Effect of solar radiation on thermoregulatory responses of Santa Inês sheep and their crosses with wool and hair Dorper sheep

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A R T I C L E   I N F O

A B S T R A C T

This study aimed to assess the thermoregulatory responses of Santa Inês (SI), Dorper x Santa Inês (CH) and White Dorper x Santa Inês sheep (CW) to direct solar radiation in Southeast Brazil. Thirty adult non-pregnant and non-lactating Santa Inês (SI) hair ewes and their crosses with Dorper (hair ewes) and White Dorper (wool ewes) were allocated into three groups n = 10 and exposed to continuous solar radiation for three consecutive days. Ocular and surface temperatures, measured by infrared thermography, the rectal temperature, respiratory rate and sweating rate were collected at 7:00, 13:00 and 20:00 h. During the experiment, the black globe temperature reached a peak at 13:00 h, reaching mean values of 43.5 °C ± 0.45 °C, representative of severe discomfort for sheep. All genotypes showed an increase in surface temperature, reaching the maximum value at 13:00 h. The wool White Dorper x Santa Inês showed significantly higher surface temperature (dorsal, ventral, and shoulder) than the other genotypes. All the genotypes showed similar rectal temperature increases, peaking at 13:00 h, with values close to 39.4 °C ± 0.12 °C. At 20:00 h, all the genotypes decreased the rectal temperature (RT), albeit not reverting to the 7:00 h values. Changes in ocular temperature values mirrored the RT. All the genotypes presented high levels of evaporative heat loss. Even though all breeds significantly increased the respiratory rate, Santa Inês exhibited significantly higher values (146 bpm) than the others (112 and 117 for CH and CW, respectively). The sweating rate was very high in all genotypes, without differences among them, and exhibiting the same trend with a maximum value at 20:00 h. This behavior reflects the continuous effort to lose heat during the day, despite the decrease in black globe temperature. This study revealed analogous thermoregulatory responses among genotypes groups studied. The three genotypes showed similar heat tolerances, albeit presenting different thermogenesis and thermolysis dynamics, as evidenced by the maintenance of rectal temperatures within physiological limits even when subjected to intense high solar radiation.

1. Introduction

With a wide geographical distribution extending from cold to tropical climates, sheep are among the most successful species, playing an essential role in several production systems. Environmental factors, such as ambient temperature, solar radiation, and relative humidity, directly and indirectly affect animals (Collier et al., 1982). Maintaining a constant body temperature is a challenge for grazing sheep in tropical regions due to the combination of high ambient temperature and relative humidity (Silanikove 2000; Sejian et al. 2017). Heat stress in animals is driven by an imbalance between thermogenesis (metabolic and environment) and thermolysis. In response to thermal stress, animals activate physical, biochemical and physiological processes to counteract the adverse effect of heat stress and maintain thermal equilibrium (Castanheira et al., 2010). High ambient temperature increases the sheep's effort to dissipate body heat, resulting in an increased

respiration rate, body temperature, and water consumption (Marai et al., 2007). In fact, increased body temperature and respiration rate are the most important signs of heat stress in sheep (Al-Hidary et al., 2012).

To alleviate heat stress, animals should develop adaptive characteristics that reduce heat acquisition from the environment and maximize the evaporative heat loss. In this way, endocrine changes, reduction in metabolic rate and consequently, decreases in performance are less likely to occur (Shkolnik and Choshniak, 2006; Pereira et al., 2008; Al-Hidary et al., 2012). Therefore, proper breed selection is crucial for sustaining animal production under an increasingly challenging environment (Silanikove, 1992; Sejian et al., 2017). Nevertheless, importing high-producing sheep breeds from temperate to tropical areas does not always work adequately due to their limited capacity of losing heat efficiently.

Studies highlighting the physiological differences between hair and wool sheep breeds in tropic environment will help farmers to choose more adequate breeds for withstanding to hot weather. Crossbreeding native sheep breeds with more productive breeds adapted to the tropics will bring additional productive versatility to sheep production systems (Castanheira et al., 2010; Correa et al., 2012). The use of heterosis to improve performance maintaining the breed's adaptability to thermal stress is crucial for increasing productivity. Crossbreeding native hair sheep with Dorper may the herd productivity (McManus et al., 2011). However, crossbreeds' sheep show a wide range of phenotypic variations, leading perhaps to significant differences in their ability to regulate body temperature, limiting the success of their use (Cloete et al., 2000, Wojtas et al., 2014).

This study aimed to assess the thermoregulatory responses of Santa Inês (SI), Dorper x Santa Inês (CH) and White Dorper (wool) X Santa Inês (CW) to direct solar radiation in southeast Brazil.

Moreover, it aimed to determine the usefulness of infrared thermography to evaluate temperature variation among genotypes and to assess the essential variables in the differentiation of these genetic groups for heat tolerance.

2. Material and methods

2.1. Location and execution period

The experiment was carried out during summer (January) at the Biometeorology, Ethology and Animal Welfare Laboratory, located at the Fernando Costa Campus of the University of São Paulo, Pirassununga, Brazil (latitude 21°59'46' S and longitude 47°25'33' W; 627 meters above sea level). The climate of the study site is humid subtropical (Cwa) according to the Köppen-Geiger classification (Kottek et al. 2006), with rainfall predominantly in summer. The average annual temperature is 22 °C and the average yearly rainfall is 1,363 mm. The Research Ethics Committee approved all procedures of the Faculty of Zootechny, Science and Food Engineering FZEA-USP (protocol number 6567160517).

