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Research article

Heat tolerance in hair sheep: individual differences on physiological, endocrine, and behavioral responses

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A B S T R A C T

Heat stress is a major factor affecting animal productivity in tropical countries, with effects on physiological, hormonal, and behavioral responses. This study aimed to assess the differences in these responses between heat-tolerant and less heat-tolerant hair sheep during heat stress. Twenty-four Santa Ines sheep were selected from a group of 80 sheep, with 12 identified as heat-tolerant and 12 as less heat-tolerant based on thermotolerance assessment. The animals were exposed to heat stress in a climatic chamber at an average temperature of 36 °C (1000–1600 h) for 8 days and maintained at 28 °C (1600–1000 h). The rectal temperature, respiration rate, sweat rate, ocular surface temperature, body surface temperature, tympanic temperature, triiodothyronine level, and insulin level were measured. Skin samples were collected on the last day of the cycle for histological analysis. The results showed that the less heat-tolerant sheep had higher rectal and body surface temperatures ($P < 0.05$). Although no differences in skin morphology were observed between the groups, less heat-tolerant sheep continued to sweat for a longer period after the end of the thermal challenge to lose heat ($P < 0.05$). Less heat-tolerant animals also presented higher rectal temperatures during cooler hours and required more time to dissipate the excess heat. These findings suggest that there are individual differences in the thermoregulatory responses within the same breed under the same environmental conditions, and that breeding programs could be employed to produce more heat-tolerant, but still productive animals in tropical conditions.

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Implications

Heat stress affects animal productivity, mainly with the advent of climate change, which causes an increase in temperature in different regions of the world. It is important to identify strategies to improve animal productivity in high-temperature environments. Findings of the present study reinforce the existence of individual differences concerning heat tolerance capacity within the same breed, which in turn, open possibility for selecting more heat- resilient sheep. In addition, breeding programs could be employed to produce more heat-tolerant, but still productive animals, especially in tropical conditions, to improve herd productivity under heat- stress conditions.

Specification table

Subject	Welfare, Behaviour and Health Management
Specific subject area	Heat stress in production animals
Type of data	Table, Figure.
How data were acquired	Climatic chamber, data logger (*HOBO® U12-013*), digital clinical thermometer (*TH150, G-Tech, ±0.2 °C*), ear thermometer (*TCI100, Incoterm, ±0.2 °C*), infrared camera (*875-2i, Testo, Germany*)
Data format	Raw data in XLSX format.

(*continued on next page*)

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[¥] in memorian.

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2772-6940/

Parameters for data collection	Data of animals were collected inside the climate chamber kept in the climate chamber with an average temperature of 36 °C (1000–1600 h) and temperature of 28 °C (1600–1000 h). Physiological, behavioral variables and blood samples were taken at temperatures considered stressful (36 °C), and thermoneutral conditions (28 °C) and the skin was collected at a temperature of 36°.
Description of data collection	Santa Ines sheep were kept for 10 days in the climate chamber. On days 9 and 10, physiological variables and blood samples were collected every 3 h. For 3 consecutive days, behavioral observations were carried out and drinking water and eating events were recorded. On the last day inside the climatic chamber, a biopsy was performed to collect a skin fragment.
Data source location	Institution: Faculdade de Zootecnia e Engenharia de Alimentos/ Universidade de São Paulo City/Town/Region: Pirassununga/São Paulo Country: Brazil Latitude and longitude (and GPS coordinates, if possible) for collected samples/data: -21.953374304979665-4 7.45195066168257
Data accessibility	Repository name: Harvard Dataverse https://dataverse.harvard.edu/ Data identification number: https://doi.org/10.7910/DVN/2OD2MO
Related research article	Pantoja, M.H.A., 2022. Thermoregulation responses in sheep: a cellular approach. Thesis (PhD), University of Sao Paulo, Faculty of Animal Science and Food Engineering, Pirassununga, Sao Paulo, Brazil. https://doi.org/10.11606/T.74.2022.tde-07022023-085254 triiodothyronine Pantoja, M.H.A., Poleti, M.D., Novais, F.J., Duarte, K.K.S., Mateescu, R.G., Mourão, G.B., Coutinho L. L., Fukumasu, H., Titto, C.G., 2024. Skin transcriptomic analysis reveals candidate genes and pathways associated with thermotolerance in hair sheep. International Journal of Biometeorology 68, 435–444. https://doi.org/10.1007/s00484-023-02602-4 Pantoja, M.H.A., Novais, F.J., Mateescu, R. G., Mourão, G.B., Poleti, M.D., Beline, M., Monteiro, P.M.,Fukumasu, H., Titto, C.G., 2024. Exploring candidate genes for heat tolerance in ovine through liver gene expression, Heliyon 10, e25692. https://doi.org/10.1016/j.heliyon.2024.e25692.

Introduction

The Santa Ines breed is a hair sheep developed in Brazil that exhibits adaptive characteristics, such as the absence of wool and short, silky hair (Ribeiro and González-García, 2016). Additionally, hair sheep exhibit primary follicles that produce thick hairs, which provide mechanical protection, while wool sheep have more numerous secondary follicles that produce fine fibers, offering thermal protection (Ansari-Renani et al., 2011). Santa Ines is a breed raised in tropical environments, and studies on its tolerance can contribute to the development of sheep farming in countries with tropical climates, where heat stress is a major factor influencing animal productivity, reproduction, health, immunity, and survival (Mandal et al., 2021).

