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ORIGINAL ARTICLE

Use of the milk composition stress-comfort ratio to monitor heat stress in cows in tropical regions[1]

Uso da razão estresse-conforto na composição do leite para monitorar o estresse por calor em vacas em regiões tropicais

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HIGHLIGHTS:

Maximum temperature and humidity index greater than 76 reduces the fat content of milk from crossbred dairy cows.
Milk fat content declined during the hottest months, with stress-to-comfort (S:C) ratios below 1.0.
The S:C ratio can be used by growers in tropical regions to monitor heat stress.

ABSTRACT: This study aimed to use the daily maximum temperature and humidity index (THImax) to calculate the ratio between average milk composition in the three hottest (stress) and three mildest months (comfort) of the year in an important milk-producing region in Brazil, with potential practical application in other tropical regions. The study was conducted in the municipalities of Araxá, Sacramento, and Uberaba in Minas Gerais State, from 2017 to 2020. THImax was calculated using daily maximum temperature and minimum relative humidity data. The three months with the largest number of days with THImax ≥ 76 were classified as the stress period, and the three months with THImax < 76 as the comfort period. Average fat, protein, and lactose content, total solids, and non-fat solids were calculated for both periods using 10,063 milk composition analyses, and the stress-to-comfort (S:C) ratio of the milk components was determined. Fat content declined during the stress period, with an S:C ratio below 1.0. Most ratios were also below 1.0 for protein and total solids, while values for lactose and non-fat solids showed minimal deviation from 1.0. THImax values ≥76 significantly alter the milk composition of crossbred dairy cows in tropical regions, largely by decreasing the fat content, resulting in an S:C ratio below 1. Determining the milk composition ratio between the hottest and mildest months enables the identification of thermal discomfort in crossbred cows in tropical regions and provides a practical tool for dairy producers to monitor heat stress.

Key words: animal welfare, dairy cows, milk quality, temperature, tropical cattle

RESUMO: Objetivou-se calcular a razão da composição média do leite entre os três meses mais quentes (Estresse) e os três meses mais amenos (Conforto) baseado no THImax diário em uma importante região produtora de leite, Minas Gerais, Brazil, o que pode servir para aplicação prática em outras regiões tropicais. Este estudo foi realizado nas regiões de Araxá, Sacramento e Uberaba, Minas Gerais, Brasil de 2017 a 2020. Calculou-se o ITUmax com dados diários de temperatura máxima e umidade relativa mínima. Os três meses com maior número de dias com ITUmax ≥76 foi considerado estresse e os três com ITU <76 como conforto. Depois, calculou-se as médias de gordura, proteína, lactose, sólidos totais e sólidos não gordurosos de leite nos períodos estresse e conforto utilizando 10.063 análises. Depois, calculou-se a razão Estresse/Conforto dos componentes do leite. Houve redução no teor de gordura durante o estresse, com razão Estresse/Conforto abaixo de 1,0, enquanto a maioria das razões para proteína e sólidos totais também ficou abaixo de 1,0. Para lactose e sólidos não gordurosos os valores diferiram pouco de 1,0. Valores de ITUmax ≥ 76 provocam alterações na composição do leite de vacas mestiças leiteiras em regiões tropicais, principalmente com redução no teor de gordura, gerando uma relação Estresse/Conforto menor que 1. A determinação da relação entre a composição média do leite nos três meses mais quentes e nos mais amenos permite identificar o desconforto térmico de vacas mestiças em regiões tropicais e pode ser utilizado pelos produtores de leite para monitorar o estresse térmico.

Palavras-chave: bem-estar animal, vacas leiteiras, qualidade do leite, temperatura, bovinos tropicais

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INTRODUCTION

The frequency, duration and severity of heat stress have intensified with climate change and prompted research on thermal comfort in cattle (Giannone et al., 2023). Dairy cows exposed to high temperatures may exhibit hyperthermia, reduced feed intake, and endocrine changes, among others, which can compromise milk production, including the synthesis of milk components (Bernabucci et al., 2015; Moore et al., 2023).