2.2. Animals, environmental data and experimental design

Thirty adult non-pregnant and non-lactating ewes, with an average body weight of 56.03 ± 7.46 kg and age between 2 and 4 years, were allocated into three groups: 10 Santa Inês (SI) (Group 1); 10 Dorper x Santa Inês (CH) (light skin and coat) (Group 2); 10 White Dorper x Santa Inês, semi-wool ewes (Group 3) with wool all over their body except for the head, hind limbs, forelimbs and belly; the wool length ranged from 10 cm to 15 cm.

The experiment lasted 6 days. The sheep were kept altogether in 1 ha pasture of Bermuda grass [*Cynodon dactilon* (L) Pers], with free access to water and mineral salt; corn silage was provided as a supplement. During the first three days - the adaptation period - sheep had free access to all the pasture (with and without shade). From the fourth to sixth days, animals remained in an unshaded area from 7:00 to 20:00 h during hours of daylight. Animals spent the night in a sheltered area for rain protection.

During the three days of the experimental phase, air temperature (AT), relative humidity (RH) and black globe temperature (BGT), were recorded every 15 minutes using a data logger (HOBO® U12;013 Data Logger) installed outside the corral.

The temperature-humidity index (THI) was calculated using the modified sheep formula (Marai et al., 2007):

$$THI = AT - \{(0.31 - 0.31\ RH) * (AT - 14.4)\},$$

where AT is the air temperature in degrees Celsius (°C) and RH is the relative humidity (RH%)/100.

THI values under 22.2 represent no heat stress; 22.2 to < 23.3 represents moderate heat stress; 23.3 < 25.6 refers to severe heat stress, and THI > 25.6 indicate extreme heat stress.

2.3. Physiological data and cortisol

2.3.1. Body temperature and respiratory rate

During the three days of the experiment, physiological data were recorded on all sheep at 7:00 h, 13:00 h, and 20:00 h. Rectal temperature (RT, °C) was measured using a digital clinical thermometer (Geratherm® rapid GT-195-1, Germany, accuracy ± 0.1 °C) inserted into the animal's rectum. The respiratory rate [RR, breaths.min^{-1} (bpm)] was estimated by counting the respiratory movement observed on the animals' flanks for 15 seconds and then converted to minutes. During all samples all animals were evaluated one by one inside a pen. They were fixed manually and the same order was always used when choosing the animals for measurement at each time (7:00, 13:00, 20:00 h). The entire process between the entry and exit of the animals lasted 30 minutes.

2.3.2. Sweating rate and cortisol

Six ewes from each group (18 animals) were randomly selected to evaluate the sweating rate (SR; g. m^{-2} h^{-1}) at 7:00, 13:00, and 20:00 h. SR recording were always carried out on the same chosen sheep, using the Schleger and Turner (1965) colorimetric method. In the median thoracic region, 20 cm below the spinal column between the 10th and 11th rib, a 3 cm^2 area was trichotomized. In this spot, three paper discs of 0.5 cm diameter each with 10% cobalt chloride solution were fixed directly on the skin with adhesive tape. After fixation, it was measured the time for the color to change from an initial blue to pink. Observations were made individually by two people trained in this procedure. Each disc was timed separately; the turning time and averages were determined in seconds. SR was calculated from the following equation (Schleger and Turner, 1965):

$$SR = (22 \times 3,600)/(2.06\ t) = 38446.6/t\ g.m^2\ h^{-1}.$$

From the same 18 animals, blood samples were taken to quantify plasmatic cortisol concentration ($\mu g.dL^{-1}$). Blood samples were collected at 14:00 h, one hour after the higher air temperature of the day, by jugular venipuncture into 10 mL heparin vacuum tubes (BD Vacutainer). The cortisol plasma concentration was determined in duplicate, using an DetectX® species independent Cortisol Enzyme Immunoassay Kit, Arbor Assays, MI, USA. Samples were tested twice if the duplicates differed by more than 10%. The kits were validated, showing parallel curves between standard concentrations and samples dilutions.

2.4. Ocular and body surface temperatures

Ocular (OT) and surface (ST) temperature measurements were achieved by infrared thermography. A Testo® 875-2i (Germany) thermographic camera was used, with an accuracy of $\pm$ 2 °C of actual temperature, thermal sensitivity (NETD) of <50 nK, infrared resolution of 160 × 120 pixels, and image resolution of 640 × 480 pixels. Thermograms with an emissivity coefficient of 0.98 were recorded from each

sheep at a distance of less than 1 meter. Images were analyzed using the software Testo IRsoft (version3.6). A circular design delimiting the eye between the upper, middle, and lower edge and the lacrimal caruncle was performed to determine the OT in the animal' eye region and the antimere (left or right), recording the maximum temperature (Hoffmann et al., 2013; Pulido-Rodríguez et al., 2017; Hooper et al., 2018). The ST was obtained by delimiting the antimere images in seven body areas and recording each area's mean temperature (Kotrba et al., 2007). The areas examined were the dorsal (ST1), ventral (ST2), shoulder (ST3), rump (ST4), forelegs (ST5), hind legs (ST6), and ribs (ST7) (Fig. 1).