The impact of heat stress is easily noticeable because it causes changes in physiological parameters, such as the accumulation of heat, which results in an increase in rectal temperature, respiration rate, sweating rate, and surface temperature (Pulido-Rodríguez et al., 2021). The oscillation of these physiological variables is influenced by the suprachiasmatic nucleus of the hypothalamus, the main function of which is to regulate oscillations in the internal medium to modulate the setpoint (Marai et al., 2007). These physiological adjustments are essential to prevent hyperthermia and maintain the internal body temperature within the limits for survival, especially in tropical regions.

Changes in behavioral responses are reflected in increased water consumption and reduced food consumption (Shilja et al., 2016) and changes in the hormonal profile, which may be responsible for reducing the release of triiodothyronine (**T3**), a hormone linked to metabolism, to reduce endogenous heat production and avoid an increase in rectal temperature (Gonzalez-Rivas et al., 2020). Finally, there is an increase in insulin production (O'Brien et al., 2010) caused by the action of heat stress on the pituitary-adrenal axis.

The effects of heat stress on physiological, hormonal, and behavioral responses are well-documented, particularly the substantial differences in heat tolerance capacity related to breed, coat, age, and physiological status (Batista et al., 2014; McManus et al., 2016). However, individuals have different heat tolerances, reflecting different thermoregulatory responses during heat stress situations, even if they belong to the same group, breed, and environment (Luna-Nevárez et al., 2021). Thus, exploring the differences between more- and less-tolerant individuals regarding thermoregulation, it will be important to know which mechanisms work on thermotolerant ability on a long-term basis.

Therefore, it is important to understand the effects of heat stress on the thermoregulatory responses of sheep with different heat tolerance levels, especially under tropical conditions, to improve herd productivity under heat- stress conditions. The aim of this study was to identify differences in physiological, endocrine, histological, and behavioral responses of Santa Ines sheep classified according to the level of heat tolerance exposed to heat stress in a climatic chamber.

Material and methods

All experimental procedures were approved by the Institutional Animal Ethics Committee of the Faculty of Animal Science and Food Engineering of the University of São Paulo (CEUA/FZEA/USP protocol no. 7498130919).

Meteorological variables

During the experimental period, the air temperature (**Tair**, °C) inside the climatic chamber varied from 28 °C to 36 °C and the relative humidity (**RH**, %) varied from 55 to 70% (RH considered ideal). Meteorological variables were monitored using a data logger (HOBO® U12-013) programmed to take readings every 15 min.

Location and animals

An initial group of 80 black-coated, non-pregnant Santa Ines female sheep with a homogeneous body condition score (BCS 3, measured on a scale of 1–5) and aged between 4 and 5 years were used to assess thermotolerance and 24 animals were selected for the study. The Santa Ines ewes came from five different herds located in two different regions of Brazil (Southeast and Northeast).

Initially, the animals were kept together in the Biometeorology, Ethology, and Animal Welfare Research Vivarium, School of Animal Sciences and Food Engineering at the Fernando Costa Campus in paddocks with free access to artificial shade under an asbestos-cement roof painted white on the upper side (1 m^2/animal) with an Aruana pasture (Panicum maximum cv. Aruana), supplemented with corn silage.

During the study, the sheep were placed in a climatic chamber at the Department of Animal Reproduction of the same university. It has an area of 56 m^2 and is fully enclosed by brick walls, cement floor, and slab. The chamber was equipped with an external temperature and humidity controller, internal thermostats, and exhaust fan. Mineral salt and corn silage were provided daily. The animals were fed once daily (0730 h) with ad libitum access to water and feed troughs.

Experimental design and thermotolerance assessment

Before the beginning of the experimental phase, the rectal temperature of the animals was measured in a thermoneutral environment (average air temperature, 24.7 ± 1.66 °C). Under these conditions, heat-tolerant and less heat-tolerant sheep had mean rectal temperature of 38.4 ± 0.51 and 38.6 ± 0.46 °C, respectively.

Eighty Santa Ines female sheep randomly distributed in sets of 20 animals at a time (4 sets) were subjected to heat stress in a climatic chamber for 10 days with 2 days of adaptation to the new environment and 8 days of heat treatment (stressor factor). During the heat treatment, a temperature of 36 °C was maintained from 1000 to 1600 h, whereas a maintenance temperature of 28 °C was maintained from 1600 to 1000 h. The relative humidity was set to 60 ± 2%. The outdoor environment, in which the animals were kept before entering the climatic chamber, was similar during the entire experiment in summer, with an average air temperature of 23 ± 5 °C and relative humidity of 60 ± 12%. Rectal temperature data were collected every 3 h from 1300 h on day 0900–1000 h on day 10 (Pantoja et al., 2024a, b).

Rectal temperature, as an indicator of the heat stress response, was analyzed using the restricted maximum likelihood method in a mixed model. The model included the fixed effects of the evaluation cycle as a block (four cycles with 20 animals each, corrected to the same base), the time effect within the evaluation cycle (two cycles, 1000–1900 h when animals gained heat and 2200–0700 h when animals lost heat), and the animal as a random effect. The best unbiased linear prediction (BLUP, no genetic relationship matrix) estimates were obtained for each ewe, and the individual heat stress response was quantified. The effect of the second evaluation cycle was used to determine the capability of each individual to lose heat after a heat-stress period and was used to rank ewes from the most heat-tolerant to the least heat-tolerant (Pantoja et al., 2024b).

After ranking heat tolerance, we selected twenty-four ewes; 12 were considered the most heat-tolerant and 12 were considered the least heat-tolerant. The animals were kept in a climatic chamber at 36 °C, starting at 1000 h until 1600 h, and maintained at 28 °C from 1600 to 1000 h for 10 days (2 days of adaptation and 8 days of heat treatment). Physiological variables, surface temperatures, and blood samples were obtained every 3 h, for a total of eight measurements per animal. On the morning of the other day, a histological skin biopsy was performed inside the climatic chamber.

