



## Influence of successive heat waves on the thermoregulatory responses of pregnant and non-pregnant ewes

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### ABSTRACT

The frequency of heat waves has increased over the last years, with an impact on animal production and health, including the death of animals. Therefore, the aim of this study was to evaluate the dynamics of thermoregulation and hormonal responses in non-pregnant and pregnant ewes exposed to successive heat waves. Twenty-four non-pregnant and 18 pregnant Santa Ines ewes with black coat color (live weight:  $55 \pm 9.03$  kg; age: 60 months) were used. Weather variables such air temperature, relative humidity, and solar radiation were continuously recorded. The rectal and tympanic temperatures and respiratory rate were measured daily. Serum triiodothyronine (T3) and prolactin were evaluated during the heat wave and thermoneutral periods. The physiological variables were higher under the heat wave conditions and were related to the activation of the thermoregulatory system for maintaining homeothermy ( $P < 0.05$ ). The core body temperature was higher during successive heat waves ( $P < 0.05$ ), as was the tympanic temperature, which are both affected by changes in air temperature ( $P < 0.05$ ). T3 and prolactin levels were not influenced by successive heat waves ( $P < 0.05$ ) and rectal temperature and respiratory rate were highest in non-pregnant ewes ( $P < 0.05$ ). Prolactin was not affected by temperature. The results indicate that the Santa Ines breed overcomes the thermal challenge during a heat wave without showing severe signs of thermal stress regardless of being pregnant or not.

### 1. Introduction

The current scenario of climate changes has increased the frequency and intensity of heat waves in different regions of the world (Sejian et al., 2013). A heat wave is defined as an extreme temperature period (Vitali et al., 2015) that can last three (Brown-Brandl et al., 2005) to nine consecutive days (Reis et al., 2019).

The increase in heat waves can affect animal health and production and can cause the death of animals because these waves occur suddenly and change the thermal environment without prior conditioning of the animals (Bishop-Williams et al., 2015; Pereira et al., 2020). Effective thermoregulatory responses to minimize the impact of rapid changes in the thermal environment on animals show the acclimation capacity (Collier et al., 2019).

Indigenous tropical breeds such as Santa Ines sheep exhibit characteristics related to heat tolerance (Lv et al., 2014). Under stressful environmental conditions, the animals increase their respiration rate, sweating rate and rectal temperature, in addition to modifications in hormonal concentrations (Morais et al., 2008; Titto et al., 2016) in order to avoid sudden death. However, even when acclimated to heat, a sudden change in the environmental temperature caused by a heat wave can represent a real challenge for sheep in terms of activation of their thermoregulatory system and coping with heat loss (Morais et al., 2008; Titto et al., 2016).

Heat waves have resulted in economic losses in different parts of the world. Despite the availability of studies on simulated heat waves and their effects on animal production, the thermoregulatory responses of sheep to sudden temperature changes, such as heat waves, are not

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sufficiently understood. Therefore, the aim of this study was to evaluate the dynamics of thermoregulation and hormonal responses in non-pregnant and pregnant female hair sheep exposed to two successive heat waves during the end of winter in a tropical climate.

## 2. Material and methods

### 2.1. Ethical approval

The procedures were approved by the Ethics Committee on Animal Experimentation (CEUA/FZEA/USP Declaration 7498130919), considering the legal and ethical issues of the interventions performed.

### 2.2. Animals

Twenty-four non-pregnant and 18 pregnant Santa Inês ewes with a black coat color (live weight:  $55 \pm 8.6$  kg; body condition score: 2.5 on a scale from 1 to 5; age: 60 months) were used. Pregnancy was confirmed by ultrasound. During the experiment, the ewes had a mean of  $86 \pm 25.2$  in the first heat wave and  $101 \pm 25.3$  days of gestation during in the second heat wave considering a mean duration of pregnancy of 150 days. The animals were kept on Aruana pasture (*Panicum maximum* cv. Aruana), with free access to artificial shade under an asbestos-cement roof painted white on the upper side ( $1 \text{ m}^2/\text{animal}$ ). They had a concentrate supplementation at 7 a.m. every day (1% of live weight) with concentrate (corn bran and soybean meal).