2.5. Statistical analysis

Previously data were tested for normality by Shapiro–Wilk test, homoscedasticity by Levene test and sphericity by Mauchly test. For RT, RR and surfaces temperatures, the model included, the genotype as fixed effect and hour of temperature recording as a repeated measurement, the interactions genotype × hour, and animals and day as random effects. For plasma cortisol concentrations, genetic groups were considered as fixed effects. Significantly different means were compared using the Tukey–Kramer test, regarded as significantly different when P < 0.05. Pearson's correlation was performed among thermography readings and physiological parameters (except cortisol). All data were presented as mean ± SEM, and the analysis was generated using SAS software, Version 9.3 of the SAS System for Windows.

3. Results

3.1. Environmental variables, animal status and cortisol

Table 1 summarizes the variables characterizing the thermal environment, namely the air temperature (AT), black globe temperature (BGT), relative humidity (RH), and THI. The meteorological variables presented values significantly higher at 13:00 h, while the values observed at 20:00 h remained higher than those at 7:00 h. The high BGT values observed at 13:00 h, above 43 °C, evidenced high solar radiation intensity. The THI values observed at 13:00 h may be considered indicative of "extreme heat stress".

During the experiment, no behavioral evidence of stress, such as those suggestive of handling stress or pre-thermoregulatory failure, was observed. All animals remained quiet and almost indifferent to the daily handling procedures related to recording of the rectal temperature and sweating rate. Plasma cortisol concentration remained within the baseline values for sheep and has not evidenced significant differences among genotypes, indicating the absence of noticeably psychological stress (P > 0.05) (Fig. 2).
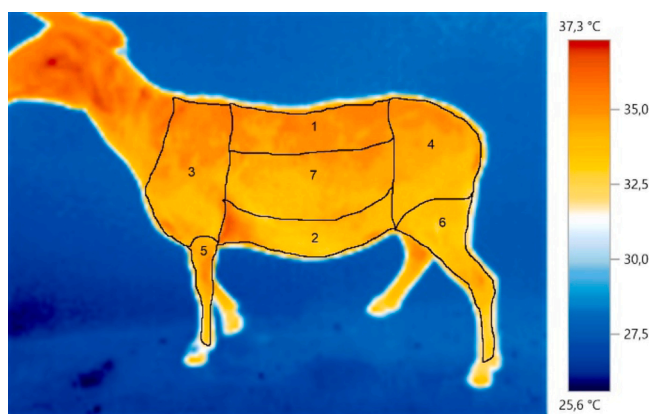

Fig. 1. Thermographic image of the antimere and the different defined regions of sheep. *Dorsal (1), ventral (2), shoulder (3), rump (4), forelegs (5) hind legs (6) and ribs (7).*

Table 1
Air temperature (AT), black globe temperature (BGT), relative humidity (RH) and temperature humidity index (THI) measured at 7:00 h, 13:00 h and 20:00 h. Values are provided as Mean ± standard error of the mean.

Environmental data	Hour			SEM	*P*-value
	7:00	13:00	20:00		
AT (°C)	21.93[c]	34.89[a]	25.54[b]	0.21	<0.001
BGT (°C)	23.33[c]	43.45[a]	24.07[b]	0.45	<0.001
RH (%)	84.00[a]	61.71[c]	69.92[b]	0.44	<0.001
THI	21.55[c]	32.41[a]	24.50[b]	0.15	<0.001

Different letter in lines represent P < 0.05.

3.2. Rectal, ocular and surface temperatures

Hour and genotype significantly influenced RT and OT (P ≤ 0.01; Tables 2 and 3) and surface temperatures (Table 4).

During the experimental period, the thermal environment variability significantly affected RT and OT throughout the day (Table 2). The thermal environment recorded at 13:00 h led to a significant increase in rectal and ocular temperatures (P < 0.001), which displayed similar tendencies. At 20:00 h, RT and OT's mean values were higher than those observed at 7:00 h, showing remaining levels of stored heat. Nevertheless, at 7:00 h, the observed RT was slightly lower than the species' range values, from 37.9 to 39.8 °C (Gomes daSilva and Minomo, 1995).

Concerning the RT mean values recorded by the different genotypes, SI's mean values were significantly lower (P < 0.001), than those of CH and CW, which did not differ. The OT displayed a similar tendency to RT.

Table 3 displays the rectal and ocular temperatures of the different genotypes throughout the day.

Regarding RT, all genotypes presented a similar variation pattern, with a significant increase in RT at 13:00 h, albeit without noticeable differences between the genotypes. At 20:00 h, despite a general reduction of the RTs (P < 0.05), no genotype presented a full RT recovery from the initial values recorded at 7:00 h. Interestingly, at 7:00 h, the SI group showed a significantly lower RT than the other genotypes (Table 3).

The OT mirrored the RT values. These values were significantly higher at 13:00 h and 20:00 h, although there were no significant differences between genotypes within the same hours (Table3).

The body surface temperatures (Table 4) followed a similar pattern across all genotypes, and presented interaction effect genotype × hour (P < 0.05).