Physiological variables

The respiration rate (breaths.min^{-1}) was measured by observing the thoracic-abdominal movements of the sheep for one min. Rectal temperature (°C) was measured using a digital clinical thermometer (TH150, G-Tech, ±0.2 °C). Sweat rate ($g/m^{-2}h^{-1}$) was determined using filter paper discs impregnated with cobalt chloride placed on the skin of the animal, the time for the color change of the cobalt chloride from blue to pink, and the sweat rate was calculated using the following equation: Sweat rate = $(22 \times 3600)/(2.06 \times t)$ (Schleger and Turner, 1965). The tympanic temperature (°C) was measured using an ear thermometer (TCI100, Incoterm, ±0.2 °C) placed in the ear canal.

The surface temperature of the ocular region (°C) was measured by IR thermography using a manually focused IR camera (875-2i, Testo, Germany) with a thermal sensitivity (NETD; Noise Equivalent Temperature Difference) of <50 mK. The camera was maintained at the level of the ocular region at approximately 0.5 m. The emissivity used was 0.98. Analysis of the ocular region was performed following previous studies, in which circular tracing was performed over the orbital region, including the eyeball and approximately 1 cm of the ocular cavity. Body surface temperature (°C) was measured using an IR thermometer (G-Tech Premium, Incoterm, ±0.2 °C).

Triiodothyronine and insulin measurement

Blood samples were collected after the assessment of all physiological variables. Restraint was carried out by positioning oneself to the side of the animal, holding its flank with one hand, and securing the jaw with the other hand to maintain containment until the blood collection was completed. Blood samples were collected from two sheep at a time by experienced people who were already familiar with the sheep prior to the experiment.

Blood samples were collected into 10 mL vacuum tubes without anticoagulant by puncture of the external jugular vein. Samples were centrifuged at 3000 rpm for 20 min, and the serum was immediately frozen at −20 °C until determination of T3 and insulin levels. These hormones were measured by enzyme immunoassay using commercial kits, according to the manufacturer's instructions (Monobind, Lake Forest, CA, USA). Both kits were validated using parallel curves between standard concentrations and serially diluted serum samples. The intra- and inter-assay CV were 3.8 and 6.3%, respectively, for T_3 and 4.5 and 6.2%, respectively, for insulin.

Skin morphology

A biopsy was performed on the right side of the animal, in the middle dorsal region. Before biopsy collection, the area was shaved, disinfected, and anesthetized using 1 mL of local anesthetic without a vasoconstrictor (2% lidocaine hydrochloride). Then, micro-fragments of skin tissue were excised using an 8 mm diameter punch. Specimens were immediately fixed in 10% buffered formalin for 48 h and stored in 70% alcohol until histological analysis using the same methods as those used in previous studies (Strefezzi et al., 2003; Pulido-Rodríguez, 2019).

Each fragment was cut into 4 μm thick sections and stained with hematoxylin and eosin for histopathological evaluation. Sections were examined under an optical microscope (Leica DM500) at 40 × magnification in an image with an area of 0.08 mm^2. Images were acquired for each section. Images were analyzed using the ImageJ software version 1.52a (National Institutes of Health, USA).

The parameters described below were determined for each section: For epidermal and dermal thicknesses (μm), 30 measurements per slide were performed at different sections of the epidermis and dermis. Sweat gland density (number of sweat glands per linear micrometer) was determined by counting sweat glands using a multipoint tool and dividing the number by the length of the epidermal surface. The glandular area (μm^2) was measured using a freehand selection tool that allowed tracing of the area of each sweat gland. Sweat gland depth (μm) was the average of four measurements from the upper and lower distant edges of the sweat glands in relation to the epidermis. The arithmetic mean of the parameters was used for statistical analysis, except for the sweat gland density.

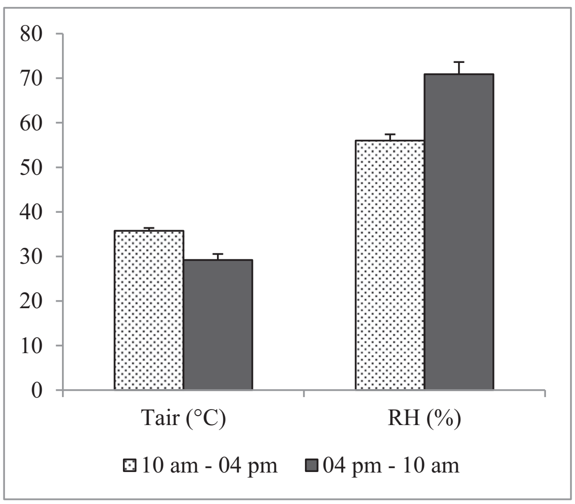


Fig. 1. Mean and SE values of air temperature (Tair) and relative humidity (RH) during thermal challenge (1000–1600 h) and without thermal challenge (1600–1000 h) imposed on heat tolerant (HT) and less heat tolerant (LHT) Santa sheep inside the climatic chamber.

Behavior

Animal behavior of the twenty-four ewes, 12 heat-tolerant ewes, and 12 less heat-tolerant ewes was observed for three consecutive days inside the climate chamber (days 5, 6, and 7). Feeding time (min), observed from the ingestion of silage with the mouth in the feeder, and water drinking events, observed by drinking water from the trough, were analyzed continuously and individually using the animal focal sampling technique. The animals were numbered with non-toxic spray paint, white in color, on both sides of the body, to facilitate animal identification.

