









# Temperature and Humidity Index (THI) during the 2021/2022 summer and its impact on dairy cattle in Rio Grande do Sul, Brazil

Índice de Temperatura e Umidade (ITU) ao longo do verão de 2021/2022 e estimativas dos impactos na bovinocultura de leite no Rio Grande do Sul, Brasil

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**Abstract:** This study aimed to calculate the temperature and humidity indexes (THIs) derived from extremely high air temperatures during the months of December 2021, January, and February 2022 in Rio Grande do Sul. The main goal was to characterize and regionalize potential impacts on dairy production. We used hourly measurements of temperature and relative humidity from 28 meteorological stations of the INMET/SIMAGRO/SEAPI network. These data cover ten ecoclimatic regions of the state. THI and Estimated Milk Loss (EML) were calculated, with the daily number of hours classified under each THI category during the trimester by municipality and region. The effects of region and month on THI and EML were evaluated through analysis of variance at a 5% significance level. Differences between means were compared using the Tukey HSD test ( $P < 0.05$ ). The probability of each daily hour having a THI in thermal discomfort ( $\text{THI} > 70$ ) was determined through analysis of variance for binomial variables by region, with the observed effect of time ( $P < 0.05$ ), and means were compared using the non-parametric Bonferroni test at 5%. To group these data, we applied the Scott-Knott test. The Baixo Vale do Uruguai stood out in the trimester with the highest THI values, indicating thermal discomfort. Conversely, no heat stress was indicated in the Serra do Nordeste. In all regions, the most frequent daily classification of thermal discomfort ranged from attention to alert, with January being particularly significant. The highest EML estimates for most of the eight production levels were recorded in January. High estimates of productivity loss occurred in cows with higher milk production potential.

**Keywords:** Thermal Comfort; Ecoclimatic Regions; Cattle; Productivity.

**Resumo:** O objetivo do estudo foi calcular o índice de temperatura e umidade (ITU), devido às temperaturas do ar extremamente elevadas durante os meses de dezembro 2021, janeiro e fevereiro 2022 no Rio Grande do Sul, para fins de caracterização e regionalização dos possíveis impactos na produção leiteira. Empregaram-se dados horários de temperatura e umidade relativa do ar de 28 estações meteorológicas da rede INMET/SIMAGRO/SEAPI, de dez regiões ecoclimáticas

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do Estado, calculando-se ITU e Perda Estimada de Leite (DPL), contabilizando-se número diário de horas em cada classificação do ITU, durante trimestre por município e região. Avaliou-se efeito da região e mês no ITU e DPL, pela análise de variância a 5% de significância. Detectadas diferenças entre médias, compararam-se pelo teste Tukey HSD ( $P < 0,05$ ). Verificou-se probabilidade de cada hora diária apresentar ITU em desconforto térmico ( $ITU > 70$ ), através da análise de variância para variáveis binomiais, por região; observado efeito de horário ( $P < 0,05$ ), médias foram comparadas pelo teste não paramétrico Bonferroni a 5%. Para agrupamento destas, usou-se o teste Scott-Knott. O Baixo Vale do Uruguai se destacou no trimestre com maiores valores do ITU, desconforto térmico, enquanto na Serra do Nordeste, não indicaram estresse calórico. Em todas as regiões, a classificação de desconforto térmico mais frequente, diariamente, foi de atenção até alerta, destacando-se o mês de janeiro. Maiores estimativas de DPL, para grande parte dos oito níveis de produção, registraram-se em janeiro. Elevadas estimativas de perda de produtividade, ocorreram em vacas com maior potencial de produção de leite.

**Palavras chaves:** Conforto Térmico; Regiões Ecoclimáticas; Bovinos; Produtividade.

## Introduction

The state of Rio Grande do Sul is characterized by high air temperatures during the summer. Climatically, the average maximum monthly temperature in December is 28.3°C, ranging from 23.8°C to 31.9°C; in January, it is 29.2°C, fluctuating between 22.7°C and 32.6°C; and in February, it is 28.6°C, with ranges of 24.8°C to 31.4°C<sup>(1)</sup>.

Since 1990, Brazil has experienced an increase in annual average air temperatures, with the two largest positive temperature deviations observed in the 1961-2022 series occurring in 2015 and 2019, both registering a value of 0.9°C above the historical average, making them the two hottest years since 1961. The year 2022 was the twentieth warmest year since 1961. The most intense heatwave occurred between January 12 and 26, 2022, with maximum air temperatures exceeding 40°C in several municipalities in Rio Grande do Sul. February was also marked by high temperatures, including a record in Uruguaiana (RS), which recorded 42.9°C on February 27, corresponding to 6°C above the average for that day. This was the highest temperature recorded in 110 years and the highest value recorded in the city since measurements began in 1912, surpassing the 42.2°C recorded on January 27, 1986<sup>(2)</sup>.

During the trimester (December 2021, January, and February 2022), air temperatures were extremely high, especially in January, exceeding the climatological average (standard climatology 1991-2020) throughout the state<sup>(3)</sup>. In 62.5% of the evaluated meteorological stations, there were periods with at least five consecutive days with an absolute maximum temperature  $\geq 5^\circ\text{C}$  above the average, characterizing this month as a heatwave and high maximum air temperatures<sup>(3)</sup>. Similarly, the months of December 2021 and February 2022 had several days with maximum air temperatures above average, with positive anomalies of 1.8°C in December<sup>(3)</sup> and 4.7°C in February<sup>(5)</sup>.

Periods of extremely hot weather and, especially, heatwaves, cause thermal discomfort, leading to heat stress that negatively impacts the health, economy, and productivity of livestock animals<sup>(3)</sup>. Animals experience heat stress when they generate more heat than

they can dissipate; to adapt, they reduce food consumption, resulting in a decline in production<sup>(6)</sup>. While there are reports that reduced consumption explains only 36% of the decrease in milk production<sup>(7)</sup>, one study suggests that the decrease in consumption explained approximately 50% of the reduction in production in dairy cows<sup>(8)</sup>. Besides consumption reductions, thermal stress in lactating cows causes significant metabolic adaptations. These changes result in reduced energy supply to the mammary gland, which appears to be one of the mechanisms responsible for the decrease in milk production and its components, affecting its quality as well<sup>(9)</sup>.

Cattle are homeothermic animals, capable of maintaining their body temperature regardless of ambient temperature variations, using physiological, metabolic, and behavioral mechanisms for thermoregulation<sup>(10)</sup>. In this sense, there is a range of ambient temperature (Thermal Comfort Zone; TCZ) within which animals exhibit minimal metabolism, showing no signs of thermal discomfort nor activating physical and chemical thermoregulation mechanisms<sup>(11)</sup>. The best climatic conditions for cattle are temperatures between 10°C and 27°C, relative humidity between 60% and 70%, and a THI (Temperature and Humidity Index) below 74<sup>(12)</sup>.

Heat stress occurs when high temperatures combined with high metabolic heat production result in excess body heat storage, and animals are unable to dissipate it into the environment<sup>(13)</sup>. Dairy cattle, especially those with high milk production potential, struggle to dissipate this body heat in unfavorable environmental conditions. Lactating cows are highly susceptible to heat stress due to the high metabolic load of milk synthesis and visceral metabolism associated with high feed intake<sup>(14)</sup>. A high-production cow expends approximately 31.1% of the energy ingested daily in heat production. More than half of this heat (53%) comes from milk synthesis, and nearly a quarter (23.5%) originates from fermentation, digestion, and excretion. Together, these percentages constitute what is known as heat increment. The remaining 23.5% corresponds to heat produced by metabolic processes necessary to maintain vital functions<sup>(15)</sup>.

Dissipation of excess body heat primarily depends on temperature fluctuations during the day and night. If nighttime temperatures do not drop below 21°C for a period of three to six hours, animals cannot effectively lose all the heat acquired during the previous day<sup>(16)</sup>. Daily average and maximum temperatures have variable effects on food intake and, consequently, on milk production, depending on relative humidity and the duration of exposure to temperatures capable of causing stress<sup>(13)</sup>. Feed intake reductions in dairy cows begin when the ambient temperature reaches 25°C, and it decreases significantly when it exceeds 40°C (20% to 40%)<sup>(17,18)</sup>.

The ability to cope with climatic variations in their environment varies among animals. Thus, indices have been developed to establish classification criteria for various environments and combinations of elements that influence animal thermal comfort, with the Temperature and Humidity Index (THI) being a prominent one<sup>(19)</sup>. This index takes into account the combined effects of temperature and relative humidity and is widely used for thermal comfort evaluation using a meteorological database<sup>(20)</sup>, as it is easily obtained.

Thermal stress, therefore, negatively affects the performance of lactating cows, resulting in significant economic losses for producers and the dairy industry. A detailed study of THI and its regionalization determines the comfort or discomfort experienced by animals, especially under extremely high air temperatures and heat waves during summer. It can assist in selecting suitable locations and means for thermal conditioning. Thus, the index becomes a valuable zootechnical resource to enhance milk production efficiency by distributing animals appropriately to specific regions<sup>(21)</sup> and establishing management strategies to mitigate environmental effects. Based on this, our goal was to assess potential impacts on dairy production in different regions of Rio Grande do Sul State (Brazil) by calculating THI during the atypical summer of 2021/2022.

## Materials and methods

Hourly data (00:00 to 23:00) of temperature and relative humidity for the months of December 2021 (Dec.), January (Jan.), and February (Feb.) 2022 were obtained from 28 meteorological stations within the network of the National Institute of Meteorology (INMET) and the Agroclimatic Monitoring and Alert System (SIMAGRO/RS) of the Department of Agriculture, Livestock, Sustainable Production, and Irrigation (SEAPI/RS). The analysis period encompassed the summer season (December, January, and February), a climatological delineation commonly adopted by other authors<sup>(22, 23)</sup>. To represent the ten Ecoclimatic Regions of the state<sup>(24)</sup>, meteorological data from three municipalities/regions were used, except the Encosta Inferior da Serra and Grandes Lagos, where two municipalities were considered, as shown in Table 1.