### 2.3. Experimental design

The experiment was designed to assess the thermoregulatory response of animals under different outdoor environmental conditions and was carried out in the end of winter of 2020 in southeastern Brazil.

Data were collected during successive heat waves, with the interval being characterized by low temperatures that occurred at the end of winter (September). The 1st heat wave occurred over four consecutive days with temperature above  $35^\circ\text{C}$ , reaching a maximum temperature of  $37.2^\circ\text{C}$ , and the 2nd heat wave occurred on seven consecutive days with air temperatures above  $37.5^\circ\text{C}$ , reaching a maximum temperature of  $39.8^\circ\text{C}$ . The interval between the 1st and 2nd heat wave was 14 days. The animals were taken to the shade area in the afternoon (4 p.m.) to the collection of physiological and blood samples on the penultimate day of each heat wave, predicted by CEPAGRI – UNICAMP (Centro de Pesquisas Meteorológicas e Climáticas Aplicadas a Agricultura de Campinas-SP).

The animals were also evaluated in winter in the absence of a heat wave, which was called the thermoneutral period (no stress). The data were collected at a mean ambient temperature of  $24.7^\circ\text{C}$  and the maximum temperature recorded on the day was  $27.2^\circ\text{C}$ . The mean maximum air temperature during winter is  $28^\circ\text{C}$  in Pirassununga-SP.

### 2.4. Meteorological variables

The air temperature ( $^\circ\text{C}$ ), air relative humidity (%), and solar radiation ( $\text{W}/\text{m}^2$ ) were continuously recorded by an automatic weather station (Davis, Vantage Pro 2, Arizona, USA). The station was programmed to take readings every 5 min. As thermal comfort evaluation criterion, the Temperature and Humidity Index (THI) specific for sheep was calculated, as proposed by Marai et al. (2007), using the following equation:  $\text{THI} = \text{AT} - \{(0.31 - 0.31 * \text{RH}) * (\text{AT} - 14,4)\}$ , where: AT is the air temperature ( $^\circ\text{C}$ ) and RH is the relative air humidity (%). The THI values obtained indicate:  $<22.2$  = absence of thermal stress;  $22.2$  to  $<23.3$  = moderate thermal stress;  $23.3$  to  $<25.6$  = severe thermal stress; and  $\geq 25.6$  = extreme severe thermal stress.

### 2.5. Physiological variables

The respiratory rate (breaths per minute), rectal temperature ( $^\circ\text{C}$ ) and tympanic temperature ( $^\circ\text{C}$ ) were measured in the afternoon (4 p.m.). The respiratory rate was obtained by observation of thoracoabdominal movements, the rectal temperature was measured with a digital clinical thermometer, and the tympanic temperature was measured with an ear thermometer (TCI100, Incoterm) inserted into the animal's ear canal.

### 2.6. Triiodothyronine (T3) and prolactin measurement

A blood sample was collected from each animal by external jugular vein puncture into vacuum tubes without anticoagulant. The samples were centrifuged at 3000 rpm for 15 min and refrigerated at  $-20^\circ\text{C}$ . Triiodothyronine and prolactin were measured by enzyme immunoassay (EIA) using commercial kits according to the manufacturer's instructions (Monobind, Lake Forest, CA, USA). Both kits were validated by demonstrating parallel curves between standard concentrations and serially diluted serum samples. The intra- and interassay coefficients of variation were, respectively, 3.8 and 6.3% for T3 and 5.5 and 7.3% for prolactin.

### 2.7. Statistical analysis

The variables were measured at a  $3 \times 2$  factorial arrangement of treatments in a completely randomized design, where the factors are the presence of heat wave (thermoneutral, 1st wave, 2nd wave) and pregnancy status of the ewes (pregnant, non-pregnant). The response variables were analyzed using a mixed model, considering a Poisson distribution for respiratory rate and a normal distribution for the other variables. The model included the random effect of sheep, considering a specific correlation matrix of better fit because of the repeated measures in the same animal, as well as the fixed effects of physiological state (pregnant or non-pregnant) and stress condition/heat wave (thermoneutral, wave 1, and wave 2) and the interaction between these effects. Means were compared by Tukey-Kramer test and significance was set at 5%. All results are reported as the mean  $\pm$  standard error of the mean. The SAS for Windows 9.4 software (2016) was used for the statistical analyses.