Each focal animal was observed from 0800 to 1800 h by six trained observers, three in the morning and three in the afternoon. They were positioned inside the climate chamber approximately 1.5 m away from the animals and positioned away from their vision behind a wall. Santa Ines sheep were accustomed to human presence, and to avoid observer interference in the behavior, sheep involved in the study were already familiar with the observers before the start of the experiment.

Statistical analysis

For the model, response traits as a function of the covariates and a specific distribution under the GLMM with a better link function were used. A better link function ensures well-fitted values, and a specific distribution is typically used for each trait. Fixed factor of the tolerance group (categorical with two levels: heat tolerant and less heat tolerant) and daytime (continuous). The interaction term was the tolerance group × daytime (similar to a cubic regression). To incorporate dependency among observations of the same animal, we used nested as an animal random intercept.

For feed intake behavior, the model included the random effect of sheep as well as the fixed effects of heat tolerance (0800–1700 h) and the interaction between these effects. Means were compared by Tukey-Kramer test, and the significance was set at 5%. All results are reported as the mean ± SE of the mean. SAS for Windows 9.4 software (2016) was used for statistical analyses.

Table 1
Skin morphological characteristics of heat tolerant and less heat tolerant Santa Ines sheep during a thermal challenge in a climatic chamber.

Variable	Heat tolerant sheep	Less heat tolerant sheep	Pooled SEM[1]	*P*-value
Sweat gland density (glands μm^{-1})	0.01	0.01	0.00	0.462
Glandular area (μm^2)	11 453.16	10 680.0	1 015.10	0.596
Sweat glands depth from epidermis (μm)	1 015.81	1 101.0	81.16	0.467
Epidermal thickness (μm)	45.90	42.44	5.59	0.666
Dermal thickness (μm)	2 193.83	2 279.0	75.46	0.432

[1] n = 12 per treatment.

Table 2
Timing of feed intake of heat tolerant and less heat tolerant Santa Ines sheep during thermal challenge in a climatic chamber.

Temperature (°C)	Hour	Heat tolerant sheep (mins)	Less heat tolerant sheep (mins)	Pooled SEM[1]	*P*-value
28.5	0800	32.92[a]	38.58[a]	3.271	1.000
28.5	0900	11.62[b]	15.00[b]	4.145	1.000
34.0	1000	25.14[a]	17.27[b]	3.722	1.000
35.8	1100	21.71[ab]	22.00[ab]	3.902	1.000
36.0	1200	16.20[b]	23.09[ab]	3.500	0.998
36.0	1300	34.00[a]	16.33[b]	3.902	0.198
36.1	1400	22.14[ab]	26.50[ab]	4.152	1.000
36.1	1500	13.60[b]	26.37[ab]	3.930	0.678
34.4	1600	24.17[a]	27.64[ab]	3.345	1.000
32.6	1700	22.73[ab]	13.33[b]	3.345	0.904

[1] n = 12 per treatment.
[a,b] Different lowercase letters indicate a significant difference in each column ($P < 0.05$); *P*-values differ between heat-tolerant and heat-less tolerant sheep.

Results

From 0100 to 0700 h, Tair was 29 ± 1.33 °C and reached values of 35.7 ± 0.64 °C from 1100 to 1600 h, when the temperature of the climatic chamber was reduced (Fig. 1). The RH values were higher when the Tair was lower.

Skin morphology did not differ between the groups ($P > 0.05$) (Table 1), with similar measurements for sweat gland density,

Table 3
Regression table of physiological and hormonal variables of sheep classified as heat-tolerant and less heat-tolerant sheep.

	Heat tolerant sheep	*P*-value	Less heat tolerant sheep	*P*-value
Respiration rate (breaths min^{-1})	$y = 3.55–0.0537*h + 0.01312*h^2 - 0.0005*h^3$	<0.001	$y = 3.65–0.1171*h + 02115*h^2 - 0.00075*h^3$	<0.001
Tympanic temperature (°C)	$y = 35.9072–0.2512*h + 0.05542*h^2 -0.00209*h^3$	0.001	$y = 35.6121–0.2287*h + 0.06028*h^2 -0.0023*h^3$	0.001
Sweat rate (g/m^{-2}h^{-1})	$y = 6.0771–0.1342*h + 0.01884*h^2 -0.00048*h^3$	0.003	$y = 5.8125–0.01884*h + 0.001986*h^2 -0.00002*h^3$	0.879
Rectal Temperature (°C)	$y = 38.7885–0.1729*h + 0.01884*h^2 - 0.00052*h^3$	<0.001	$y = 39.0478–0.1454*h + 0.16733*h^2 - 0.00048*h^3$	<0.001
Body surface temperature (°C)	$y = 36.6581–0.2624*h + 0.04577*h^2 -0.00163*h^3$	0.001	$y = 36.7710-–0.2658*h + 0.05363*h^2-0.00197*h^3$	0.001
Ocular temperature (°C)	$y = 38.8251–0.286* h + 0.03971*h^2 -0.00123*h^3$	0.001	$y = 39.0891–0.2957*h + 0.0441*h^2 -0.00144*h^3$	0.001
T3 (ng/mL)	$y = 1.1234 + 0.01316*h-0.00166*h^2 + 0.000048*h^3$	0.453	$y = 1.1709–0.029*h + 0.001794*h^2-0.00004*h^3$	0.555
Insulin (μIU/mL)	$y = 16.6880–0.3079*h + 0.02965*h^2-0.00113*h^3$	0.746	$y = 29.1356–3*h + 0.3384*h^2-0.01013*h^3$	0.009

Abbreviations: T3 = triiodothyronine.