There are different environmental indices for assessing thermal discomfort, the temperature and humidity index (THI) being the most widely used given the ease in measuring air temperature and humidity (Wankar et al., 2021; Giannone et al., 2023). In temperate regions, the ratio between performance in summer and winter (S:W) has also been used to assess heat stress (Flamenbaum & Galon, 2010). However, calculating S:W in tropical regions can lead to errors since the seasons are not well defined. As such, a more appropriate strategy in the tropics is to divide the performance in the quarter of the year with the largest number of heat stress days (stress, S) divided by the performance in the quarter with the highest frequency of thermal comfort days (Comfort, C).

Comparing milk components between warm and mild periods may encourage producers to adopt strategies that mitigate the harmful effects of high temperatures and monitor the efficiency of existing measures (Flamenbaum & Galon, 2010). With the use of adequate mitigating measures, the producer may receive monetary bonuses on the sale of milk, considering that most Brazilian companies that process milk and dairy products adopt payment programs based on their composition, particularly fat and protein percentages. Nevertheless, few studies investigate the applicability of S:W in monitoring heat stress in tropical regions and the influence of the ratio on milk composition, especially in crossbred cows, which account for an important portion of dairy animals in Brazil.

Our hypothesis is that the ratio between milk composition in the hottest (S) and mildest (C) months will be less than 1, which will negatively affect milk component percentages. Thus, the aim was to use the daily maximum THI (THImax) to calculate the ratio between average milk composition in the three hottest (S) and three mildest months (C) of the year in Minas Gerais State (MG), Brazil, an important dairy milk producing region, with practical application in other tropical regions.

MATERIAL AND METHODS

The study was carried out from 2017 to 2020 in the municipalities of Araxá (19° 35’ 36” S, 46° 56’ 27” W, 1008 m.a.s.l.), Sacramento (19° 51’ 55” S, 47° 26” 47” W, 832 m.a.s.l.) and Uberaba (19° 45’ 27” S, 47° 55’ 36” W, 752 m.a.s.l.), in the state of Minas Gerais, Brazil. The climate in these municipalities is classified as Cwa (warm subtropical with dry winters), typical of southeastern Brazil, with a dry season from April to September and a rainy season from October to March (Alvares et al., 2013).

Daily maximum air temperature (Tmax; °C) and minimum relative humidity (RHmin; %) data were used to calculate THImax, according to Ouellet et al. (2019).

These data were obtained from the National Institute of Meteorology - INMET (INMET, 2021) and used to calculate THImax via the equation recommended by Berman et al. (2016) Eq. 1:

$$\begin{aligned} \mathrm{THImax} &= 3.43 + 1.058 \times \mathrm{Tmax} - 0.293 \times \mathrm{RUmin} + \\ &+ 0.0164 \times \mathrm{Tmax} \times \mathrm{RUmin} + 35.7 \end{aligned} \qquad (1)$$

where: Tmax is the daily maximum temperature and RUmin the daily minimum relative humidity. This equation was chosen because it is best suited to identifying heat stress in crossbred dairy calves in a tropical environment, as demonstrated in research conducted in the Triângulo Mineiro region, where the municipalities studied here are located (Nascimento et al., 2019).

Thermal discomfort was defined at a THImax greater than or equal to 76 and thermal comfort at less than 76 in accordance with Azevedo et al. (2005), who estimated THI thresholds in ½, ¾ and 7/8 Holstein-Zebu cows in Brazil. Next, the three months of each year with the largest number of days with THImax ≥76 were calculated to represent summer (stress, S) and the three

Table 1. Quarterly means of the minimum, average and maximum values of the maximum daily temperature and humidity index (THImax) in the three months of the year with the highest frequency of THImax ≥ 76 (Stress - S) and the three months with the greatest frequency of THImax < 76 (Comfort - C) in the municipalities of Araxá, Sacramento and Uberaba (2017 to 2021), Minas Gerais, Brazil