**Table 1.** Altitude and geographical coordinates (latitude and longitude) of the municipalities encompassed in this study, for ten Ecoclimatic Regions of Rio Grande do Sul, Brazil.

Region	Municipality	Altitude (m)	Latitude (South)	Longitude (West)
Planalto Médio	Passo Fundo	680	28°15'40"	52°24'30"
	Ibirubá	400	28°37'48"	53°05'25"
	Getúlio Vargas	644	27°52'34"	52°13'16"
Serra do Sudeste	Caçapava do Sul	430	30°30'59"	53°29'12"
	Encruzilhada do Sul	348	30°31'37"	52°31'06"
	Pinheiro Machado	419	31°34'37"	53°23'06"
Serra do Nordeste	Veranópolis	693	28°54'03"	51°33'10"
	Vacaria	960	28°30'39"	50°55'47"
	Bento Gonçalves	671	29°10'26"	51°31'07"
Alto e Médio Vale do Uruguai	Frederico Westphalen	535	27°21'27"	53°23'40"
	Santa Rosa	268	27°52'16"	54°28'55"
	Porto Vera Cruz	168	27°44'17"	54°54'08"
Baixo Vale do Uruguai	Itaqui	64	29°09'09"	56°33'03"
	São Borja	74	28°40'58"	55°58'39"
	Maçambará	88	29°08'25"	56°04'26"
Depressão Central	Campo Bom	22	29°40'49"	51°03'13"
	Santa Maria	139	29°41'29"	53°48'03"
	Porto Alegre	22	30°01'40"	51°13'43"

Campanha	Alegrete	76	29°47'05"	55°46'33"
	Uruguaiana	56	29°44'58"	57°05'18"
	Bagé	214	31°19'43"	54°06'26"
Missioneira	São Luiz Gonzaga	260	28°24'31"	54°57'41"
	Santiago	354	29°10'23"	54°51'21"
	Bossoroca	221	28°42'37"	54°53'42"
Grandes Lagos	Capão do Leão	15	31°46'03"	52°26'55"
	Jaguarão	23	32°33'37"	53°22'52"
Encosta Inferior da Serra	Teutônia	47	29°26'56"	51°48'48"
	Sobradinho	363	29°25'22"	53°01'57"

Temperature and Humidity Index (THI) was calculated using the following formula<sup>(19)</sup>:

$THI = Tave + (0.36Tdp + 41.5)$ , where: Tave = daily average air temperature and Tdp = dew point temperature.

$$Tdp = ((RH/100)^{(1/8)} * (112 + (0.9 * Tave))) + (0.1 * Tave) - 112$$

THI was divided into four classes adapted from classification by Rosenberg, Biad, and Verns (1983)<sup>(25)</sup>, identifying thermal comfort/discomfort ranges, namely:

THI1 =  $\leq 70$ , non-stressful condition, within the thermal comfort range;

THI2 = 71-78, thermal stress condition (71-75 attention and 75-78 alert situation);

THI3 = 79-83, severe thermal stress condition (danger situation);

THI4 =  $\geq 84$ , critical thermal stress condition (emergency).

Hourly air temperature and average relative humidity data were used to calculate the hourly THI for each municipality, and with these values, daily and monthly averages were calculated. Subsequently, monthly averages were calculated for each ecoclimatic region. The daily number of hours within each THI classification during the trimester was counted, and averages were calculated for each municipality and region.

To estimate the effects of meteorological variables, using THI values, on the estimation of milk production in the evaluated ecoclimatic regions, the following equation for lactating Holstein cows<sup>(26)</sup>, adapted by Hahn (1993)<sup>(27)</sup>:

$EML = -1.075 - 1.736 \times PN + 0.02474 \times SP \times THI$ , where: EML is the estimated milk loss (kg day<sup>-1</sup>), and SP is the standard production (kg day<sup>-1</sup>).

Eight milk production levels were used as references: 5, 10, 15, 20, 25, 30, 35, and 40 kg day<sup>-1</sup>, assuming that the animals were in a thermoneutral situation, meaning their normal production without stress. For the analysis and characterization of critical periods, the four THI classes were considered.

To assess the effect of ecoclimatic region and month on THI and EML, the data were subjected to analysis of variance at a 5% significance level ( $P < 0.05$ ) using the "lmer" function

of the “lme4” package, using the R statistical software (v.4.1.1). The statistical model included fixed effects of region, month, and their interaction. Municipality was considered a random effect. When differences between means were detected, they were compared using the Tukey HSD test ( $P < 0.05$ ).

To determine the probability of each hour of the day having a THI within the thermal discomfort range ( $THI > 70$ ), the data were first subjected to analysis of variance for binomial variables per region, using the “glm” function of the R statistical software (v.4.1.1). If a significant effect of time was observed ( $P < 0.05$ ), a comparison of means was performed using the non-parametric Bonferroni test at the same significance level. Additionally, a Scott-Knott analysis was conducted to group the means.

## Results and Discussion

The results of the analysis of variance indicated a significant effect ( $p < 0.05$ ) of the ecoclimatic region, month, and their interaction on THI values and the daily number of hours within the four thermal comfort classifications during the summer of 2021/2022.

Averages, standard deviation, as well as minimum and maximum THI values for December, January, and February in the ten ecoclimatic regions of Rio Grande do Sul are presented in Table 2. The Baixo Vale do Uruguai region stood out in all three consecutive months with the highest average THI values, while the Serra do Nordeste region had the lowest values. In December, THI ranged from  $67.1 \pm 0.7$  to  $73.4 \pm 0.7$ , in January from  $70.0 \pm 0.7$  to  $77.2 \pm 0.7$ , and in February from  $68.6 \pm 0.7$  to  $74.0 \pm 0.7$ .

**Table 2** Temperature and Humidity Index (THI) averages and standard deviation, minimum and maximum values, during the summer months (December 2021; January and February 2022), in ten Ecoclimatic Regions of Rio Grande do Sul, Brazil.

Region	THI average and standard deviation			Minimum THI			Maximum THI		
	Dec	Jan	Feb	Dec	Jan	Feb	Dec	Jan	Feb
Baixo Vale do Uruguai	73,4±0,7a/B	77,2±0,7a/A	74±0,7a/B	71,9	75,7	72,5	74,9	78,7	75,5
Alto e Médio Vale do Uruguai	73,1±0,7ab/B	75,9±0,7a/A	73,4±0,7a/B	71,6	74,4	71,9	74,5	77,4	75
Missioneira	72,4±0,7abc/B	75,1±0,7ab/A	72,6±0,7ab/B	70,9	73,6	71,1	73,9	76,6	74,1
Depressão Central	71,0±0,9abcde/C	74,5±0,9ab/A	72,7±0,9ab/B	69,2	72,7	70,8	72,8	76,3	74,5
Campanha	70,9±0,7abcd/B	74,5±0,7ab/A	71,7±0,7abc/B	69,4	73,1	70,2	72,4	76	73,2
Encosta Inferior da Serra	70,5±0,9abcde/B	73,9±0,9abc/A	71,6±0,9abc/B	68,7	72	69,7	72,3	75,7	73,4
Planalto Médio	69,9±0,7bcde/B	71,6±0,7bc/A	69,8±0,7bc/B	68,1	70,1	68,3	71,1	73,1	71,3
Grandes Lagos	69,0±0,9cde/C	73,3±0,9abc/A	70,8±0,9abc/B	67,2	71,4	69	70,9	75,1	726
Serra do Sudeste	68,2±0,7de/C	72,2bc/A	69,8±0,7bc/B	66,7	707	68,3	69,7	73,1	71,3
Serra do Nordeste	67,1±0,7e/C	70,0 ±0,7c/A	68,6±0,7c/B	65,6	68,5	67,1	68,6	71,4	70,1

Means followed by the same uppercase letter in the row and lowercase in the column do not differ according to the Tukey HSD test at a 5% significance level.

Considering the entire season, the average THI calculated ranged from  $67.1 \pm 0.7$  in the Serra do Nordeste (December 2021) to  $77.2 \pm 0.7$  in the Baixo Vale do Uruguai (January 2022) (Table 2). In all ecoclimatic regions, the month of January had the highest average THI values ( $P < 2.2 \times 10^{-16}$ ). Air temperatures were high in all regions, with the occurrence of an intense heatwave in which maximum temperatures were extremely high, even by the standards considered normal for the summer months (Dec-Jan-Feb) in Rio Grande do Sul<sup>(28)</sup>. Table 3 shows the average values of the Standard Climatological Norm (1991-2020) for average and maximum air temperatures in the months of December, January, and February in regions of RS. During the summer of 21/22, several days with maximum temperatures above  $35^{\circ}\text{C}$  were recorded in all ecoclimatic regions evaluated, as well as temperatures above  $40^{\circ}\text{C}$  in approximately 60% of the regions (Depressão Central, Encosta Inferior da Serra do Nordeste, Alto e Médio Vale do Uruguai, Missioneira, Baixo Vale do Uruguai and Campanha)<sup>(3)</sup>.

**Table 3** Standard Climatological Norm (1991-2020) for Average Air Temperature and Maximum Air Temperature in the months of December, January, and February, in municipalities of Rio Grande do Sul, Brazil.