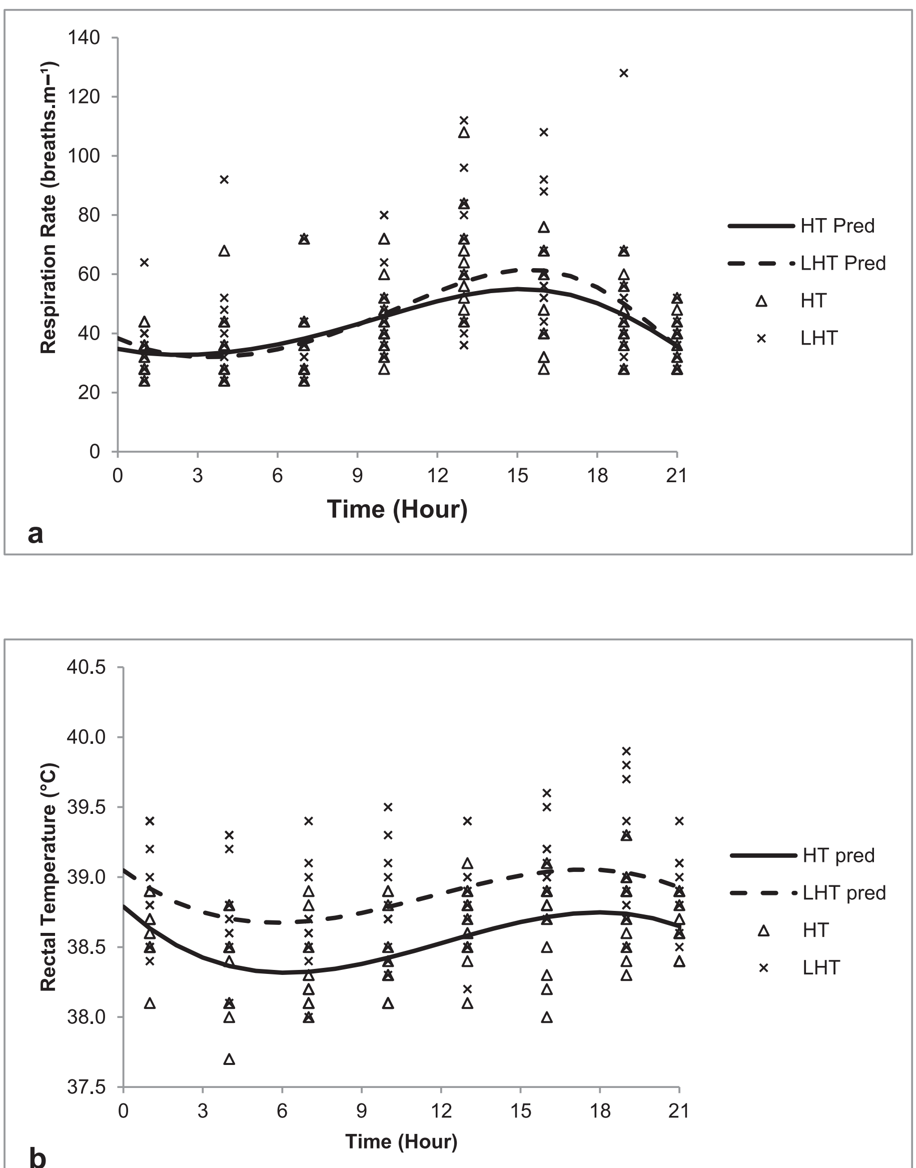
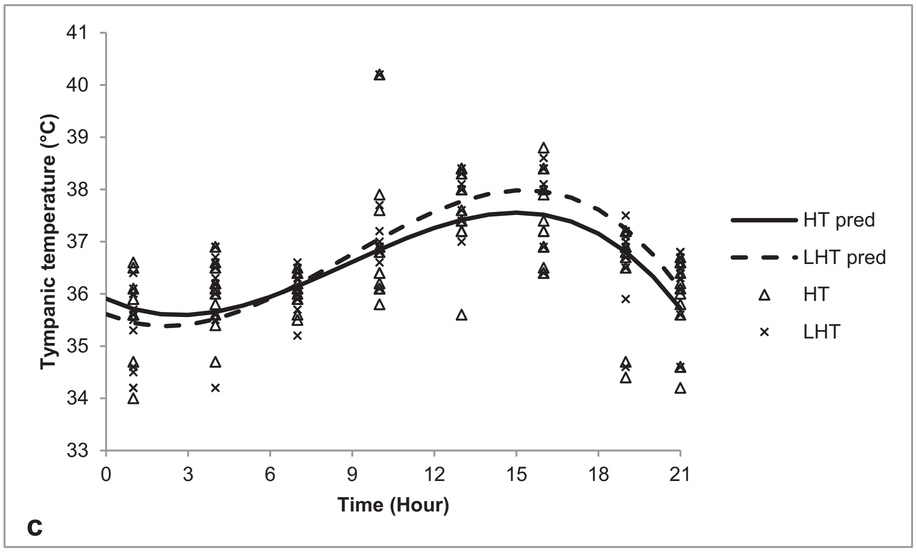


Fig. 2. Predicted (pred) and observed (Δ and X) **a** respiration frequency, **b** rectal temperature, **c** tympanic temperature, and **d** sweat rate of heat tolerant (HT) and less heat tolerant (LHT) Santa Ines sheep throughout the day.



