., 2018). In such geographical location, the angle of solar declination results in a more constant pattern of solar irradiance, air temperature, and photoperiod (Silva, 2006). In fact, when compared with the magnitude of thermal fluctuations in temperate zones (Silva et al., 2015), annual climate changes in equatorial regions are much smaller as observed by meteorological data collected in the present study between 2014 and 2015. Even that, for both years, the means of solar irradiance, ambient air temperature, and day length began to increase from September. Perhaps, these environmental zeitgebers influence and synchronize changes in hair coat and skin traits of sheep breeds throughout the year. Such changes are referred to as phenotypic acclimatization (Lovegrove, 2005). Our results revealed that annual changes of hair density, diameter, length, and thickness,

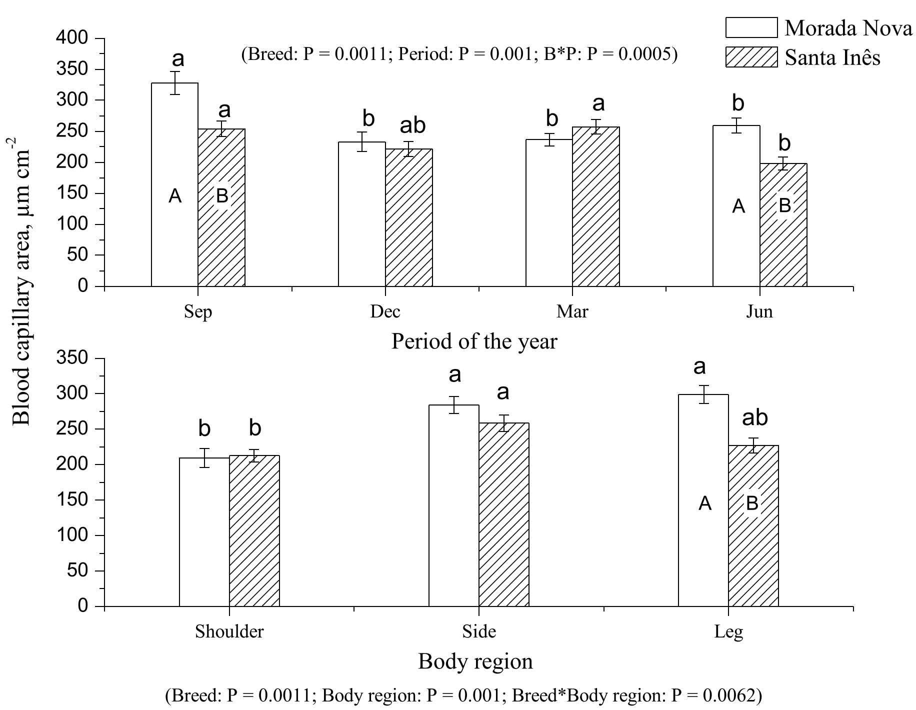

Fig. 6. Annual pattern and body region differences for blood capillary area of Morada Nova and Santa Inês sheep raised in an equatorial semi-arid region. Uppercase letters correspond to the effect of period of the year, and lowercase letters correspond to the breed effect. Sep = September 2014; Dec = December 2014; Mar = March 2015; Jun = June 2015; B*P = Interaction between the fixed factors (breed and period of the year). Data are presented with mean ± SEM.

plus the skin blood capillaries and sweat gland area were observed in both breeds. For instance, it was clearly evidenced that for Morada Nova sheep, lower overall cutaneous thermal insulation was observed in September, which was coupled with lower hair density, coat thickness, and higher sweat gland and blood capillary area.

The animal thermal insulation revealed how easily heat and mass are exchanged with their environment. The evolution of the hair sheep breeds in equatorial semiarid environment has probably driven the cutaneous surface traits (fur and skin) for two main purposes: protection of the skin tissue against shortwave solar radiation and high thermal conductance of the body cutaneous surface (Marai et al., 2007; Silva, 2008). The thermal conductivity of the hair coat is related to the structure of a non-homogeneous system constituted by the hairs and the air trapped among them that is influenced by hair density, diameter, length, and hair angle normal to the skin (Maia et al., 2009). In addition, the set of skin characteristics like pigmentation, dermal and epidermal thickness, blood capillary, and sweat glands could indicate the level of skin protection and capacity to eliminate body heat, as well.

The approach used in this study to estimate the effective thermal conductivity of hair coat took into account only conduction processes through the fur layer. Our results showed that the overall means of effective thermal conductivity was a lightly higher in the Morada Nova (0.0391 $W m^{-1} K^{-1}$) than in Santa Ines sheep (0.0320 $W m^{-1} K^{-1}$), which them are much less than hair fiber conductivity (0.26 $W m^{-1} K^{-1}$), and closer to the air conductivity (0.026 $W m^{-1} K^{-1}$). Moreover, while there were annual changes in hair density, diameter, length, and thickness, they resulted in minor changes on the effective thermal conductivity both for Morada Nova and Santa Ines sheep (Fig. 4). However, the portion of metabolic heat dissipated to the environment by conduction processes through the hair fiber is minor when compared with other routes like surface convection and radiation (Hammel, 1955). For instance, the thermal insulation provide by the fur is affected strongly by wind passing over the coat surface, especially in the coats where the trapped layer of air can easily be disturbed (Tregear, 1965; Boyles and Bakken, 2007). Therefore, the absence of convection and radiation mechanisms in our approach could impose an apparent limitation on discussion of the annual changes in body thermal insulation.