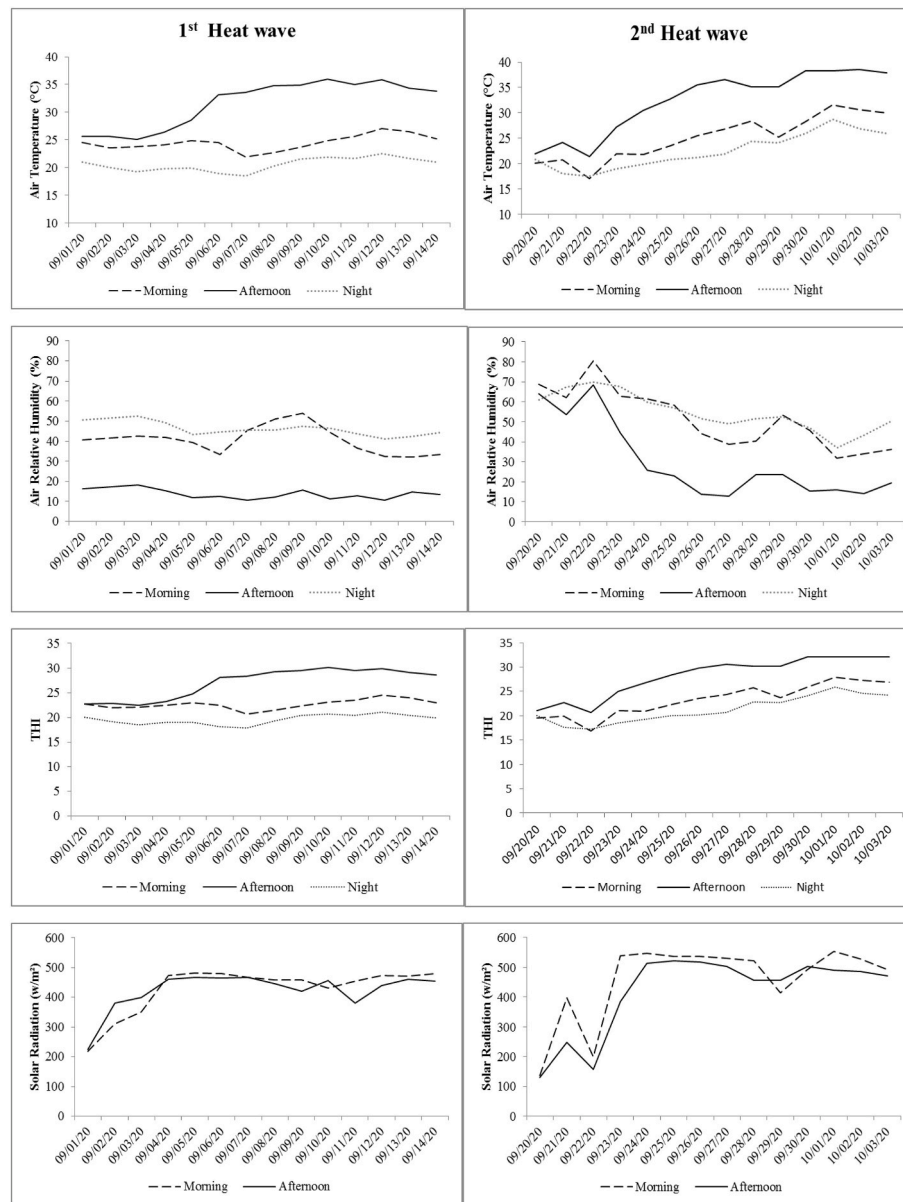
## 3. Results

The mean afternoon air temperature fluctuated to around  $35.2$  and  $37.5^\circ\text{C}$  in the 1st and 2nd heat wave respectively, while the mean afternoon RH was 13% in the 1st heat wave and 19% in the 2nd heat wave. The mean THI values were close to 23.6 during the morning and 29.2 in the afternoon indicating severe to extreme heat stress in the 1st heat wave and the average THI value during the 2nd heat wave was 26 in the morning and 31.3 in the afternoon, indicating extreme stress for sheep (Fig. 1).