Region	Municipality	Average Temperature ( $^{\circ}\text{C}$ )			Maximum Temperature ( $^{\circ}\text{C}$ )		
		December	January	February	December	January	February
Campanha	Bagé	22,7	23,8	23,1	28,8	29,7	28,9
	Uruguaiana	24,6	25,8	24,7	30,9	32,1	30,8
Serra do Nordeste	Bom Jesus	18,6	19,4	19,2	25,2	25,6	25,4
Planalto Médio	Cruz Alta	23,3	23,7	23,0	29,7	30,0	29,2
	Passo Fundo	22,0	22,3	21,8	28,4	28,4	27,8
Serra do Sudeste	Encruzilhada do Sul	21,7	22,8	22,3	28,4	29,2	28,5
Depressão Central	Porto Alegre	24,0	25,0	24,7	30,0	31,0	30,5
	Santa Maria	24,2	25,0	24,2	30,4	31,0	30,2
Missioneira	São Luiz Gonzaga	25,4	26,0	25,2	32,1	32,7	31,9
Região dos Grandes Lagos	Pelotas	22,2	23,5	23,2	27,4	28,6	28,4

Source: INMET (2023)

Maximum average temperatures in January in the Baixo Vale do Uruguai region, which had the highest average THI values, exceeded  $35^{\circ}\text{C}$ , with records of  $36^{\circ}\text{C}$  in Itaquí,  $35.7^{\circ}\text{C}$  in Quaraí, and  $35.7^{\circ}\text{C}$  in Maçambará<sup>(28)</sup>. These temperatures are extremely high concerning animal thermal comfort, as the thermoneutral range for cattle is from  $10^{\circ}\text{C}$  to  $27^{\circ}\text{C}$ <sup>(12)</sup>.

On the other hand, the Serra do Nordeste was the only region where, for most of the quarter, there was no thermal stress imposed on the animals, with average THI values  $\leq 70$ . The maximum average temperatures recorded in January in the region ranged from  $29^{\circ}\text{C}$  in Vacaria to  $31.4^{\circ}\text{C}$  in Bento Gonçalves<sup>(28)</sup>. Following the behavior of average and maximum air temperatures, only in the month of January did the maximum average THI value exceed 71.4 (Serra do Nordeste) in all regions, indicating a condition of stress that requires the attention of farmers, especially for lactating cows.

Although extremely high temperatures were recorded during the season, the maximum average THI value in any of the evaluated regions suggested a condition of severe (THI3=79-83) or critical (THI4 =  $\geq 84$ ) thermal stress, putting the animals in a situation of danger to emergency. The maximum average THI value (78.7) occurred in the Baixo Vale do Uruguai region in January, indicating a thermal alert condition, and the minimum (65.6), a non-stressful condition, in the Serra do Nordeste in December 2021 (Table 2).

The regions of Baixo Vale do Uruguai, Alto e Médio Vale do Uruguai, and Missioneira were the only ones that presented minimum average THI values within the discomfort range during the evaluated quarter.

The highest average THI values in December occurred in the Baixo Vale do Uruguai and Alto e Médio Vale do Uruguai regions, but they did not differ from the others, except for the Planalto Médio, Grandes Lagos, Serra do Sudeste, and Serra do Nordeste, where they did not exceed 69.9. The maximum average temperatures recorded in these three regions in December were below 32°C<sup>(3)</sup>.

In January, except for the Serra do Nordeste, all THI values were elevated and within the discomfort range. In seven regions, the values were higher than in the Planalto Médio, Serra do Sudeste, and Serra do Nordeste. In these three regions, the maximum average temperatures ranged from 29°C to a maximum of 34.1°C<sup>(28)</sup>.

In February, on the other hand, the average THI values were also elevated and within the discomfort range in seven regions, which did not occur again in the Planalto Médio, Serra do Sudeste, and Serra do Nordeste. The maximum average air temperatures in February in these three regions were below 31.3°C<sup>(5)</sup>.

Since there are large thermal variations between the minimum and maximum air temperatures that occur in the summer in Rio Grande do Sul and considering that the calculation of THI uses the average values of temperature and relative humidity, it is important to consider the number of hours during the day when lactating cows were exposed to different comfort/discomfort ranges. The average data and standard deviation of the daily number of hours in the four THI classes during the evaluated quarter are shown in Table 4.

**Table 4** Average data and standard deviation of the daily number of hours in four Temperature and Humidity Index (THI) classes during December 2021, and January and February 2022, for ten Ecoclimatic Regions of Rio Grande do Sul, Brazil.

Region	Average daily number of hours			
	THI1	THI2	THI3	THI4
DECEMBER/2021				
Serra do Nordeste	14,8±1,07a/A	6,5±0,9b/B	0,03±0,5c/B	0,0a/A
Planalto Médio	12,5±1,07ab/A	10,7±0,9ab/A	0,7±0,5bc/B	0,0a/A
Serra do Sudeste	15,5±1,08a/A	7,8±1,0ab/B	0,2±0,5c/B	0,0a/A
Encosta Inferior da Serra	10,8±1,31abc/A	11,3±1,2ab/A	1,3±0,6bc/B	0,0a/B
Grandes Lagos	12,9±1,31ab/A	8,6±1,2ab/B	0,16±0,6c/B	0,0a/A



Campanha	10,5±1,08abc/A	11,3±1,0a/A	1,4±0,5bc/B	0,07±0,3a/B
Depressão Central	10,2±1,31abc/A	11,9±1,2a/B	1,2±0,6bc/B	0,0a/B
Missioneira	7,8±1,09bc/A	11,7±1,0a/A	2,8±0,5ab/B	0,1±0,3a/B
Alto e Médio Vale do Uruguai	6,8±1,08bc/A	11,7±1,0a/A	2,9±0,5ab/AB	0,3±0,3a/B
Baixo Vale do Uruguai	7,3±1,07c/A	12,4±0,9a/A	3,9±0,5a/B	0,5±0,3a/C
JANUARY/2022				
Serra do Nordeste	11±1,08a/B	8,5±1,0a/A	1,7±0,5b/A	0,0c/A
Planalto Médio	9,5±1,07ab/B	9,8±1,0a/A	2,7±0,5b/A	0,2±0,3bc/A
Serra do Sudeste	8,5±1,07abc/C	10,9±1,0a/A	2,5±0,5b/A	0,4±0,3bc/A
Encosta Inferior da Serra	5,7±1,31abcd/A	12,3±1,2a/A	3,4±0,6ab/A	1,2±0,4bc/A
Grandes Lagos	5,4±1,31abcd/B	12,7±1,2a/A	2,5±0,6b/A	0,3±0,4bc/A
Campanha	5,2±1,07bcd/B	11,8±1,0a/A	3,7±0,5ab/A	1,0±0,3bc/A
Depressão Central	4,7±1,31bcd/B	12,4±1,2/a/B	3,3±0,6ab/A	1,3±0,4bc/A
Missioneira	4,5±1,07bcd/B	11,8±1,0a/A	3,8±0,5ab/A	1,6±0,3b/A
Alto e Médio Vale do Uruguai	3,4±1,09cd/B	9,7±1,0a/B	3,0±0,5ab/A	1,5±0,3bc/A
Baixo Vale do Uruguai	2,9±1,07d/B	11,5±1,0a/A	5,2±0,5a/A	3,6±0,3a/A
FEBRUARY/2022				
Serra do Nordeste	14,5±1,08a/A	9,0±1,0a/A	0,3±0,5c/B	0,0a/a
Planalto Médio	12,6±1,08abc/A	10,1±1,0a/A	1,2±0,5bc/B	0,0a/a
Serra do Sudeste	12,9±1,08ab/A	10±1,0a/A	0,9±0,5bc/B	0,01±0,3a/a
Encosta Inferior da Serra	9,00±1,33abcd/B	12,4±1,2a/A	2,0±0,6abc/B	0,1±0,4a/b
Grandes Lagos	8,8±1,33abcd/C	13,4±1,2a/A	0,7±0,6bc/B	0,0a/a
Campanha	8,8±1,08bcd/A	12,3±1,0a/A	2,3±0,5abc/B	0,3±0,3a/b
Depressão Central	6,9±1,33cd/B	14,3±1,2a/A	2,3±0,6abc/ab	0,2±0,4a/b
Missioneira	7,2±1,08d/A	11,6±1,0a/A	2,8±0,5ab/b	0,4±0,3a/b
Alto e Médio Vale do Uruguai	6,7±1,12d/A	9,3±1,0a/B	1,9±0,5abc/b	0,7±0,3a/b
Baixo Vale do Uruguai	5,5±1,08d/A	12,3±1,0a/A	4,2±0,5a/b	1,2±0,3a/b

Means followed by the same lowercase letter (region effect) and uppercase letter (month effect) in the column do not differ according to the Tukey HSD test with a 5% probability. TH11 ( $\leq 70$ ), TH12 (71-78), TH13 (79-83), TH14 ( $\geq 84$ ).

In December, non-stressful thermal conditions (TH11  $\leq 70$ ) were registered along the day in a greater number of regions. Seven of them had values above ten hours, while three had between six and eight hours. The longest daily non-stressful periods occurred in the Serra do Sudeste (15.5±1.08h) and Serra do Nordeste (14.8±1.07h), while the shortest was in the Alto e Médio Vale do Uruguai (6.8±1.08h) (Table 4). This result aligns with the THI values recorded in December in their respective ecoclimatic regions (Table 2).

Periods within the discomfort thermal range (TH12=71-79), requiring attention to alert farmers regarding animal thermal comfort, lasted for more than 10 hours in seven regions, with the longest recorded in Baixo Vale do Uruguai (12.4±0.9 h) and the shortest in Serra do Nordeste (6.5±0.9h).

In all regions, brief periods during the day within the severe thermal discomfort range (THI<sub>3</sub>=79-83) were observed. The higher values registered in Baixo Vale do Uruguai, Alto e Médio Vale do Uruguai, and Região Missioneira, which differed from the other regions but did not exceed four hours a day. Emergencies were registered in these three regions, as well as in Campanha, but they lasted for a truly short time, not exceeding one hour.