The more significant increases in body temperature were found in regions with the highest direct solar radiation incidence at 13:00 h. In contrast, the front and rear limbs showed no significant differences among hours. The regions with the highest surface temperatures were the dorsal and shoulder. At 13:00 h, the CW animals showed significantly higher values in the regions most exposed to direct solar radiation compared with other genotypes, suggestive of higher wool's absorbance. Moreover, in the CW's crossbreds, the regions showing significantly higher values at 13:00 h were the same that showed the lowest temperatures at 20:00 h (P < 0.01) when compared with the corresponding regions of the other genotypes. These different temperature variations presume different interactions between radiation and surfaces covered by hair or wool.

3.3. Respiratory rate and sweating rate

The fixed factors hour and genotype significantly influenced the respiratory and sweating rates (Table 5). There was a significant interaction to respiratory rate (P = 0.007).

Even though the evaporative heat loss pathways showed somewhat different behaviors throughout the day, relevant thermoregulatory responses from both respiratory and sweating rate were evident. Table 6
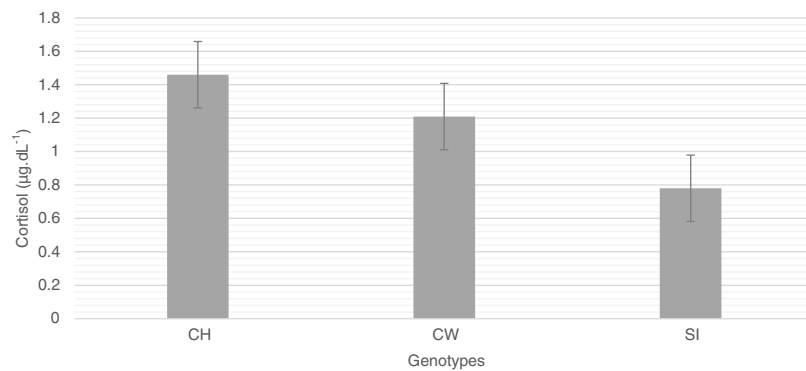
Fig. 2. Plasmatic cortisol concentration in different genotypes SI – Santa Inês; CH – Dorper crossbreed hair; CW – White Dorper crossbreed wool. (P > 0.05).

Table 2
Values of rectal and ocular temperature (Mean ± standard error of the mean) on hours and genotypes Santa Inês breed (SI), Dorper crossbreed hair (CH) and White Dorper crossbreed wool (CW) sheep.

Physiologic parameters	Hour			SEM	P value	Genotypes			SEM	P- value
	7:00	13:00	20:00			SI	CH	CW		
RT (°C)	38.04[b]	39.23[a]	39.05[a]	0.07	<0.001	38.59[b]	38.86[a]	38.87[a]	0.07	0.01
OT (°C)	37.46[c]	39.00[a]	38.07[b]	0.07	<0.001	38.01[b]	38.22[a]	38.31[a]	0.07	0.009

RT – Rectal Temperature; OT – Ocular Temperature; SI – Santa Inês; CH – Dorper x Santa Inês; CW – White Dorper x Santa Inês. Different letter at 7:00, 13:00,18:00 hours, and SI, CH, CW group animal represents significant differences P < 0.05.

Table 3
Values (mean ± standard error of the mean) of rectal temperature (RT), ocular thermography temperature (OT) of the three the genotypes of sheep over the day.

Physiologic variables	Hour	Genotypes			SEM	P- Value
		SI	CH	CW		
RT(°C)	7:00	37.67[aA]	38.11[aB]	38.36[aA]		
	13:00	39.14[bA]	39.34[bB]	39.23[bB]	0.10	0.04
	20:00	38.96[aA]	39.15[aA]	39.04[abA]		
OT (°C)	7:00	37.31[aA]	37.47[aB]	37.62[aB]		
	13:00	38.77[bA]	39.11[bA]	39.14[bA]	0.09	0.03
	20:00	37.95[bA]	38.08[bA]	38.17[bA]		

SI: Santa Inês; CH: Dorper x SI crossbreed hair; CW: White Dorper x SI crossbreed wool. For each group animal, mean values with different small letters are different among hours (P < 0.05). For each hour, mean values with different capital letters are different among group animal (P < 0.05).

Table 4
Values (Mean ± standard error of the mean) of the surface temperatures (°C) measured by infrared thermography of the body regions of the three the genotypes of sheep over the day.