The level of wind penetration into the coat is dependent on the spacing between the hairs and the coat thickness (Tregear, 1965). The overall mean for hair coat density was greater for Morada Nova sheep, and the opposite was observed for thickness. Moreover, for both Morada Nova and Santa Inês sheep, the highest hair density was observed in June (501 hairs cm^{-2}), which was almost 20% higher when compared with the other periods of the year. Also, the Morada Nova sheep presented greater fur thickness in March and June, and differences with other periods was minor (~ 1.0 μm); while the coat thickness of Santa Ines did not change over the year. Façanha et al. (2010) investigated aspects of phenotypic acclimatization in Holstein cows at equatorial latitude (~ 5 °S), and observed higher hair density in June, plus non-significant alterations in the fur thickness. Similarly, Pantoja et al. (2017) observed that the coat thickness of Morada Nova, Santa Ines, and Dorper sheep presented minimal variation throughout the year in a latitude close to 21° S.

The present study showed that annual changes in hair coat density and thickness are smaller than the seasonal changes that have been reported in species living in more temperate regions. For example, Hart (1956) observed that winter changes in these coat traits can reach 50% for some wild species. Similar pattern was observed by Dowling (1958) who investigated the winter and summer coats of Holstein cattle managed at 25° S latitude. Obviously, the larger climate fluctuations in temperate zones may explain these differences in the level of annual phenotypic acclimatization for hair coat traits. Nevertheless, our results showed that the highest hair density coincided with the period of lower means of solar irradiance and ambient air temperature. That time was also the period of highest rainfall. Then, what is the impact of this on heat and mass transfer processes?

Firstly, it is important to mention that the large circadian oscillation for air temperature in semi-arid equatorial regions, and therefore, combination of lower air temperature, wind displacement, and precipitation during the night, can present challenges for animal thermal regulation (Fonseca et al., 2014). The convection mechanism is described as transfer heat from natural or forced fluid displacement at the body surface (McArthur, 1987). In still air conditions, Tregear (1965) determined no consistent relation between hair density and fur conductance, but for wind displacement close to 2 $m s^{-1}$, the positive relation between hair density and thermal insulation was obvious. As the hair density increases the penetration of wind into the boundary layer decreases and so the air and moisture trapped between hairs is less disturbed, thereby decreasing mass and heat transfer to the environment (McArthur, 1991; Maia et al. 2003, 2015). Moreover, as the effectiveness of evaporative cooling depends on water molecules reaching the skin surface, less space between hairs serves as a barrier that prevents evaporative heat loss, as well as to prevent water contact with skin, in the case of a rain event.

On the other hand, as the hair density decreases, mass and heat are more easily transferred to the environment from the skin surface. Our findings showed that lower hair density was recorded in September, both for Morada Nova and Santa Ines, concomitant with increasing means of ambient air temperature, and solar irradiance. Likewise, for Morada Nova sheep, higher dermal thickness, blood capillaries and sweat gland area was also detected in the same period. Otherwise, skin traits of Santa Ines sheep showed minimal variation throughout the year. As we expect a proportional relationship between anatomical aspects of sweat gland parameters and sweating activity (Costa et al., 2014), it seems that the capacity to dissipate body heat for Morada Nova sheep increased in September. However, interestingly, the dermal thickness was also higher in this period. Possibly, even that speculative, this adjustment could be related to the protection of deep tissues against the transmission of ultraviolet solar radiation (Silva, 2008).

5. Conclusion

This study provides some basic and preliminary evidence regarding phenotypic acclimatization in fur and skin traits of hair coat sheep breeds that have been raised in an equatorial semi-arid environment. Even in this region, phenotypic acclimatization in morphological traits of cutaneous surface and skin can modify the overall thermal insulation of these sheep breeds differently. Nevertheless, more investigations dealing with this aspect are important to elucidate the environmental factors influencing these changes, and which environmental factor(s) (e.g., day length, air temperature, solar radiation) provide the stimulus for the changes.

Acknowledgments

The authors would like to express their sincere appreciation to Robyn Hetem and Shane Maloney for their relevant scientific contributions to the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.06.007.

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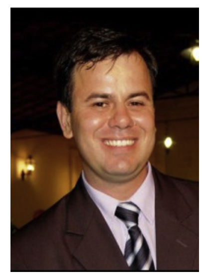

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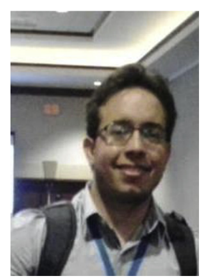

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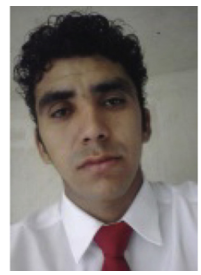

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