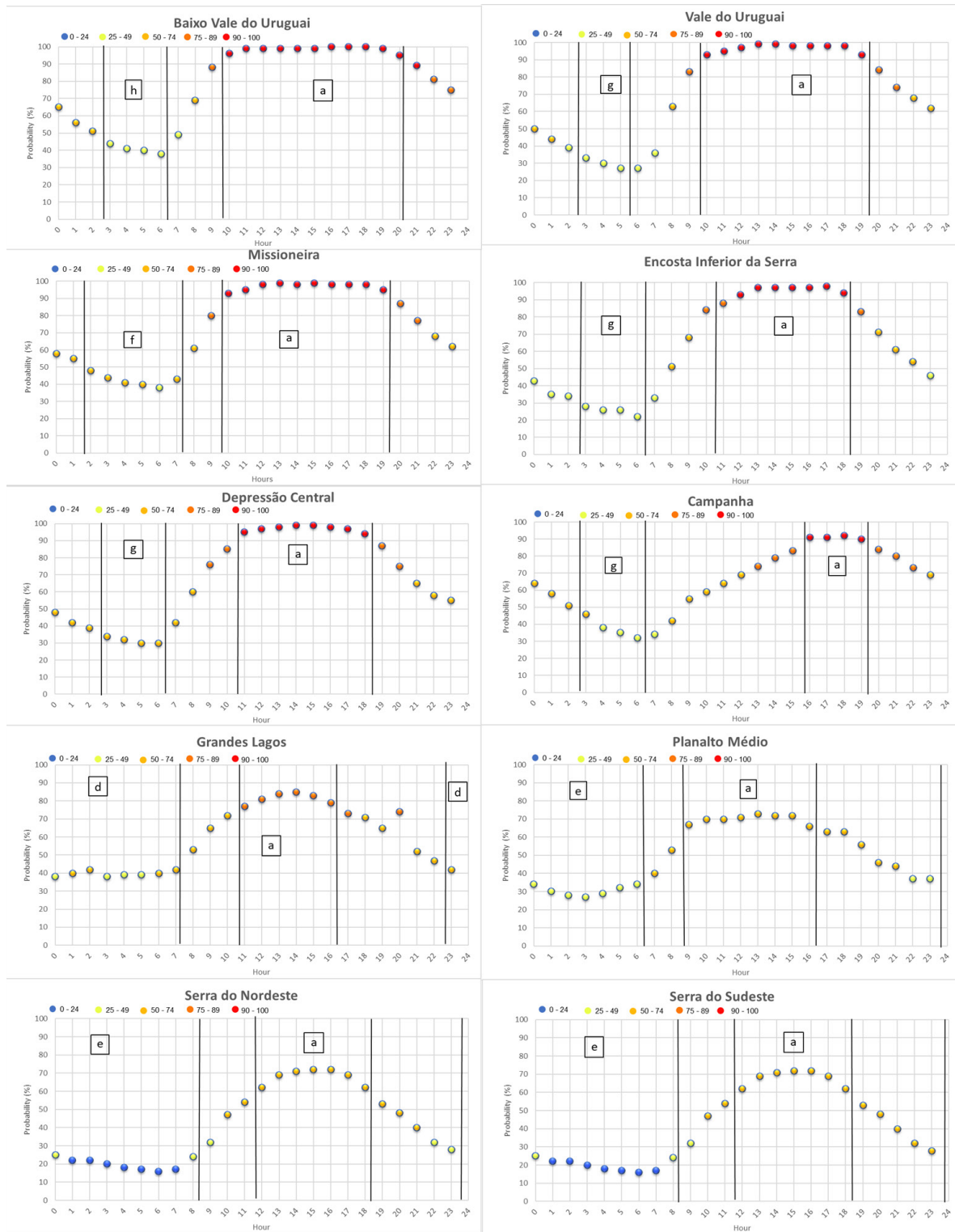
As lactating cows require from three to six nighttime hours within the thermal comfort zone to dissipate heat accumulated during the day, in December, despite instances of thermal stress, the animals probably managed to activate their physiological thermoregulation mechanisms, maintaining their physiological body temperature (38°C to 39°C), which might not have affected milk production.

In January, when the highest THI values were recorded, only in the Serra do Nordeste did the average number of daily hours without thermal stress exceed ten, similar to Planalto Médio and Serra do Sudeste, with a minimum of eight hours. However, the other seven regions experienced periods without thermal comfort lasting less than the required six hours to restore normal body temperature, with an extremely brief period of thermal comfort in Alto e Médio Vale do Uruguai (3.4±1.09h) and Baixo Vale do Uruguai (2.9±1.07h). This month was also when the most hours of severe and critical stress were observed in all regions, except for Serra do Nordeste.

In February, only in three regions, the daily period without thermal stress exceeded 12 hours (Serra do Nordeste and Serra do Sudeste and Planalto Médio; Table 4). Except for Baixo Vale do Uruguai, the other regions experienced periods without stress exceeding six hours, possibly allowing for animal thermoregulation. Severe thermal stress was observed in all regions but for short periods during the day, less than four hours. Emergencies were also recorded in seven regions but for less than one hour.

A study with Holstein-Argentine cows with an average production between 29 and 32 kg day<sup>-1</sup> reported a stressful condition when THI values were higher than 68, with exposure to 8.5±1.09 hours of daily heat stress, using only February to represent the summer season, which aligns with our findings.

When summarizing results in Table 4 for the trimester, all regions showed the most frequent thermal discomfort classification during the day for THI<sub>2</sub>, with values between 71-75. This situation requires farmers' attention in terms of thermal conditioning for lactating cows. January was the most concerning month and, to avoid female heat stress, animals had to be managed to mitigate thermal effects on milk production. Therefore, to assist farmers in making viable and economically sound decisions to manage thermal stress situations, it is essential to determine when during the day there was a higher probability of THI values compatible with stress (i.e., THI >70), according to the classification adopted in this study. The results are presented graphically for each region in Figure 1.



**Figure 1** Probabilities of thermal stress occurrence throughout the day in December 2021, January, and February 2023, in ten ecoclimatic regions of Rio Grande do Sul, Brazil. Different letters in the figure indicate statistically significant differences in higher and lower probability values according to the Bonferroni non-parametric test (5%).

In all regions, there was a probability of thermal stress occurrence during the day in the 2021/22 summer, reaching extremely high values (100% in Baixo Vale do Uruguai) and low values, such as 16% recorded in the Serra do Nordeste and Serra do Sudeste. The time of the day with the highest probabilities of stress occurrence in most regions was between 10:00 AM

and 8:00 PM. Probabilities above 93% were recorded in Baixo Vale do Uruguai, Alto e Médio Vale do Uruguai, Depressão Central, Região Missioneira, and Encosta Inferior da Serra. In these regions, the lowest probabilities were observed between 2:00 AM and 6:00 AM, ranging from 22% at 6:00 AM in Encosta Inferior da Serra to 48% at 2:00 AM in the Região Missioneira.

In the Baixo Vale do Uruguai, the probability of thermal stress was extremely high between 10:00 AM and 8:00 PM, ranging from 95% to 100%. The lowest probability, below 44%, was recorded between 3:00 AM and 6:00 AM. This region also had the highest average, maximum, and minimum THI values, as well as the highest number of daily hours under thermal stress (Table 4), and therefore the highest probability of occurrence, reaching 100% between 4:00 PM and 6:00 PM.

The Alto e Médio Vale do Uruguai region also had high probabilities of stress occurrence, with values between 93% and 99%, during the period from 10:00 AM to 7:00 PM. Just as for Baixo Vale do Uruguai, the lowest probability of animals being in a thermal discomfort zone occurred between 3:00 AM and 6:00 AM (below 33%). A similar pattern was observed in the Região Missioneira, but the lowest probabilities of occurrence, below 48%, corresponded to the period between 2:00 AM and 6:00 AM.

In the Campanha region, the highest probability of thermal stress was observed between 4:00 PM and 7:00 PM, ranging from 90% to 92%. In turn, the lowest probability occurred at 6:00 AM (32%). In the other regions, maximum values of potential occurrence ranged from 85% at 2:00 PM in the Grandes Lagos to 72% in the Serra do Nordeste and Serra do Sudeste at 3:00 PM. These latter regions recorded the lowest probability at 6:00 AM (16%).

Considering the meteorological conditions during the 2021/22 summer in Rio Grande do Sul State, our findings revealed that in all ten ecoclimatic regions of the state, thermal stress occurrence was probable throughout the day to a greater or lesser extent. Comparable results were found in a study of THI geospatialization for Rio Grande do Sul State, which considered two climatological normals (1961-1990 and 1981-2010). The study showed that 100% of the state's territory is under thermal discomfort due to heat during the summer ( $\text{THI} > 74$ ), and up to 27% of the area is under extremely hot environmental conditions ( $\text{THI} > 79$ ) in January. However, this study did not consider the number of daily hours within thermal discomfort ranges<sup>(30)</sup>.

Cattle tend to consume more dry matter after 4:00 PM when temperatures are cooler. However, thermal stress probabilities remained high between 4:00 PM and 11:00 PM, dropping below 50% only in specific regions, including Serra do Nordeste, Serra do Sudeste, Planalto Médio, Grandes Lagos, Depressão Central, and Encosta Inferior da Serra. In this sense, our findings raise concerns about the thermal comfort of lactating cows, particularly during their peak dry matter intake period and evening milking, as the likelihood of thermal stress persists beyond 6:00 PM in numerous regions of the state.

Regionalized milk production loss estimates play a crucial role in providing alerts and assisting technicians and farmers in fine-tuning their management strategies. This specific approach helps mitigate the impact of environmental factors on animal productivity and, in

turn, prevents more substantial economic losses. The climatic conditions in Rio Grande do Sul State during the 2021/22 summer, which are characterized by heatwaves and drought, revealed significant effects ( $P < 0.05$ ) of region, month, and their interaction on the eight milk production levels across all regions assessed in the study.