	Hour	Genotypes		
		SI	CH	CW
Dorsal	7:00	33.18 ± 0.46[Ba]	32.91 ± 0.46[Bab]	30.80 ± 0.46[Bb]
	13:00	38.75 ± 0.46[Ab]	38.26 ± 0.47[Ab]	42.56 ± 0.46[Aa]
	20:00	33.24 ± 0.46[Ba]	33.54 ± 0.46[Ba]	31.47 ± 0.47[Bb]
Ventral	7:00	34.94 ± 0.28[Ca]	33.64 ± 0.28[Cb]	33.29 ± 0.28[Cb]
	13:00	37.92 ± 0.28[Ab]	37.33 ± 0.29[Ab]	38.79 ± 0.28[Aa]
	20:00	36.16 ± 0.28[Ba]	35.41 ± 0.28[Bb]	35.09 ± 0.29[Bb]
Shoulder	7:00	34.59 ± 0.39[Ba]	33.60 ± 0.40[Ba]	32.54 ± 0.40[Cb]
	13:00	38.50 ± 0.39[Ab]	38.08 ± 0.40[Ab]	41.77 ± 0.39[Aa]
	20:00	35.43 ± 0.39[Ba]	34.63 ± 0.40[Ba]	34.56 ± 0.40[Ba]
Rump	7:00	33.76 ± 0.33[Ca]	32.45 ± 0.33[Cb]	31.55 ± 0.33[Cb]
	13:00	38.64 ± 0.33[Ab]	37.99 ± 0.34[Ab]	42.43 ± 0.33[Aa]
	20:00	34.67 ± 0.33[Ba]	33.97 ± 0.33[Ba]	32.92 ± 0.34[Bb]
Forelegs	7:00	33.44 ± 0.27	32.79 ± 0.28	32.18 ± 0.30
	13:00	37.03 ± 0.27	36.53 ± 0.28	36.36 ± 0.28
	20:00	34.82 ± 0.28	34.28 ± 0.30	34.22 ± 0.28
Hind legs	7:00	33.26 ± 0.29	32.25 ± 0.28	31.52 ± 0.28
	13:00	37.01 ± 0.27	36.95 ± 0.28	36.89 ± 0.27
	20:00	34.28 ± 0.29	34.17 ± 0.33	33.33 ± 0.29
Ribs	7:00	33.50 ± 0.28[Ca]	32.53 ± 0.28[Ca]	31.67 ± 0.28[Cb]
	13:00	38.08 ± 0.28[Ab]	37.14 ± 0,29[Ab]	40.71 ± 0.28[Aa]
	20:00	34.75 ± 0.28[Ba]	34.16 ± 0.28[Ba]	33.60 ± 0.29[Bb]

SI – Santa Inês; CH – Dorper x SI crossbreed hair; CW – White Dorper x SI crossbreed wool. For each group animal, mean values with different capital letters are different among hours (P < 0.05). For each hour, mean values with different small letters are different among groups of animals (P < 0.05).

summarizes the RR and SR values over the day observed in the different genotype.

The most significant differences between RR and SR responses were associated with the time they persist throughout the day. A significant RR increase was observed mainly at 13:00 h when the ambient temperature and radiation were higher. The RR mean values decreased significantly at 7:00 h and 20:00 h.

The SR response displayed consistently high values throughout the day, showing the maximum values at 20:00 h, significantly higher than those observed at 7:00 h and 20:00 h.

The RR and SR mean values significantly differed between genotypes. CH presented lower RR compared to SI or CW (P ≤ 0.001), which did not differ from each other. On the other hand, SI showed SR mean values lower than others (P ≤ .001).

According to the evaporative heat loss pathways, the breeds displayed different patterns over the hours. The RR increased significantly (P < 0.001) until 13:00 h, with increments of 4.3, 3.5, and 2.6 times for SI, CH, and CW, respectively. Thereafter, the RR showed a notable decrease until 20:00 h, returning to the values observed at 7:00 h. At 13:00 h, the SI exhibited a significantly higher RR than the other

genotypes, which no longer occurred at 20:00 h. Contrastingly, the RR remained significantly higher in the CW at 20:00 h when compared to the others.

Concerning SR, this parameter showed a quite distinct pattern from RR, with an overall trend for SR increases throughout the day. At 20:00 h, both the CW and CH showed significantly higher SR than at 7:00 h and 13:00 h. Moreover, SI exhibit significantly lower SR at 20:00 h than CW and CH, being the only genotype that kept a similar SR throughout the

Table 5

Effect of the hour and genotype on the respiratory and sweating rates of the three genotypes of sheep (Mean ± standard error of the mean).

Physiologic parameters	Hour			SEM	P value	Genotypes			SEM	P value
	7:00	13:00	20:00			SI	CH	CW		
RR (bpm)	37[b]	125[a]	41[b]	3.9	<0.001	73[a]	59[b]	72[a]	3.9	0.02
SR (g m^{-2} h^{-1})	466.3[b]	457.1[b]	604.1[a]	32.91	<0.001	481.5[b]	545.0[a]	501.1[a]	30.91	0.04

RR – Respiratory Rate; SR – sweating rate; SI – Santa Inês; CH – Dorper x SI crossbreed hair; CW – White Dorper x SI crossbreed wool. Different letter at 7:00 h, 13:00 h, 18:00 h, and SI, CH, CW group animal represents significant differences P < 0.05.

Table 6

Respiratory (RR) and sweating (SR) rates of the genotypes of sheep over the day (Mean ± standard error of mean).

Physiologic parameters	Hour	Genotypes			SEM	P value
		SI	CH	CW		
RR (bpm)	7:00	34[aB]	32[aA]	44[aA]	6,7	0.007
	13:00	146[bA]	112[bB]	117[bB]		
	20:00	37[aA]	33[aA]	53[aB]		
SR (g. m^{-2} h^{-1})	7:00	435.5[aA]	486.6[aA]	476.6[aA]	56,1	0.74
	13:00	449.5[aA]	517.6[aA]	405.5[aA]		
	20:00	559.6[aA]	630.9[bB]	621.1[bB]		

SI – Santa Inês; CH – Dorper x SI crossbreed hair; CW – White Dorper x SI crossbreed wool. For each group animal, mean values with different small letters are different among hours (P < 0.05). For each hour, mean values with different capital letters are different among group animal (P < 0.05).

day (P > 0.05). Table 7 presents the correlations among the environmental parameters and the variables measured in the animals.