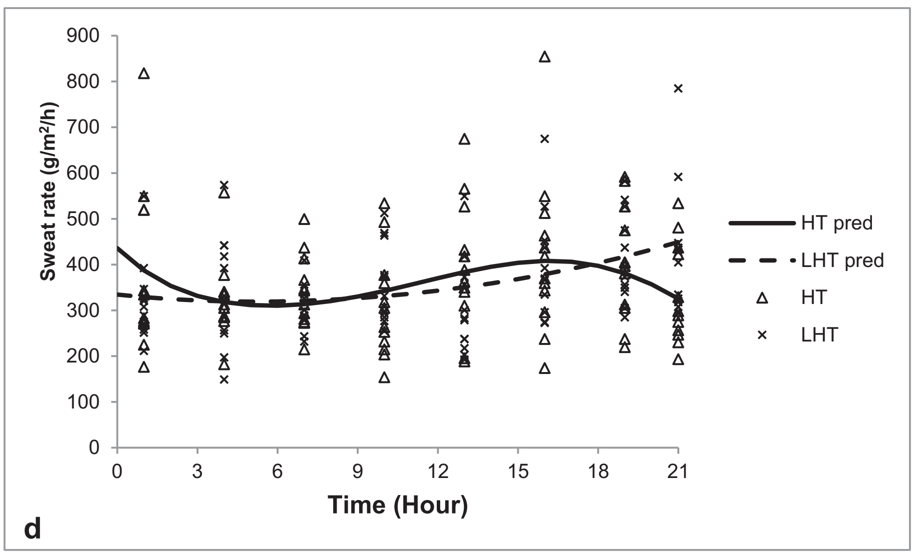


Fig. 2 (*continued*)

glandular area, sweat gland depth from the epidermis, epidermal thickness, and dermal thickness.

There was no difference in the duration of feed intake per hour between heat-tolerant (20.69 ± 1.072 min) and less heat-tolerant sheep (20.73 ± 1.035 min) ($P > 0.05$). However, the time of feed intake of heat-tolerant and less heat-tolerant sheep varied throughout the day ($P < 0.05$; Table 2). There was no significant difference in water intake during all observation days between the groups, with a total of 18 min for heat tolerance and 23 min for low heat tolerance ($P = 0.429$).

The cubic regression was significant for respiration rate and tympanic temperature over time in both heat-tolerant ($P < 0.0001$) and less heat-tolerant sheep ($P < 0.05$) (Table 3). An increase in respiration rate and tympanic temperature was observed, mainly between 1000–1600 h, when the thermal challenge was applied (Fig. 2ac). After this period, these physiological variables decreased in both heat-tolerant and less heat-tolerant animals but remained higher in less heat-tolerant animals (Fig. 2ac). The cubic regression for the sweating rate of the less heat-tolerant animals over time was not significant ($P = 0.8789$; Table 3). Although the sweating rate of the less heat-tolerant animals did not vary throughout the day, an increase was observed after the conclusion of heat treatment (Fig. 2d). The rectal temperature of the less heat-tolerant animals was higher during the heat challenge along the day ($P < 0.0005$; Fig. 2b).

Body surface temperature and ocular temperature showed significant cubic regression over time for both heat-tolerant and less heat-tolerant groups ($P < 0.0001$; Table 3). The body surface temperature (Fig. 3a) and ocular temperature (Fig. 3b) varied throughout the day, peaking between 1000–1600 h, when the environmental temperature was the highest, with low temperatures in heat-tolerant sheep.

Insulin concentration did not increase during the thermal challenge or throughout the day in heat-tolerant sheep (Fig. 4a); therefore, cubic regression was not significant ($P < 0.7459$; Table 3). However, the insulin concentration in less heat-tolerant sheep varied over time ($P < 0.0094$; Table 3). In contrast, the variation in T_3 concentration over time was similar between heat-tolerant and less heat-tolerant sheep, and showed no variation throughout the day (Fig. 4b).

Author's points of view

- This study demonstrated variation in the thermoregulatory mechanisms of Santa Ines sheep during heat challenge. Although evaporative and non-evaporative losses were similar