Tables 5 and 6 present the average data and standard deviation of estimated milk production losses at eight levels across ten ecoclimatic regions in Rio Grande do Sul State. For cows producing between 5 kg and 20 kg per day, average losses ranged from a minimum of  $1.3 \pm 0.07$  kg per day in December, observed in the Encosta Inferior da Serra, to a maximum of  $5.1 \pm 0.2$  kg per day in January, recorded in the Baixo Vale do Uruguai. In the case of cows producing between 25 kg and 40 kg per day, the minimum drop of  $2.7 \pm 0.3$  kg per day was estimated in the Serra do Nordeste, also in December, while the maximum loss of  $9.1 \pm 0.4$  kg per day occurred in January, specifically in the Baixo Vale do Uruguai region.

**Table 5** Estimated average milk production loss (kg per day) at four production levels during December (2021), January (2022), and February (2022) across ten ecoclimatic regions of Rio Grande do Sul State, Brazil.

Region	Milk production level (kg.day <sup>-1</sup> )			
	5	10	15	20
DECEMBER/2021				
Serra do Nordeste	1,4±0,06b/B	1,7±0,1b/B	2,0±0,2b/B	2,3±0,2b/B
Planalto Médio	1,5±0,06ab/B	2,0±0,1ab/B	2,5±0,2ab/B	3,0±0,2ab/B
Serra do Sudeste	1,4±0,06b/B	1,7±0,1b/B	2,0±0,2b/B	2,4±0,2b/B
Encosta Inferior da Serra	1,3±0,07b/B	2,0±0,1ab/B	2,5±0,2ab/B	3,0±0,3ab/B
Grandes Lagos	1,6±0,07bcde/B	1,6±0,1b/C	1,9±0,2b/C	2,1±0,3b/C
Campanha	1,6±0,05ab/B	2,1±0,1ab/B	2,6±0,1ab/B	3,0±0,2ab/B
Depressão Central	1,5±0,07abc/B	2,0±0,1ab/B	2,4±0,2ab/B	3,0±0,3ab/B
Missioneira	1,7±0,06a/A	2,3±0,1a/B	3,0±0,2a/B	3,7±0,2a/B
Alto e Médio Vale do Uruguai	1,7±0,05a/A	2,4±0,1a/B	3,0±0,2a/B	3,7±0,2a/B
Baixo Vale do Uruguai	1,8±0,05a/B	2,5±0,1a/B	3,2±0,2a/B	4,0±0,2a/B
JANUARY/2022				
Serra do Nordeste	1,5±0,06e/A	2,0±0,1e/A	3,0±0,2 e/A	3,0±0,2e/A
Planalto Médio	1,6±0,05cde/A	2,2±0,1cde/A	3,0±0,2cde/A	3,4±0,2cde/A
Serra do Sudeste	1,6±0,05de/A	2,2±0,1de/A	2,7±0,2de/A	3,2±0,2de/A
Encosta Inferior da Serra	1,7±0,07bcde/A	2,4±0,1bcde/A	3,1±0,2bcde/A	4,0±0,3bcde/A
Grandes Lagos	1,6±0,07bcde/C	2,2±0,1bcde/A	2,8±0,2bcde/A	3,3±0,3 bcde/A
Campanha	1,8±0,06abcd/A	2,6±0,1abcd/A	3,3±0,2abcd/A	4,1±0,2 abcd/A
Depressão Central	1,8±0,07bcde/A	2,5±0,1bcde/A	3,2±0,2bcde/A	4,0±,3 bcde/A
Missioneira	1,9±0,05abc/A	2,7±0,1abc/A	3,5±0,2abc/A	4,3±0,2abc/A
Alto e Médio Vale do Uruguai	1,9±0,06ab/B	2,8±0,1ab/A	3,6±0,2ab/A	4,5±0,2ab/A

Baixo Vale do Uruguai	2,1±0,05a/A	3,1±0,1a/A	4,1±0,2a/A	5,1±0,2a/A
FEBRUARY/2022				
Serra do Nordeste	1,5±0,06c/AB	1,9±0,1c/AB	2,3±0,2c/A	2,7±0,2c/AB
Planalto Médio	1,6±0,06abc/AB	2,1±0,1abc/AB	2,6±0,2abc/AB	3,1±0,2abc/AB
Serra do Sudeste	1,5±0,06c/B	1,9±0,1c/A	2,3±0,2c/B	2,7±0,2c/B
Encosta Inferior da Serra	1,6±0,7abc/B	2,2±0,1abc/B	2,8±0,2abc/B	3,3±0,3abc/B
Grandes Lagos	1,5±0,073bc/B	1,9±0,1bc/B	1,9±0,2bc/C	2,7±0,3bc/B
Campanha	1,6±0,06abc/B	2,1±0,1abc/B	2,7±0,2abc/B	3,0±0,2abc/B
Depressão Central	1,6±0,07abc/B	2,2±0,1abc/B	2,8±0,2abc /B	3,3±0,3abc/B
Missioneira	1,7±0,05abc/B	2,3±0,1abc/B	3,0±0,1abc/B	3,6±0,2abc/B
Alto e Médio Vale do Uruguai	1,8±0,05ab/B	2,5±0,1ab/B	3,2±0,2ab/B	4,0±0,2ab/B
Baixo Vale do Uruguai	1,8±0,05a/B	2,5±0,1a/B	3,3±0,2a/B	4,0±0,2a/B

Means followed by the same lowercase letter (region effect) and uppercase letter (month effect) in the column do not differ according to the Tukey HSD test, with a 5% probability.

**Table 6** Estimated average milk production loss (kg per day) at four production levels during December (2021), January (2022), and February (2022) across ten ecoclimatic regions of Rio Grande do Sul State, Brazil.

Region	Milk production level (kg.day <sup>-1</sup> )			
	25	30	35	40
DECEMBER/2021				
Serra do Nordeste	2,7±0,3b/B	3,0±0,3b/B	3,3±0,4c/B	3,6±0,4b/B
Planalto Médio	3,4±0,3ab/B	3,9±0,3ab/B	4,4±0,4ab/B	4,8±0,4ab/B
Serra do Sudeste	2,8±0,3b/B	3,1±0,3b/B	3,4±0,4b/B	3,8±0,4b/B
Encosta Inferior da Serra	3,4±0,3ab/B	3,1±0,4ab/B	4,4±0,5ab/B	4,9±0,5ab/B
Grandes Lagos	2,4±0,3b/C	2,7±0,4b/C	3,0±0,5b/C	3,2±0,5b/C
Campanha	3,6±0,3ab/B	4,0±0,3ab/B	4,5±0,4ab/B	5,0±0,4ab/B
Depressão Central	3,3±0,3ab/B	3,7±0,4ab/B	4,2±0,5ab/B	4,7±0,5ab/B
Missioneira	4,3±0,3a/B	4,9±0,3a/B	5,5±0,4a/B	6,2±0,4a/B
Alto e Médio Vale do Uruguai	4,3±0,3a/B	5,0±0,3a/B	5,6±0,4a/B	6,3±0,4a/B
Baixo Vale do Uruguai	4,7±0,3a/B	5,4±0,3a/B	6,1±0,4a/B	6,8±0,4a/B
JANUARY/2022				
Serra do Nordeste	3,4±0,3e/A	3,9±0,3e/A	4,3±0,4e/A	4,8±0,4e/A
Planalto Médio	4,0±0,3cde/A	4,5±0,3cde/A	5,0±0,4cde/A	5,6±0,4cde/A
Serra do Sudeste	3,8±0,3de/A	4,3±0,3de/A	4,9±0,4de/A	5,4±0,4de/A
Encosta Inferior da Serra	4,5±0,3bcde/A	5,1±0,4bcde/A	5,8±0,5bcde/A	6,5±0,5bcde/A
Grandes Lagos	3,9±0,3bcde/A	4,5±0,4bcde/A	5,0±0,5bcde/A	5,6±0,5bcde/A
Campanha	4,8±,3abcd/A	5,6±0,3abcd/A	6,3±0,4abcd/A	7,1±0,4abcd/A
Depressão Central	4,6±0,3bcde/A	5,3±0,4bcd/A	6,0±0,5bcde/A	6,7±0,5bcde/A
Missioneira	5,1±0,3abc/A	6,0±0,3abc/A	6,8±0,4abc/A	7,5±0,4abc/A
Alto e Médio Vale do Uruguai	5,4±0,3ab/A	6,2±0,3ab/A	7,0±0,4ab/A	7,9±0,4ab/A

Baixo Vale do Uruguai	6,1±0,3a/A	7,1±0,3a/A	8,1±0,4a/A	9,1±0,4a/A
FEBRUARY/2022				
Serra do Nordeste	3,1±0,3c/AB	3,6±0,3c/A	4,0±0,4c/A	4,4±0,4c/B
Planalto Médio	3,6±0,3abc/AB	4,1±0,3abc/AB	4,6±0,4abc/AB	5,1±0,4abc/AB
Serra do Sudeste	3,2±0,3c/B	3,6±0,3c/B	4,0±0,4c/B	4,4±0,4c/B
Encosta Inferior da Serra	4,0±0,3abc/B	4,4±0,4abc/B	4,9±0,5abc/B	5,5±0,5abc/B
Grandes Lagos	3,1±0,3bc/B	3,6±0,4bc/B	4,0±0,5bc/B	4,4±0,5bc/C
Campanha	3,8±0,3abc/B	4,3±0,3abc/B	4,8±0,4abc/B	5,4±0,4abc/B
Depressão Central	4,0±0,3abc/B	4,4±0,4abc/B	5,0±0,5abc/B	5,5±0,5abc/B
Missioneira	4,3±0,3abc/B	4,9±0,3abc/B	5,5±0,4abc/B	6,1±0,4abc/B
Alto e Médio Vale do Uruguai	4,6±0,3ab/B	5,3±0,3ab/B	6,0±0,4ab/B	6,7±0,4ab/B
Baixo Vale do Uruguai	4,7±0,3a/A	5,5±0,3a/B	6,2±0,4a/B	6,9±0,5a/B

Means followed by the same lowercase letter (region effect) and uppercase letter (month effect) in the column do not differ according to the Tukey HSD test, with a 5% probability.