The environmental parameters (AT, BGT and THI) correlated positively and significantly with rectal, ocular, and surface temperatures. In contrast, the relative humidity (RH) showed negative and significant correlations with different environmental parameters and, consequently, negative correlations with animals' variables. Rectal and eye temperatures were positively and significantly correlated, with a coefficient of 0.71. The RT and OT showed higher correlation coefficients with AT (0.61; 0.80) than BGT (0.53; 0.78) or THI (0.60; 0.79), although the coefficients are very similar. Regarding surface temperatures, the highest correlation coefficients are related to AT (0.79–0.86), except only for the dorsal region, whose highest correlation coefficient was associated with BGT (0.83).

The RR showed positive and significant correlations with AT (0.85), BGT (0.88), and THI (0.84), contrasting with the sweating rate, where no significant correlation was found. In opposition to the RT, OT and RR are more closely correlated to BGT (0.88) than with AT (0.85) or THI (0.84). Moreover, RR showed a stronger correlation with OT (0.71) than

with RT (0.54).

4. Discussion

Air temperature, humidity, solar radiation, and wind-speed are important meteorological factors affecting livestock productivity. Each factor affects thermoregulation in sheep; still, their importance depends on the fleece or hair characteristics, determining the relative relevance of solar radiation and relative humidity on heat load. Previous studies have established that the increased body temperature is the best indicator of thermal stress (Monty et al. 1991; Silanikove, 2000).

Psychological stress must be minimized when studying the effects of high ambient temperatures on animal physiology. The blood cortisol concentrations in animals can give information about their stress status. Psychological stress increases plasmatic cortisol concentrations, positively influencing thermogenesis. When psychological stress is kept at a minimum, physiological reactions and endocrine changes are mostly related to intensity of thermal stress. In our study, the constant low blood cortisol levels, suggest relatively low stress in animals (Wojtas et al., 2014). It has been shown that early and continuous handling and training of animals before the experiment fostered the animals' low reactivity towards the contact persons, with a positive reflex on the animal welfare during the experimental period in the climatic chambers (Boissy, 1998; Pereira et al., 2008). On the other hand, the absence of high plasma cortisol levels did not reveal acute thermal stress and reinforces the idea of adequately acclimatized animals (Alvarez and Johnson, 1971). Blood cortisol concentration increases in animals suddenly exposed to high temperatures and gradually decreases during long-term exposure to these temperatures. The decrease in cortisol levels during chronic heat stress results from the animal's adaptation to the acute thermal conditions (McFarlane et al., 1995; Silanikove, 2000).

In the current study, all genotypes increased RT with maximum values at 13:00 h. However, these increments can be considered modest, as they approached the reference range of temperatures for sheep, which vary from 38.3 to 40 °C (Cook, 1996; Piccione et al., 2002; Marai et al., 2007). RT registered herein are below those reported by (Dias e Silva

Table 7

Pearson's correlation coefficients among environmental parameters and animal's responses.

	OT	RT	RR	SR	ST1	ST2	ST3	ST4	ST5	ST6	ST7	AT	RH	BGT
RT	0.71*													
RR	0.72*	0.54*												
SR	−0.03	0.14	−0.14											
ST1	0.65*	0.43*	0.68*	−0.01										
ST2	0.67*	0.54*	0.67*	−0.04	0.79*									
ST3	0.70*	0.47*	0.69*	−0.07	0.87*	0.86*								
ST4	0.71*	0.47*	0.72*	−0.04	0.91*	0.83*	0.91*							
ST5	0.74*	0.56*	0.69*	0.01	0.71*	0.76*	0.76*	0.75*						
ST6	0.70*	0.53*	0.70*	0.01	0.79*	0.79*	0.76*	0.83*	0.81*					
ST7	0.70*	0.48*	0.68*	−0.04	0.86*	0.81*	0.91*	0.91*	0.74*	0.79*				
AT	0.80*	0.61*	0.85*	−0.01	0.82*	0.79*	0.81*	0.86*	0.81*	0.85*	0.82*			
RH	−0.79*	−0.74*	−0.74*	−0.12	−0.69*	−0.77*	−0.74*	−0.76*	−0.80*	−0.80*	−0.75*	−0.92*		
BGT	0.78*	0.53*	0.88*	−0.12	0.83*	0.74*	0.80*	0.85*	0.76*	0.80*	0.80*	0.96*	−0.82*	
THI	0.79*	0.60*	0.84*	−0.0	0.83*	0.79*	0.81*	0.86*	0.81*	0.85*	0.82*	0.99*	−0.91*	0.96*

RT: rectal temperature; RR: respiratory rate; SR: Sweating rate; ST1: surface temperature dorsal; ST2: surface temperature ventral; ST3: surface temperature shoulder; ST4: surface temperature rump; ST5: surface temperature forelegs; ST6: surface temperature hind legs; AT: air temperature; RH: relative humidity; BGT: Black Globe temperature; THI: Temperature-Humidity Index. * = P < 0.05.

et al., 2016), in similar conditions with Morada Nova sheep where RT was above 40 °C at 14:00 h.