Ewes in the 1st and 2nd heat waves had higher respiratory rate, rectal and tympanic temperatures ( $P < 0.05$ ; Table 1) than those in the thermoneutral environment. The physiological and hormonal variables showed no significant interaction ( $P > 0.05$ ).

Despite a reduction in T3 from thermoneutral to first and second waves and an increase on prolactin levels, both were not influenced by successive heat waves ( $P > 0.05$ ; Table 2).

The rectal temperature and respiratory rate were significantly higher ( $P < 0.05$ ; Table 3) in non-pregnant ewes than in pregnant ewes. Pregnant animals exhibited higher levels of prolactin ( $P > 0.05$ ) when compared to non-pregnant ewes. Additionally, there was not difference ( $P > 0.05$ ) in tympanic temperature and T3 levels between non-pregnant and pregnant ewes.



**Fig. 1.** Average of maximum values of air temperature, air relative humidity, THI, and solar radiation during successive heat waves: morning (6 a.m.-12 p.m.), afternoon (12 p.m.-6 p.m.), and night (6 p.m.-6 a.m.).

**Table 1**

Mean and standard error values of respiratory rate (breaths.m<sup>-1</sup>), Rectal temperature (°C), Tympanic temperature (°C) in Santa Ines ewes measured during successive heat waves and in the thermoneutral environment.

	Respiratory rate	Rectal temperature	Tympanic temperature
Thermoneutral	47.30 ± 3.80 C	38.94 ± 0.06 B	34.53 ± 0.19 C
1st Heat wave	84.02 ± 3.80 B	39.76 ± 0.06 A	38.80 ± 0.19 A
2nd Heat wave	114.18 ± 3.86 A	39.81 ± 0.06 A	37.05 ± 0.19 B

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

**4. Discussion**

The average air temperatures during winter in southeastern Brazil are generally within the thermoneutral range for wool sheep, with temperatures below 31 °C. According to Van Wettere et al. (2021), thermal stress causes an impact on the performance and welfare of sheep

**Table 2**

Mean and standard error values of triiodothyronine (ng.mL<sup>-1</sup>) and prolactin (ng.mL<sup>-1</sup>) in Santa Ines ewes measured during successive heat waves and in the thermoneutral environment.

	T3	Prolactin
Thermoneutral	26.2 ± 0.11 A	13.1 ± 0.25 A
1st Heat wave	23.7 ± 0.11 A	16.2 ± 0.25 A
2nd Heat wave	23.0 ± 0.11 A	15.7 ± 0.26 A

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

when temperatures fall below 12 °C (lower critical temperature) or rise above 25–31 °C (upper critical temperature), situations that trigger the mechanisms of thermoregulation and challenge the capacity of sheep to maintain homeothermy.

During the successive heat waves in the end of winter, the rapid change in ambient temperature exceeded the upper critical limit for sheep. The temperatures reached a maximum of 37.2 °C in the 1st heat

**Table 3**

Mean and standard error values of physiological variables, triiodothyronine, and prolactin in non-pregnant and pregnant Santa Ines ewes.

Variable	Non-pregnant	Pregnant	P value
Respiratory rate (breaths.m <sup>-1</sup> )	86.72 ± 2.87	76.95 ± 3.35	0.029
Rectal temperature (°C)	39.58 ± 0.05	39.42 ± 0.06	0.048
Tympanic temperature (°C)	36.86 ± 0.14	36.73 ± 0.16	0.564
T3 (ng.mL <sup>-1</sup> )	2.52 ± 0.09	2.33 ± 0.09	0.145
Prolactin (ng.mL <sup>-1</sup> )	1.21 ± 0.21	1.80 ± 0.21	0.050

wave and of 39.8 °C in the 2nd heat wave. Therefore, the challenge for sheep to regulate their body temperature was different between the 1st and 2nd heat waves. In addition, the THI in the second wave was higher than in the first wave, demonstrating environmental conditions of extreme stress throughout the day.