The study indicates that the highest estimates of milk production loss, across most of the eight production levels analyzed, occurred in the month of January. However, in the Planalto Médio and Serra do Sudeste regions, there was no significant difference from the month of February (Tables 5 and 6). These findings align with the pattern of elevated temperatures and THI (Temperature-Humidity Index) values calculated in the ecoclimatic regions. Furthermore, it is worth noting that the high estimates of productivity loss were predominantly observed in cows with higher milk production potential.

When THI values reach or exceed 72, a steeper decline in milk production has been reported in high-production and Holstein cows alike<sup>(31, 32, 33)</sup>. Conversely, for European-origin high-production dairy cows, a decline in milk production began at lower THI values, around 68<sup>(34)</sup>. These findings align with our milk production loss estimates, considering the average THI values calculated for the eight production levels during the 2021/2022 summer in Rio Grande do Sul.

In terms of milk production, Holstein cows raised in the central region of Arizona/USA (hot and dry climate), with the critical minimum, mean, and maximum THI values of 64, 72, and 76, respectively, reduced their production between 11.5 to 16.0 kg daily during the summer months compared to periods of milder temperatures<sup>(35)</sup>. Some reports have indicated that for THI values of 70 or lower, dairy cows show almost no thermal discomfort, although at 75 or higher, milk production and feed intake are seriously affected<sup>(36)</sup>.

A study conducted with crossbred Holstein Zebu cows in waiting rooms in Piauí (Brazil) identified that milk production decreased by 2.46 kg in cows that were exposed to two hours of solar radiation with a THI of 73 when compared to other environments (THI below 69). Physiological parameters for this group of animals indicated that they were outside the thermoneutral zone<sup>(37)</sup>.

Feed intake reductions in milking cows in dairy cattle are reported to occur regardless of their production stage when subjected to challenging environments, compromising the efficiency of nutrient utilization from the diet<sup>(38)</sup>. Some authors indicated that voluntary

feed intake reductions have been the main reason for decreases in milk production in cows subjected to heat stress<sup>(39, 40)</sup>.

Indeed, when the temperature remains above 30°C for an extended period, exceeding 6 hours, a cow producing 27 kg of milk per day can experience a substantial reduction of up to 4 kg in daily milk production, resulting in significant losses for the producer<sup>(41)</sup>. As temperatures approach 25.5°C, cows encounter challenges in dissipating excess heat, which can lead to a decrease in feed intake<sup>(42,43)</sup>. This decrease in feed consumption can, in turn, result in lower milk fat content and an increased likelihood of digestive disorders<sup>(44)</sup>. To mitigate daily heat production when the environmental temperature rises to 35°C, an increase in water intake is expected. However, temperatures beyond this range can lead to a depression in water consumption, reduced physical activity, decreased rumination time, an elevated respiratory rate, and a decrease in feed intake by as much as 30%<sup>(44)</sup>.

Stressed animals exposed to an average temperature of 38°C exhibited a significant reduction of 49% in total dry matter digestibility and a 55% decrease in crude protein digestibility compared to animals kept in thermal comfort conditions, where the environment maintained an average temperature of 21°C throughout the entire experimental period<sup>(45)</sup>.

THI values recorded during the spring and summer seasons in three regions of Croatia frequently exceeded 72, highlighting the susceptibility of cows to heat stress in these areas. Significant differences in milk production ( $p < 0.01$ ) between periods with and without stress have been reported, underscoring that production in regions with varying microclimates can be significantly affected when THI levels reach stressful thresholds<sup>(46)</sup>.

A study investigating the impact of heat stress on different cattle breeds has found that for each one-unit increase in THI, milk production decreased by 0.69 kg in Holstein cows and 0.45 kg in Jersey cows<sup>(47)</sup>. This suggests that selection for higher milk production leads to a more substantial negative impact from heat stress<sup>(47)</sup>. Furthermore, an experiment conducted with Holstein cows, which assessed the influence of the season on milk production, revealed a significant decrease of 10% to 40% during the summer months compared to the winter<sup>(48)</sup>, highlighting the substantial seasonal variations in milk production due to heat stress.

This study highlights that the Baixo Vale do Uruguai ecoclimatic region, encompassing municipalities such as Itaqui, São Borja, and Maçambará, experienced significant heat stress conditions, especially in January. These conditions had the potential to adversely affect dairy productivity. While this region is primarily known for beef cattle production, dairy producers should remain vigilant about the environmental challenges their animals face, especially those with high milk production levels during the summer season. These recommendations should also be extended to the Alto e Médio Vale do Uruguai and Missioneira regions. Since the former plays a substantial role in dairy production in the state, it is highly advisable to implement management measures aimed at ensuring the thermal comfort of animals during the summer, thus preventing significant losses in productivity.

In broad terms, during the summer of 2021/2022, the Planalto Médio, Serra do Nordeste, and Serra do Sudeste regions generally offered thermal comfort conditions or



the ability for animals to dissipate the excess body heat they produced throughout the day. In these regions, the recorded THI values during the 2021/2022 summer were generally the lowest in terms of average, maximum, and minimum values. Additionally, the daily periods within the thermal discomfort range were shorter, and estimated milk production losses were comparatively lower. It is noteworthy that these regions also have the highest average altitudes among the municipalities evaluated in this study, with altitudes of 575m, 775m, and 399m, as shown in Table 2.

The observation above aligns with the bioclimatic zoning conducted in the state of Rio Grande do Sul, which identified very to extremely hot environmental conditions during the spring/summer period, with greater severity in lower-altitude regions<sup>(49)</sup>. Furthermore, a study conducted between the summers of 2000 and 2020 identified three THI classes in Rio Grande do Sul, with higher-altitude regions, such as parts of the Northeast and Northwest mesoregions, having lower THI values (between 68 and 66), while lower-altitude regions, bordering Argentina and the coast, had higher THI values ( $\text{THI} > 72$ )<sup>(50)</sup>. As mentioned by some authors and consistent with our findings, a  $\text{THI} > 70$ , which is prevalent in most of Rio Grande do Sul's territory, indicates conditions ranging from thermal discomfort to heat stress. These conditions should be assessed for each animal category, as highlighted in previous studies<sup>(41, 51, 52)</sup>.

Management strategies aimed at minimizing thermal stress situations are crucial to prevent reproductive and productive performance losses. As indicated by the results presented so far, thermal stress conditions were observed throughout the day, particularly in January. This necessitates the implementation of measures to restore thermal comfort for the animals. These measures focus on effectively controlling the environment, utilizing natural and artificial mechanisms to enhance heat dissipation from the animals' bodies. Among these, key strategies include increasing air movement, wetting the animal's surface, evaporative air cooling (using systems like fans, misters, and evaporative panels), providing shade to mitigate the direct effects of solar radiation<sup>(53, 54, 55)</sup>, and adjusting diets to reduce caloric intake<sup>(13)</sup>.

When selecting a practice to be implemented on the farm, it is essential to consider the animals' needs, the environmental impact of the chosen technologies, the level of property management, available capital, and the cost-benefit relationship of the selected technology<sup>(56)</sup>.

In this sense, raising dairy cattle under Integrated Crop-Livestock-Forest Systems (ICLFS) represents an economically attractive and sustainable option. It provides shade for the animals and enhances productivity by creating a more favorable microclimate, which benefits heat exchange and maintains thermal comfort<sup>(57)</sup>. Studies have shown an increase in milk production ranging from 9.7%<sup>(58)</sup> to 15%<sup>(59, 60)</sup>, with conception rates improving by up to 20%. Additionally, there has been a reduction in the number of services per conception by as much as 50% in ICLFS systems in Brazil.

Furthermore, the pursuit of crossbreeding between breeds that can maintain a high standard of production and immunological status, even in challenging environmental conditions with extremely hot summers, is an alternative to mitigate the negative impacts of climate on milk production<sup>(61)</sup>.

## Conclusion

The Baixo Vale do Uruguai region stood out with the highest average THI values falling within the thermal discomfort range for three consecutive months, while the Serra do Nordeste region did not indicate heat stress. Throughout the quarter, the classification range for thermal discomfort was frequent in all regions, ranging from attention to alert levels for farmers regarding animal thermal comfort daily. During the assessed months, periods of heat stress were observed throughout the day, with January being particularly noteworthy due to the highest daily hours spent in severe and critical heat stress situations in all regions except for the Serra do Nordeste. In all regions of the state of Rio Grande do Sul, during the summer of 2021/22, there was a probability of heat stress occurring throughout the day, reaching extremely high values in the Baixo Vale do Uruguai and low values in the Serra do Nordeste and Serra do Sudeste. The highest milk production reductions for most of the eight levels considered in this study were recorded in January, except for the Planalto Médio and Serra do Sudeste regions, following the pattern of elevated temperatures and THI values calculated in the ecoclimatic regions. Moreover, high estimates of productivity loss occurred in cows with higher milk production potential.

## Conflict of Interest Statement

The authors declare no conflicts of interest.

## Authors' contributions

Ivone Fatima Tazzo: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Management, Supervision, Validation, Visualization, Original Drafting, Writing (Review and Editing);

Adriana Kroef Tarouco: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Original Drafting, Writing (Review and Editing);

Paulo Henrique Correia Allen Junior: Data Curation, Original Drafting;

Carolina Bremm: Análise formal, Methodology;

Loana Silveira Cardoso: Original Drafting, Writing (Review and Editing);

Amanda Heemann Junges: Original Drafting, Writing (Review and Editing).