Regarding the influence of solar radiation, even though the RT rose at 13:00 h, its decline rate until 20:00 h did not complete approach the values observed at 7:00 h. These results agree with Piccione et al. (2002), who reported that RT measured in the evening was consistently higher than that in the morning. This difference is a natural consequence of the daily rhythm of body temperature previously described in sheep by Bligh et al. (1965) and Mohr and Krzywanek (1995). Moreover, the SI, had an unusual low RT at 7:00 h, suggestive of adaptive hypothermia. Adaptive hypothermia is a more common strategy in sheep adapted to arid regions as a form of water savings. The animals take advantage of the most favorable thermal gradient at night to lose extra sensible heat, then starting the day with a lower-body temperature that allows them to store more heat before triggering evaporative thermolysis (Silanikove, 1992; Louw, 1993).

Infrared thermography is a noninvasive remote sensing method used in measuring changes in heat transfer and blood flow, through the detection of small changes in body temperature (Roberto et al., 2014). When an animal becomes stressed the sympathetic stimulation and the hypothalamic-pituitary-adrenocortical axis are activated, and heat is produced due to rises in catecholamines and cortisol concentrations. Besides, peripheral blood flow increases and changes in heat production and heat loss may affect animal skin temperature (Schaefer et al., 2002). The OT was positively and significantly correlate with the RT (0.71) corroborating the results presented in several studies (Stewart et al., 2007; McManus et al., 2015; Hooper et al., 2018), but this value was lower than the 0.81 observed in cattle by Hooper et al. (2018). A possible explanation for the smaller OT might be the lower response to stress displayed by the sheep. The high OT is usually associated with the activation of the hypothalamic-pituitary-adrenal axis and subsequent increase of catecholamine and cortisol level (Stewart et al., 2010), which seems not to have occurred in our study. McManus et al. (2016) refer to the idea of the OT inflection point, which represents the moment when the physiological mechanisms can no longer maintain the core temperature under control. Higher values correspond to high heat tolerance, with 25.5 °C and 26.4 °C for Dorper and Santa Inês, respectively.

There are two factors to consider concerning surface temperatures: the surfaces covered by hair or wool, and the regions more or less exposed to direct solar radiation. In hair sheep, the hair structure can protect the skin against direct solar radiation and promote convection and heat loss by evaporation. The crucial characteristics include coat reflectance, hair length, and the number of hairs per unit area (McManus et al., 2009). The fleece performs a crucial role in improving the adaptive flexibility of sheep to extreme environmental conditions. The wool acts as a protective boundary against radiation but making it also more difficult to sweat evaporate, reducing evaporative heat loss's rate (Wojtas et al., 2014). For SI and CH sheep, the effects of solar radiation were similar in the various regions of the animals' bodies. In both genotypes, solar radiation increased body surface temperatures, mainly in the dorsal area, shoulder and rump regions and to a lesser extent in the ventral and ribs regions. Despite differences in coat colors, there were no significant differences in any surface temperature among the three genotypes used herein. These results contrast with previous studies where animals with darker coats had higher absorption of thermal radiation, a high surface temperature, and were more susceptible to heat stress than those with a lighter colored coat (McManus et al., 2011; Fadare et al., 2012; Leite et al., 2018). However, Titto et al. (2016), using Santa Ines hair sheep of different coat color, did not find differences between them, which could be explained by another relation between heat absorbance and transmittance in those coats. A lower transmittance allowed radiant heat to be absorbed only in the outer surface, which was quickly removed by convection with less interference in the epidermis. In CW sheep, wool determined surface temperature variations compared to SI and CH genotypes. Despite the white color of wool, the regions most exposed to solar radiation (dorsal, shoulder, and ribs) body temperature

considerably increase until 13:00 h, showing significantly higher values than the other genotypes at the same hour. These results confirm those obtained in Merino Branco under solar radiation in a Mediterranean climate, where fleece surface temperature reached 45 °C and the skin temperature was 39.6 °C (Haddade et al., 2018). In these cases, the effects of absorbed radiation remained mainly at the fleece's surface due to low transmittance, whereas the heat absorbed at the surface is dissipated by long-wave radiation and convection (MacFarlane, 1968; McArthur, 1980). This combination of sensible heat losses may partially explain why the same body surfaces had the highest temperature at 13:00 h presented the lowest temperatures at 20:00 h. Limbs were less influenced by solar radiation; their temperatures result from heat flows from the environment, with a lesser contribution of solar radiation and the heat flow emitted by the skin surface. The absence of significant differences among genotypes throughout the day reveals a steady balance between these heat flows.

Sweating and panting are two primary responses to heat stress (Marai et al., 2007). Sweating in wool sheep tend to be less relevant than respiratory evaporation because of the presence of a wool coat. Nevertheless, Ghosal et al. (1981) refer that native sheep can use relatively more cutaneous evaporation than exotic breeds during thermal stress at high ambient temperature, as observed with Rambouillet, Chokla and Malpura sheep. Besides sweating, sheep can also dissipate heat from the body's surface due to water diffusion through the skin as insensible perspiration (Marai et al., 2007; Gaughan and Cawdell-Smith, 2015).