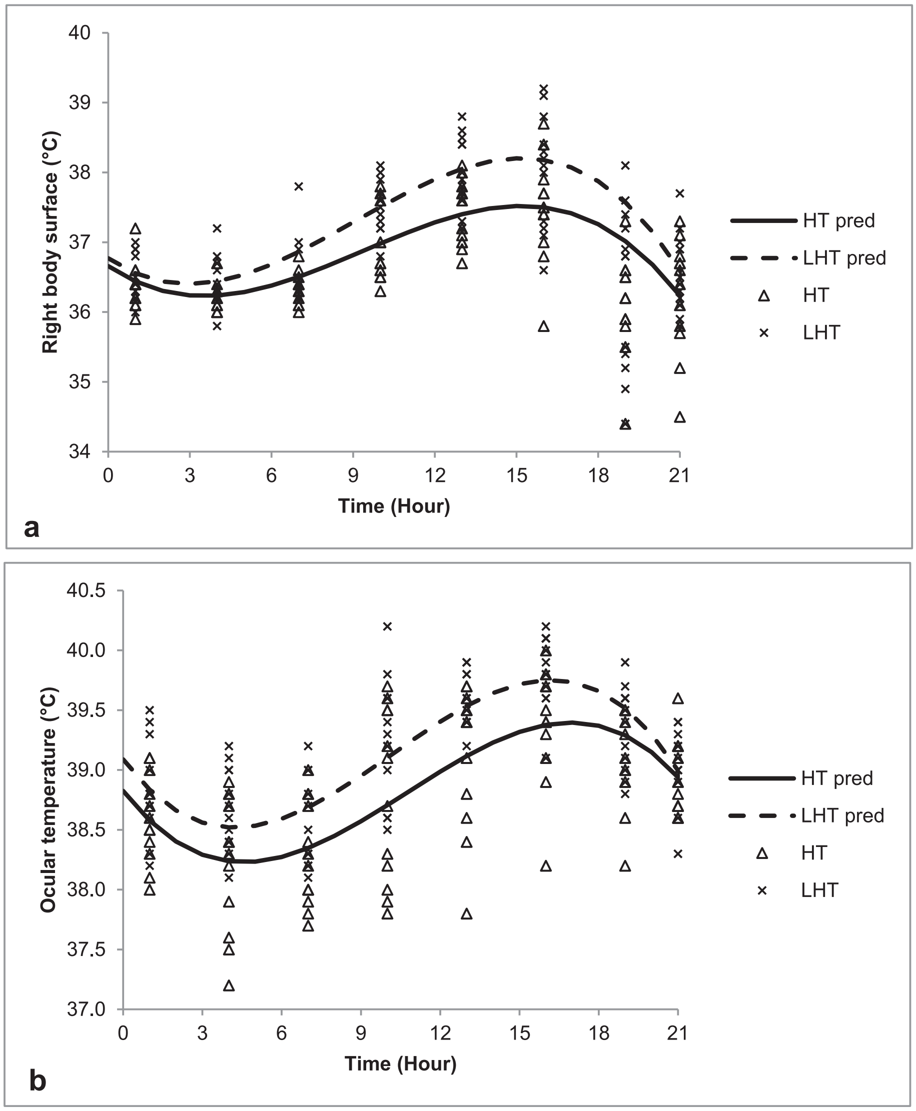


Fig. 3. Predicted (pred) and observed (Δ and X) **a** body surface temperatures r and **b** ocular surface temperatures of heat tolerant (HT) and less heat tolerant (LHT) Santa Ines sheep throughout the day.

on the day's average, it was possible to classify the sheep into heat-tolerant and less heat-tolerant groups. We observed that animals of the same breed, considered well adapted to tropical climates, under the same environmental conditions did not show the same heat loss efficiency, as evidenced by regression. When exposed to prolonged heat stress, less heat-tolerant Santa Ines sheep stored more body heat and were more reliant on evaporative cooling mechanisms through panting and sweating than those grouped as more heat-tolerant.

- Even during hours of reduced air temperature, less heat-tolerant animals did not reach the basal rectal temperature of 38.6 °C. In contrast, the heat-tolerant group was able to dissipate heat more efficiently, showing rectal temperatures of 38.3 °C between 0300 and 1000 h. The basal rectal temperature of the heat-tolerant animals was 38.4 °C. These findings demonstrate the influence of environmental temperature according to Terrien et al. (2011) and the need for thermoregulation as an important strategy for maintaining internal body temperature within the ideal range (De et al., 2017).
- The lower rectal and body surface temperatures in heat-tolerant animals explained the individual differences between the groups, as the lower rectal temperature before the beginning of the increase in air temperature could dissipate body heat more efficiently, as observed by the lower body surface temperature during the hottest hours, the same observed by Titto et al. (2016a). Related to rectal temperature, we consider this finding relevant because it reflects the deleterious effects of heat stress on animals (Shilja et al., 2016) and their welfare (Caulfield et al., 2014; Joy et al., 2022).
- The higher body surface temperatures observed in less heat-tolerant sheep indicate their attempt to dissipate excess heat via vasodilation, which increases blood flow from the body core to the skin surface (Morrison and Nakamura, 2011; Mota-Rojas et al., 2021), ultimately resulting in increased skin temperature and more heat loss via radiation and convection (Gesualdi Júnior et al., 2014) because of a difference in the temperature gradient between the skin and the environment, according to Macías-Cruz et al. (2016).
- Respiration rate is known to be the most important mechanism for dissipating heat in sheep (Marai et al., 2007), and sweating has been shown to be as important as painting, confirming the arguments cited by Kahwage et al. (2018). In our study, it was observed that less heat-tolerant animals continued to sweat after the period of thermal challenge, probably to dissi-