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## References

1. Rio Grande do Sul. Secretaria da Agricultura, Pecuária e Agronegócio. Fundação Estadual de Pesquisa Agropecuária. Atlas climático do Rio Grande do Sul. Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação. 2011. Disponível em: <https://www.agricultura.rs.gov.br/agrometeorologia>. Acesso em: 08 abr. 2023.
2. Instituto Nacional de Meteorologia - INMET. Estado do Clima no Brasil em 2022. Edição Digital. Brasília 2023. Disponível em: <https://portal.inmet.gov.br/uploads/notastecnicas/Estado-do-clima-no-Brasil-em-2022-OFICIAL.pdf#page=1&zoom=auto,-99,842> Acesso em: 06 dez. 2023
3. Junges, AH, Tazzo, IF, Cardoso, LS, Cera, JC. Avaliação da onda de calor ocorrida em janeiro de 2022 no Rio Grande do Sul. *Agrometeoros*. 2022. Nov 3;30. DOI: <http://dx.doi.org/10.31062/agrom.v30>.
4. Junges AH, Varone F, Cardoso LS, Tazzo IF. Condições meteorológicas ocorridas em dezembro de 2021 e situação das principais culturas agrícolas no estado do Rio Grande do Sul. *Comunicado Agrometeorológico*. 2021.

Dez. 33, 6–26. Disponível em: <https://www.agricultura.rs.gov.br/agrometeorologia>. Acesso em: 08 abr. 2023.

5. Tazzo IF, Varone F, Cardoso LS, Junges AH. Condições meteorológicas ocorridas em fevereiro de 2022 e situação das principais culturas agrícolas no Estado do Rio Grande do Sul. Comunicado Agrometeorológico. 2022. Fev. 35, 6–26. Disponível em: <https://www.agricultura.rs.gov.br/agrometeorologia>. Acesso em: 08 abr. 2023.

6. Bond TE, Kelly CF, Ittner NR. Radiation studies of pointed shade materials. *Agricultural Engineering*. 1954. 35(6):389–92.

7. Rhoads ML, Rhoads RP, VanBaale MJ, Collier RJ, Sanders SR, Weber WJ, et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *Journal of Dairy Science*. 2009. May;92(5):1986–97.

8. Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH. Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science*. 2010. Feb 93(2):644–55. DOI: <https://doi.org/10.3168/jds.2009-2295>

9. Lage CFA, Malacco VMR, Lombardi MCL, Reis RB, Coelho SG. Efeitos do estresse térmico materno no final do período seco e lactação: estresse calórico, saúde, desenvolvimento, produção de leite, composição do leite. *Nutritime Revista Eletrônica*. 2018. Feb, 15(1):8097–106. Disponível em: <https://www.nutritime.com.br/wp-content/uploads/2020/02/Artigo-459.pdf>. Acesso em: 15 jan. 2023.

10. Perissinoto M. Sistemas de climatização em galpões tipo “freestall” para confinamento de gado leiteiro[Dissertação]. [Escola Superior de Agricultura “Luiz de Queiros, Universidade de São Paulo]; 2003. p. 140. Disponível em: <https://www.teses.usp.br/teses/disponiveis/11/11131/tde-16042004-161110/publico/mauricio>. Acesso em: 20 fev. 2023.

11. Sousa Júnior SC de, Morais DAEF, Vasconcelos Ângela M de, Nery KM, Morais JHG, Guilhermino MM. Características Termorreguladoras de Caprinos, Ovinos e Bovinos em Diferentes Épocas do Ano em Região Semi-Árida. *Rev. Cient. Prod. Anim.* [Internet]. 7º de setembro de 2010 [citado 24º de janeiro de 2024];10(2). Disponível em: <https://periodicos.ufpb.br/ojs/index.php/rcpa/article/view/42731>Acesso em: 20 fev. 2023.

12. Baêta FC, Souza CF. *Ambiência em Edificações Rurais: conforto térmico animal*. Viçosa: Editora UFV; 1997.

13. Azevêdo DMMR, Alves AA. Bioclimatologia aplicada à produção de bovinos leiteiros nos trópicos. Teresina: Embrapa Meio-Norte; 2009. Disponível em: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/664507/bioclimatologia-aplicada-a-producao-de-bovinos-leiteiros-nos-tropicos>. Acesso em: 20 fev 2023.

14. Kadzere CT, Murphy MR, Silanikove N, Maltz E. Heat stress in lactating dairy cows: a review. *Livestock Production Science*. 2002. Oct, 77(1):59–91. DOI: [https://doi.org/10.1016/S0301-6226\(01\)00330-X](https://doi.org/10.1016/S0301-6226(01)00330-X).

15. Coppock CE, West JW. Nutritional adjustments to reduce heat stress in lactating dairy cows. In: *Proc Georgia Nutrition Conference for the Feed Industry*. Atlanta; 1986.

16. Silanikove N. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science*. 2000. Dec, 67(1-2):1–18. DOI: [https://doi.org/10.1016/S0301-6226\(00\)00162-7](https://doi.org/10.1016/S0301-6226(00)00162-7).

17. Hahn GL. Dynamic responses of cattle to thermal heat loads. *Journal of Animal Science*. 1999. Feb, 77(suppl\_2):10–20. DOI: [https://doi.org/10.2527/1997.77suppl\\_210x](https://doi.org/10.2527/1997.77suppl_210x).

18. Zang CJ. Effect of dietary cation-anion balance on production performance and blood biochemistry indicators of dairy cows in the condition of heat stress. Xinjiang: Xinjiang Agricultural University; 2008.

19. Thom EC. The discomfort index. *Journal of Animal Science*. Washington: Eatherwise; 1958.

20. Buffington DE, Collazo-Arocho A, Canton GH, Pitt D. Black globe-humidity index (BGHI) as comfort equation for dairy cows. *Transactions of the ASAE*. 1981. 24(3):711–4.

21. Melo A, Moreira JM, Ataídes DS, Guimarães RAM, Loiola JL, Sardinha HC. Efeitos do estresse térmico na produção de vacas leiteiras: Revisão. *Pubvet* [Internet]. 28º de setembro de 2016 [citado 24º de janeiro de 2024];10(10). Disponível em: <https://ojs.pubvet.com.br/index.php/revista/article/view/1414>.

22. Junges AH. Caracterização climática da temperatura do ar em Veranópolis, Rio Grande do Sul. *Agrometeoros*.

2020. Mar, 3, 26(2):299–306. DOI: <http://dx.doi.org/10.31062/agrom.v26i2.26411>.

23. Berlatto MA, Cordeiro APA. Sinais de mudanças climáticas globais e regionais, projeções para o século XXI e as tendências observadas no Rio Grande do Sul: uma revisão. *Agrometeoros*. 2017. 25(2):273–302. Disponível em: <https://seer.sct.embrapa.br/index.php/agrometeoros/article/view/25884/14084>. Acesso em: 15 jan. 2023.

24. Tazzo IF, Tarouco AK, Cardoso LS, Junges AH, Allem Junior PHC, Pinto GM, da Silva Y. Biometeorologia aplicada à bovinocultura de leite no Rio Grande do Sul: condições meteorológicas, índice de temperatura e umidade (conforto térmico) e estimativa de efeitos na produção de leite no inverno de 2022. *Comunicado Agrometeorológico* 44. 2022. Jan, 6-37. Available from: <https://www.agricultura.rs.gov.br/agrometeorologia>. Acesso em: 08 abr. 2023.

25. Rosenberg LJ, Biad BL, Verns SB. Human and animal biometeorology. In: *Microclimate: the biological environment*. New York: Wiley- Interscience Publication; 1983. p. 423–67.

26. Berry IL, Shanklin MD, Johnson HD. Dairy Shelter Design Based on Milk Production Decline as Affected by Temperature and Humidity. *Transactions of the ASAE*. 1964. 7(3):0329–31.

27. Hahn GL. *Bioclimatologia e instalações zootécnicas: aspectos teóricos e aplicações*. Jaboticabal: FUNEP; 1993.

28. Cardoso LS, Varone F, Junges AH, Tazzo IF. Condições meteorológicas ocorridas em janeiro de 2022 e situação das principais culturas agrícolas no estado do Rio Grande do Sul. *Comunicado Agrometeorológico* 34, 2022. Jan. 6–29. Disponível em: <https://www.agricultura.rs.gov.br/agrometeorologia>. Acesso em: 08 abr. 2023.

29. Lendez PA, Cuesta LM, Farias ME, Vater AM, Ghezzi MD, Mota-Rojas D, et al. Alterations in TNF- $\alpha$  and its receptors expression in cows undergoing heat stress. *Veterinary Immunology and Immunopathology*. 2021. Maio, 1, 235(235):110232–2. DOI: <https://doi.org/10.1016/j.vetimm.2021.110232>.

30. Boff de Oliveira Z, Bottega EL, Boff de Oliveira M, Moares da Silva C, Link TT. Análise do conforto térmico no Estado do Rio Grande do Sul utilizando técnicas Geoestatísticas e dados das normais climatológicas. *Engenharia na Agricultura*. 2019. Jun. 19, 27(3):195–203. DOI: <https://doi.org/10.13083/reveng.v27i3.935>.

31. Johnson H. Environmental management of cattle to minimize the stress of climatic change. *Internacional Journal of Biometeorology*. 1980. 24(1):65–78.

32. Bertocelli P, Martin T, Ziech MF, Paris W, Cella P. Conforto térmico alterando a produção leiteira. *Enciclopédia Biosfera*. 2013. Dez. 1, 9(17):762–77. Disponível em: <https://conhecer.org.br/ojs/index.php/biosfera/article/view/3061>. Acesso em: 15 mar. 2023.