In both heat waves, the animals exhibited a substantial increase of evaporative heat loss by significantly increasing the respiratory rate in an attempt to minimize the tendency for increases in body temperature (Souza et al., 2008). The maximum temperature and THI recorded was higher during the 2nd heat wave than during the 1st wave and therefore triggered a greater effort of thermoregulation by increasing the respiratory rate to dissipate excessive heat.

The increase in the respiratory rate during the two heat waves was important to maintain the thermal balance. The high respiratory rates limited increases in body temperature and therefore minimized the potential consequences of thermal stress. Even the respiratory rate is high but the animal was efficient in dissipating heat, maintaining homeothermy, some consequences of thermal stress may not occur (Eustáquio Filho et al., 2011), a situation observed in the present study. Although the sheep showed an increase in rectal temperature of 1.1 and 1.5 °C during first and second heat waves respectively compared to the thermoneutral environment, which indicates relevant levels of heat storage, this temperature was below the maximum limit for the species, which is 39.9 °C (Liu et al., 2012). Additionally, the higher respiratory rate observed in the second wave occurred because the thermal challenge was greater, which can be proven by the high air temperature and the THI values that indicated extreme stress. Therefore, the higher respiratory rate was a mechanism to dissipate excess body heat and maintain the rectal temperature within the ideal value for the species (Macías-Cruz et al., 2018).

The thermoregulatory responses associated with the preconditioning of the animals to the 1st heat wave reduced the impact of heat stress on the animal, which was able to maintain the core body temperature in the appropriate range. Another factor that may have contributed to maintaining the rectal temperature within the limits for sheep was sweating. Although not measured in the present study, heat loss by sweating was probably important to maintain homeothermy, especially during the 2nd heat wave that was more challenging with THI reaching values of 32.1 (extreme heat stress), as sheep activate heat loss mechanisms (respiratory rate and sweating) to maintain a stable body temperature when subjected to high solar radiation (Pantoja et al., 2017; Pulido-Rodríguez et al., 2021).

Another important parameter was the tympanic temperature that increased during the heat wave, demonstrating the influence of ambient temperature on this variable and its rapid response to changes in air temperature (Guidryr and McDowell, 1966). In fact, the tympanic temperature reflects more faithfully the temperature of the hypothalamus and has a lower thermal inertia than the rectal temperature. Despite the increase in tympanic temperature during the heat wave, this variable remained below the rectal temperature values (Boere et al., 2003). This difference in rectal and tympanic temperatures is to be expected and only tends to vary in terms of its magnitude. Whenever sheep utilize high respiratory rates, there is the side effect of selective cooling of the brain. Countercurrent heat exchange occurs between venous blood cooled at the level of the nasal turbinates and blood of the carotid artery

at the level of the circle of Willis (Wang et al., 2016). This heat exchange allows the blood supply to the brain and thus to the tympanum to occur at a lower temperature than the body temperature. This can be demonstrated by the greater differences between rectal and tympanic temperatures in the 2nd heat wave, when the highest respiratory rates were also recorded (Meiners and Dabbs, 1977), resulting in low temperatures in the brain and in the tympanum.

The brain temperature is usually a priority in the cooling of the body and therefore oscillates little, whereas in animals with selective cooling of the brain the body temperature frequently increases beyond the reference values when the animals are exposed to adverse climate conditions (Eustáquio Filho et al., 2011; Maloney et al., 2001). Furthermore, the animals may have been preconditioned to the high temperature during the 1st heat wave. Thus, even under more severe heat stress conditions during the 2nd heat wave, the thermoregulatory mechanisms responded better due to preconditioning, eliciting a more intense and prolonged response characterized by a greater capacity to limit the increases in body temperature (Yadav et al., 2019).

In addition to changes in physiological parameters, hormonal alterations may occur during heat stress. Despite the high THI in the heat wave demonstrating extreme stress conditions and T3 is responsible for the regulation of thermogenesis (Silva, 2006), there was an absence of T3 response during the heat wave, which may be due to the Santa Inês breed being a native breed and considered heat tolerant, this has already been seen by Pereira et al. (2008) that cattle with greater heat tolerance did not show T3 reduction under heat stress.