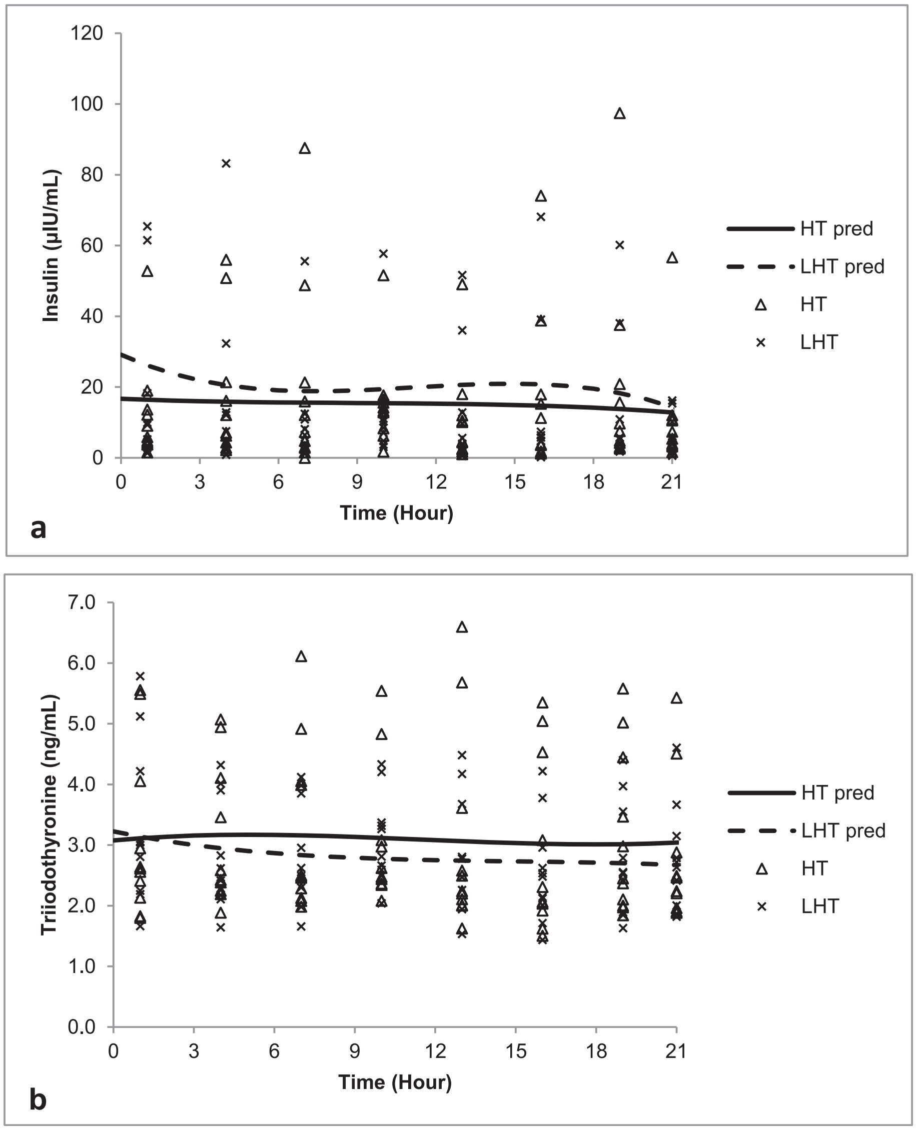


Fig. 4. Predicted (pred) and observed (Δ and X) **a** triiodothyronine and **b** insulin levels in heat tolerant (HT) and less heat tolerant (LHT) Santa Ines sheep throughout the day.

pate excess heat. The sweating rate remained high after 1600 h (end of the thermal challenge) until nighttime, when the air temperature was markedly lower, demonstrating that sweating was important for the re-establishment of thermal balance in the less heat-tolerant group, as similar findings were reported by Titto (2016b).

- Cubic regression showed that ocular temperature was higher throughout the day, mainly in less heat-tolerant sheep at the time of thermal challenge (1000–1600 h). Previous studies have shown a positive correlation between this variable and tympanic temperature (Boere et al., 2003) and rectal temperature (Schaefer et al., 2007; Kahwage et al., 2017; Arfuso et al., 2022), making it a noninvasive method for evaluating heat stress.
- Less heat-tolerant animals had higher insulin concentrations, probably because they were more affected by stress. During stress, the sympathetic nervous system releases catecholamines that stimulate α-adrenergic receptors to increase insulin secretion, as suggested by Alvarez et al. (1989). However, the T3 concentration was not influenced by the increase in air temperature. Although it is expected that T3 levels would be lower during periods of warmer temperatures to ensure a reduction in heat production, according to the findings of Pantoja et al. (2017), the eight-day challenge could have been a limitation in our study, not long enough to present changes in T3.
- As mentioned in other studies, a reduction in feed intake is expected (Luna-Nevárez et al., 2020; Luna-Nevárez et al., 2021). In contrast, the less heat-tolerant animals in the present study showed no reduction in food intake, possibly because of the thermal challenge inside the climate chamber, which differed from day to night, as the temperature was 10 °C lower at night and was set at 36 °C for 6 h during the day. The absence of solar radiation could be a limitation of our study, which is less challenging compared to animals in the field under pasture conditions (Marcone et al., 2021; Santos et al., 2021).
- We found some individual differences between Santa Ines sheep, which could be an easy tool to classify and select more thermotolerant sheep, demonstrating the usefulness of using individual classifications based on rectal temperature to define the phenotypic differences involved in tolerance to thermal stress. Animals of the same breed and under the same conditions can respond differently to stress, which can result in greater productivity and ease of combating the deleterious effects of heat stress.

- For further investigations, both surface heat exchange and sweating rate are important for understanding thermoregulatory responses in hair sheep. The use of surface heat exchange (peripheral vasodilation) by less heat-tolerant sheep likely occurred because these individuals required more time and effort to dissipate excess heat, despite the use of sweating, as discussed by Starling et al. (2002), which can be assumed to be a good thermoregulation mechanism in hair sheep. A larger sample size would be valuable for further studies to investigate the extremes of high and low heat tolerance and to find differences in cellular responses and gene expression. The results of this study can be used as reference values for Santa Ines sheep subjected to heat stress.

Ethics approval

All experimental procedures were approved by the Institutional Animal Ethics Committee of the Faculty of Animal Science and Food Engineering of the University of São Paulo (CEUA/FZEA/USP protocol no. 7498130919).

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) did not use AI- or AI-assisted technologies.

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Declaration of interest

The manuscript is an original contribution approved by all authors, all permissions to reproduce copyrighted material have been obtained, and any conflicts of interest are present.

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M.H.A. Pantoja: Data curation, Writing – review & editing. **G.B. Mourão:** Conceptualization, Formal analysis. **M.C.S. Ferreira:** Data curation. **E.A.L. Titto:** Conceptualization, Writing. **R.F. Strefezzi:** Writing. **S.B. Gallo:** Writing. **C.G.Titto:** Conceptualization, Funding acquisition, Formal analysis, Writing, Writing – review & editing.

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