33. Martello LS. *Interação animal: ambiente: efeito do ambiente climático sobre as respostas fisiológicas e produtivas de vacas holandesas em freestall [tese]*. [Pirassununga (SP) Universidade de São Paulo] 2006. Disponível em: <https://www.teses.usp.br/teses/disponiveis/74/74131/tde-05102006-091637/pt-br.php>. Acesso em: 15 mar. 2023.

34. Dalcin VC. *Parâmetros fisiológicos em bovinos leiteiros submetidos ao estresse térmico [Dissertação]*. [Universidade Federal do Rio Grande do Sul. Faculdade de Agronomia. Programa de Pós-Graduação em Zootecnia.]; 2013. 1–49. Disponível em: <https://lume.ufrgs.br/handle/10183/89729>. Acesso em: 22 mar. 2023.

35. Igono MO, Bjrtvedt G, Sanford-Crane HT. Environmental profile and critical temperature effects on milk production of Holsteins cows in desert climate. *International Journal of Biometeorology*. 1992. 36:77–87.

36. Oliveira E, Delgado R, Rosa S, Souza PJ, Neves L. Efeitos do estresse térmico sobre a produção de bovinos de leite no município de MARILÂNDIA- ES . *Enciclopédia Biosfera*. 2023. Mar, 9 9(16). Disponível em: <https://conhecer.org.br/ojs/index.php/biosfera/article/view/3417>. Acesso em: 15 mar. 2023.

37. Dias e Dias TP, Oliveira RG, Sousa Junior SC, Santos KR. Efeito da exposição à radiação solar sobre parâmetros fisiológicos e estimativa do declínio na produção de leite de vacas mestiças (Holandês X Gir) no sul do estado do Piauí. *Comunicata Scientiae*. 2012. 3(4):299–305.

38. BACCARI JÚNIOR, F. *Manejo ambiental da vaca leiteira em climas quentes*. Londrina: Editora da Universidade Estadual de Londrina, 2001. 142p.

39. Chen KH, Huber JT, Theurer CB, Armstrong DV, Wanderley RC, Simas JM, et al. Effect of protein quality and evaporative cooling on lactational performance of Holstein cows in hot weather. *Journal of Dairy Science*. 1993;76(3):819–25.

40. McDowell RE, Hooven NW, Camoens JK. Effect of Climate on Performance of Holsteins in First Lactation. *Journal of Dairy Science*. 1976. Maio. 59(5):965–71. DOI: <https://doi.org/10.3168/JDS.S0022-0302%2876%2984305-6>.
41. de Souza BB, Silva IJ de O, Mellace EM, Santos RFS, Zotti CA, Garcia PR. Avaliação do ambiente físico promovido pelo sombreamento sobre o processo termorregulatório em novilhas leiteiras. *Agropecuária Científica no Semi-Árido*. 2010. Out. 13 ,6(2). DOI: <https://doi.org/10.30969/acsa.v6i2.69>.
42. da Cruz LV, Angrimani DSR, Rui BR, da Silva MA. Efeitos do estresse térmico na produção leiteira: revisão de literatura. *Revista Científica Eletrônica de Medicina Veterinária*. 2011. 9(16). Disponível em: [http://faef.revista.inf.br/imagens\\_arquivos/arquivos\\_destaque/3Kbw8tpmlajpspv\\_2013-6-26-10-55-41.pdf](http://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/3Kbw8tpmlajpspv_2013-6-26-10-55-41.pdf). Acesso em: 15 mar. 2023.
43. Dash S, Chakravarty AK, Singh A, Upadhyay A, Singh M, Yousuf S. Effect of heat stress on reproductive performances of dairy cattle and buffaloes: A review. *Veterinary World*. 2016. Mar, 9(3):235–44. DOI: <https://doi.org/10.14202%2Fvetworld.2016.235-244>.
44. Silva JCPM, et al. Bem-estar do gado leiteiro. 1st ed. Viçosa: Aprenda Fácil; 2012.
45. Passini R, Ferreira FA, Borgatti LMO, et al. Estresse térmico sobre a seleção da dieta por bovinos. *Acta Scientiarum Animal Sciences*. 2009;31(3):303–9.
46. Gantner V, Mij P, Kuterovac K, Solić D, Gantner R. Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo*. 2011;61(1):56–63.
47. West JW. Nutritional strategies for managing the heat-stressed dairy cow. *Journal of Animal Science*. 1999. 77(suppl\_2):21–35. DOI: [https://doi.org/10.2527/1997.77suppl\\_221x](https://doi.org/10.2527/1997.77suppl_221x).
48. Du Preez JH, Hattingh PJ, Giesecke WH, Eisenberg BE. Heat stress in dairy cattle and other livestock under southern African conditions. III. Monthly temperature-humidity index mean values and their significance in the performance of dairy cattle. *Onderstepoort J Vet Res*. 1990 Dez;57(4):243–8.
49. de Oliveira ZB, Bottega EL, Silva CM, Rodrigues LR, Knies AE. Zoneamento bioclimático do Estado do Rio Grande do Sul para o conforto térmico de animais e do trabalhador rural. *Ambiência*. 2017. 13(2):423–38. Disponível em: <https://revistas.unicentro.br/index.php/ambiencia/article/view/4688>. Acesso em: 18 jan. 2023.
50. Boff de Oliveira Z, Bottega EL, Boff de Oliveira M, Moares da Silva C, Link TT. Análise do conforto térmico no Estado do Rio Grande do Sul utilizando técnicas geoestatísticas e dados das normais climatológicas. *Revista Engenharia na Agricultura - REVENG*. 2019. Jun, 19, 27(3):195–203. DOI: <https://doi.org/10.13083/reveng.v27i3.935>.
51. Mendes AM de P, de Azevedo M, Lopes PMO, Moura GB de A. Zoneamento bioclimático para a raça ovina Dorper no Estado de Pernambuco. *Pesquisa Agropecuária Brasileira*. 2014. Dez. 1 ,49(12):986–93. DOI: <https://doi.org/10.1590/S0100-204X2014001200009>.
52. Silva Júnior JLC. Zoneamento da região sudeste do Brasil, utilizando o índice de temperatura e umidade, para o gado leiteiro [Tese]. [Universidade Federal de Viçosa. Programa de Pós – Graduação Meteorologia Agrícola]; 2001. p. 73. Disponível em: <https://www.locus.ufv.br/handle/123456789/8134>. Acesso em: 12 fev. 2023.
53. Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL, Collier RJ. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal*. 2011. Dez. 8 6(05):707–28. Disponível em: [www.sciencedirect.com/science/article/pii/S1751731111002448?via%3Dihub](http://www.sciencedirect.com/science/article/pii/S1751731111002448?via%3Dihub). Acesso em: 22 fev. 2023. DOI: <https://doi.org/10.1017/S1751731111002448>.
54. West JW. Effects of Heat-Stress on Production in Dairy Cattle. *Journal of Dairy Science*. 2003. Jun. 86(6):2131–44. DOI: [https://doi.org/10.3168/jds.S0022-0302\(03\)73803-X](https://doi.org/10.3168/jds.S0022-0302(03)73803-X).
55. Pennington JA, Vandevender K. Heat Stress in Dairy Cattle – DAIRExNET. 2004. Disponível em: <https://dairy-cattle.extension.org/heat-stress-in-dairy-cattle>. Acesso em: 12 fev. 2023.
56. Pires M de FA, Campos AT. Modificações ambientais para reduzir o estresse calórico em gado de leite. Comunicado Técnico 42. Juiz de Fora: Embrapa Gado de Leite; 2004. Dez. p. 6. Disponível em: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/65410/1/COT-42-Modificacoes-ambientais.pdf>. Acesso em: 05 mai. 2023.
57. Giro A, Pezzopane JRM, Barioni Junior W, Pedroso A de F, Lemes AP, Botta D, et al. Behavior and body surface temperature of beef cattle in integrated crop-livestock systems with or without tree shading. *Science of The Total Environment*. 2019. Set. 20, 684(684):587–96. DOI: <https://doi.org/10.1016/j.scitotenv.2019.05.377>.

58. Paciullo DSC, Pires MFA, Aroeira LJM, Morenz MJF, Maurício RM, Gomide CAM, et al. Sward characteristics and performance of dairy cows in organic grass-legume pastures shaded by tropical trees. *Animal*. 2014. 8(8): 1264–71. DOI: <https://doi.org/10.1017/S1751731114000767>.
59. Pires MFA, Paciullo DSC, Pires JAA. Conforto animal no sistema integração lavoura-pecuária-floresta. Informe Agropecuário. Belo Horizonte, Minas Gerais: Epamig; 2010. p. 91–8. Disponível em: [http://alerta.cpac.embrapa.br/publicacoes/2011/alerta20022011/informe\\_agropecuario\\_31\\_257\\_2010.pdf](http://alerta.cpac.embrapa.br/publicacoes/2011/alerta20022011/informe_agropecuario_31_257_2010.pdf). Acesso em: 16 mai. 2023.
60. Ricci GD, Orsi AM, Domingues PF. ESTRESSE CALÓRICO E SUAS INTERFERÊNCIAS NO CICLO DE PRODUÇÃO DE VACAS DE LEITE- REVISÃO. *RVZ* [Internet]. 2º de agosto de 2022 [citado 24º de janeiro de 2024];20(3):381-90. Disponível em: <https://rvz.emnuvens.com.br/rvz/article/view/1028> Acesso em: 12 fev. 2023.
61. Buckley F, Lopez-Villalobos N, Heins BJ. Crossbreeding: implications for dairy cow fertility and survival. *Animal*. 2014. 8(Suppl 1):122–33. DOI: <https://doi.org/10.1017/S1751731114000901>.