Regarding prolactin, some authors have shown that the ambient temperature is responsible for stimulating the release of this hormone under adverse environmental conditions (Alamer, 2011; Pereira et al., 2019). However, there were no differences in prolactin levels in response to the temperature increase caused by the successive heat waves. This finding can be explained by the fact that Santa Ines sheep are considered a naturalized breed and are therefore better adapted to overcome the thermal challenges imposed by the environment, showing a superior thermolytic performance due to specific adaptive morphological and physiological characteristics and thus being more tolerant to heat (Titto et al., 2016; Pantoja et al., 2017). Scharf et al. (2010) also reported the lack of a prolactin response to heat stress in heat-tolerant breeds. In that study, prolactin production was not increased in heat-tolerant animals, while animals that were more susceptible to heat increased the production of prolactin in the environment with high air temperature compared to the thermoneutral environment.

On the other hand, the higher concentration of prolactin in pregnant ewes in relation to non-pregnant ewes may be related to the time of pregnancy, Brunet and Sebastian (1991) observed that in addition to the influence of the season of the year, higher concentrations of prolactin in pregnant animals occurs from day 61 to day 140 of pregnancy. In the present study, the ewes had an average of 90 days of gestation, which may be one of the causes of the higher concentration of prolactin.

Regarding rectal temperature and respiratory rate in pregnant ewes, these physiological variables were expected to be higher in pregnant animals due to the increased metabolic rate resulting from pregnancy and also because the developing fetus loses heat through the fetal circulation and uterine wall, and fetal heat production represents an extra thermal load on the mother (Yaqub et al., 2017). However, in the present study the rectal temperature was seen to be slightly lower in pregnant ewes, it is believed that it may be part of a physiological response to help protect the developing fetus from exposure to elevated temperatures in the uterus during the last trimester of pregnancy, even that the fetus is reasonably well developed at this time (Godfrey et al., 2017). This lower rectal temperature seen in pregnant ewes may have been due to heat dissipation mainly through sweating and sensitive heat exchange and to a lesser extent through respiratory rate.

Understanding how sheep respond from the point of view of physiological and hormonal response during extreme events is important for the selection of animals with better thermolytic capacity for rearing in



high temperature environments and a strategy to maintain productivity in the face of climate change. Also, heat waves during a cold season seems to be worst than heat waves during summer, related to acclimation (Collier et al., 2019).

The greater thermolytic capacity of the animals guarantees a better thermoregulatory response in a thermal challenge situation and when it is combined with the use of different forms of shading and access to water, it contributes to alleviating the thermal stress of the animals, in addition to favoring the dissipation of heat and reducing the risk of death mainly of animals raised on pasture.

## 5. Conclusions

The present results showed similar thermoregulatory responses in pregnant and non-pregnant Santa Ines sheep, even during successive heat waves. The thermoregulatory response of sheep in the 2nd heat wave indicates an additional capacity for heat loss, permitting the maintenance of body temperature stability even under more severe heat conditions. Despite differences in the extent of evaporative heat loss, both groups of sheep were efficient and were able to overcome the thermal challenge posed by the two heat waves.

## CRedit authorship statement

Cristiane Gonçalves Titto: Conceptualization, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Project administration, Funding acquisition, Messy Pantoja: Conceptualization, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Supervision. Alfredo Pereira: Conceptualization, Writing - Original Draft. Jessica Campos: Investigation. Douglas Almeida: Investigation. João Negrão: Resources, Formal analysis, Writing - Original Draft, Gerson Mourão: Formal analysis, Writing - Original Draft.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

link DOI

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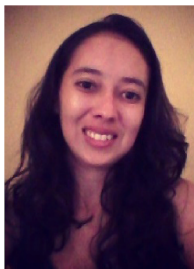
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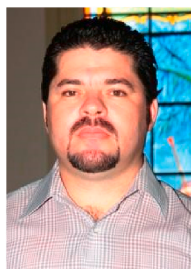
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