In sheep, a primary response to heat load is an increase in RR (Silanikove, 2000); the variations in respiratory frequencies can be described in two phases of panting (Hales and Webster, 1967). The first-phase panting is characterized by rapid shallow panting associated with an increase in RR, resulting in a rise in the respiratory volume. On the other side, the second-phase panting is characterized by slower, deeper respirations associated with open-mouthed panting and an increased respiratory volume more significant than that observed in the first-phase panting (Hales and Webster, 1967). In this study, none of the sheep presented second-phase panting, suggesting moderate thermal distress. The evaporative heat loss pathways together provide the answers to limit increases in body temperature. Even though the proportion of evaporative heat loss pathway employed by the three genotypes was somewhat different, in all the cases homeothermy was maintained throughout the day. There was a RR supremacy in the SI, which showed values higher at 13:00 h. These results contrast with those of Titto et al. (2016), in which the Santa Inês showed lower RR and better thermal tolerance than the wooled sheep breeds, which differs the thermoregulatory responses compared to hair crossbred sheep.

On the other hand, SI exhibited the lowest sweating rates, keeping the values stable throughout the day. Some authors argue that some sheep breeds favor respiratory heat loss as a water-saving route. Some breeds in arid regions have water-saving processes as a reduction in sweating rate, favoring tachypnea and selective brain cooling, which enables controlled hyperthermia and, at last, adaptive hypothermia (Silanikove, 1992; Mitchell et al., 2002). There were some similarities between CH and CW sheep in the dynamics of the thermolysis pathways' responses. Both showed RR with peak values at 13:00 h and increasing SR throughout the day. Woolly and hair sheep breeds often show differences in how they respond to heat stress. Hair sheep breeds tend to show higher sweating rates and effective sweat evaporation rates due to high convection effects. In this study, there is only no clear advantage of CH, but there is some tendency to higher SR values, particularly at 20:00 h. The maintenance of high SR throughout the day seems contrary the hypothesis that sweating sweat glands appear to exhibit fatigue or a reduction in the output when exposed to 3 to 12 hours of high temperatures but recover overnight in the cool (Hofmeyr et al., 1969; Rai et al., 1979a).

In CW sheep, the presence of fleece may contribute to reducing the efficiency of heat loss through sweat. With a cumulative increase in the vapor partial pressure inside the wool, there is a reduction in sweat's

evaporation speed, reducing the amount of heat lost per unit of time (Thwaites, 1967, 1985). On the other hand, if accompanied by a tendency towards hyperthermia, this can lead to over-stimulation of the sweat glands to produce more sweat (Rai et al., 1979b). This possible inefficiency could explain the maintenance of higher RR values at 20:00 h, values somewhat above those mentioned as standard (20–38 bpm) in thermoneutral conditions for sheep (Silva et al., 2002).

RT and OT presented higher correlation coefficients for air temperature relative to the BGT and THI, suggesting that both radiation and relative humidity had no extra influence on animal temperature. While the sweating rate showed no significant correlations with the other variables, the RR presented positive correlations with all temperatures measured in the animals as well as with AT, BGT, and THI. It should be strengthened that the main correlation coefficient was established with BGT, stressing the importance of solar radiation and peripheral heating on the onset and development of changes in the RR. Bligh (1963) and Hales and Webster (1967) demonstrated that thermal polypnea occurs in the absence of any increase of nuclear temperature and can be stimulated only by peripheral thermosensors. Thus, RR operates both as a response to hyperthermia and an anticipatory measure of imminent heat storage (Jessen, 2001).

5. Conclusion

The correlations between rectal and ocular temperatures enable the application of infrared thermography as a non-invasive methodology to detect changes in animals' core temperature. In periods of higher environmental temperature, the apparent limitations in the sweating rate of Santa Inês were compensated mainly by the higher respiratory rate, allowing to stabilize body temperature. The higher body surface temperatures observed in woolen animals did not lead per se to higher body temperature increases, emphasizing the lower fleece's conductance. Wool animals tend to extend a higher respiratory rate until the end of the day, probably due to the skin's low heat loss efficiency. Although presenting different thermogenesis and thermolysis dynamics, the three genotypes showed similar heat tolerances, evidenced by the maintenance of rectal temperatures within physiological limits even when subjected to high solar radiation intensities.

Author declaration

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

The authors confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

The authors confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

The authors further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

The author Lina Pulido-Rodríguez did the conception and design of the study, together with Prof. Cristiane Gonçalves Titto and Prof. Alfredo Pereira. Lina Pulido-Rodríguez, Mariana Frezarin Fuloni, Gabriela de Andrade Bruni, Fábio Luís Henrique, Gustavo Froge and did the acquisition of the experimental data. The analysis and interpretation of data were done by Lina Pulido-Rodríguez, Prof. Cristiane Gonçalves Titto,

Prof. Alfredo Manuel Franco Pereira, Gabriela Bruni and Gustavo Froge. The manuscript preparation was done by Lina Pulido-Rodríguez, Cristiane Gonçalves Titto, Gabriela Bruni and Prof. Alfredo Pereira. The final drafting of the article was done by Prof. Cristiane Gonçalves Titto and Prof. Alfredo Pereira.

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