City	Year	Minimum		Average		Maximum	
		S	C	S	C	S	C
Araxá	2017	69.30	63.30	76.90	69.60	81.00	74.00
	2018	70.3	67.6	78	71.3	81.3	75
	2019	65.3	62.3	77.4	70.3	81.7	75
	2020	68	61.6	77.3	70	82.3	74.6
Sacramento	2017	70.3	61.7	78.2	71	82.3	74.7
	2018	73	63.7	79	73.2	82.3	77
	2019	72.7	61	79.3	71.8	83	76.7
	2020	69	64.3	79.2	72	88.3	76
Uberaba	2017	70	61	79.1	71.3	83.3	77
	2018	75	67	80.6	73.7	83.7	78.3
	2019	74.3	60.7	80.5	73	84	78.3
	2020	74	66.7	80.5	74.4	84	79.7

Blue - Thermal comfort (THImax < 76); Orange - Heat stress (THImax ≥ 76).

months with the largest number of days with THImax <76 to represent winter (comfort, C) since this region does not have well-defined seasons.

For each of the S and C groups, mean values of the minimum, average and maximum THImax were calculated in each year and for each municipality to better assess the thermal environment, using the SPSS program (Table 1).

The fat, protein, lactose, total solids and solids-not-fat (SNF) contents of refrigerated raw milk were provided by a dairy company. In total, 10,063 results were provided for each of the variables, divided as follows: 2607 from Araxá, 2814 from Sacramento, and 4642 from Uberaba, recorded from 2017 to 2020 in the periods previously defined as stress (S) and comfort (C).

Samples were collected monthly from the expansion tank and comprised the combined milk of animals from each of the rural properties. The samples were refrigerated (4 to 7°C) and sent for analysis within 48 h at the Clínica do Leite Laboratory, using the ClinicaLog sample delivery service of the Luiz Queiroz College of Agriculture at the University of São Paulo (ESALQ/USP), in Piracicaba, São Paulo state, Brazil. The laboratory is accredited by the Ministry of Agriculture, Livestock and Supply (MAPA) as a member of the Brazilian Network of Milk Quality Comfort Laboratories (RBQL). Milk components were determined using the ISO 9622 method, in a CombiFoss™ 7 DC device from Foss Analytical A/S (Denmark).

The main genetic composition of the cows from the properties included in this study was crossbred Holstein × Zebu and the predominant rearing system in all three municipalities was semi-intensive. In the rainy season, the animals were raised on natural or cultivated pastures and troughs supplemented with roughage in the dry season. A concentrate was offered daily according to the level of milk production.

The average fat, protein, lactose, total solids and SNF contents for the months classified as S and C were determined for each municipality per year. Next, the stress-to-comfort ratio (S:C) was determined for each of the components of these variables by dividing the mean of the S group by the mean of the C group for each year and in each municipality (Flamenbaum & Galon, 2010). When the stress and comfort means were statistically equal, S:C was considered 1.0.

Using SPSS software, the quarterly mean and standard deviation were calculated for fat, protein, lactose, total solids and SNF (per year and municipality) and the variances compared by the F test to assess homoscedasticity of the data. Next, the Student's t test was applied to compare the stress and comfort periods. Significance was set at $p \leq 0.05$.

Finally, the Shapiro-Wilk test was performed ($p \leq 0.01$) for the mean THImax and milk component values. Since these data exhibited normal distribution, Pearson's correlation coefficient was used to measure the strength of the linear relationship between means at $p \leq 0.01$.

Results and Discussion

For the three municipalities studied here, the quarterly means of the minimum THImax values in the stress and comfort periods were lower than the critical value (76), whereas the average THImax values were higher than 76 in the S months and lower in the C periods (Table 1). The quarterly means of the maximum THImax values in the S group were greater than 81 and above 74 in the C group (Table 1).

In the three municipalities and the years analyzed, the maximum THImax means in the S group were ≥ 81 (Table 1). However, quarterly minimum THImax means below 76 were recorded even in S periods, indicating thermal comfort on some days (Table 1). A maximum of 25 days of thermal comfort was observed in the S group in Araxá in 2020 (Figure 1D). These data demonstrate the occurrence of a few days of thermal comfort in the warmest quarters, with long-lasting and high-intensity heat stress that, along with sex, age, breed and stage of production, influence the responses of the animal organism (Wankar et al., 2021).

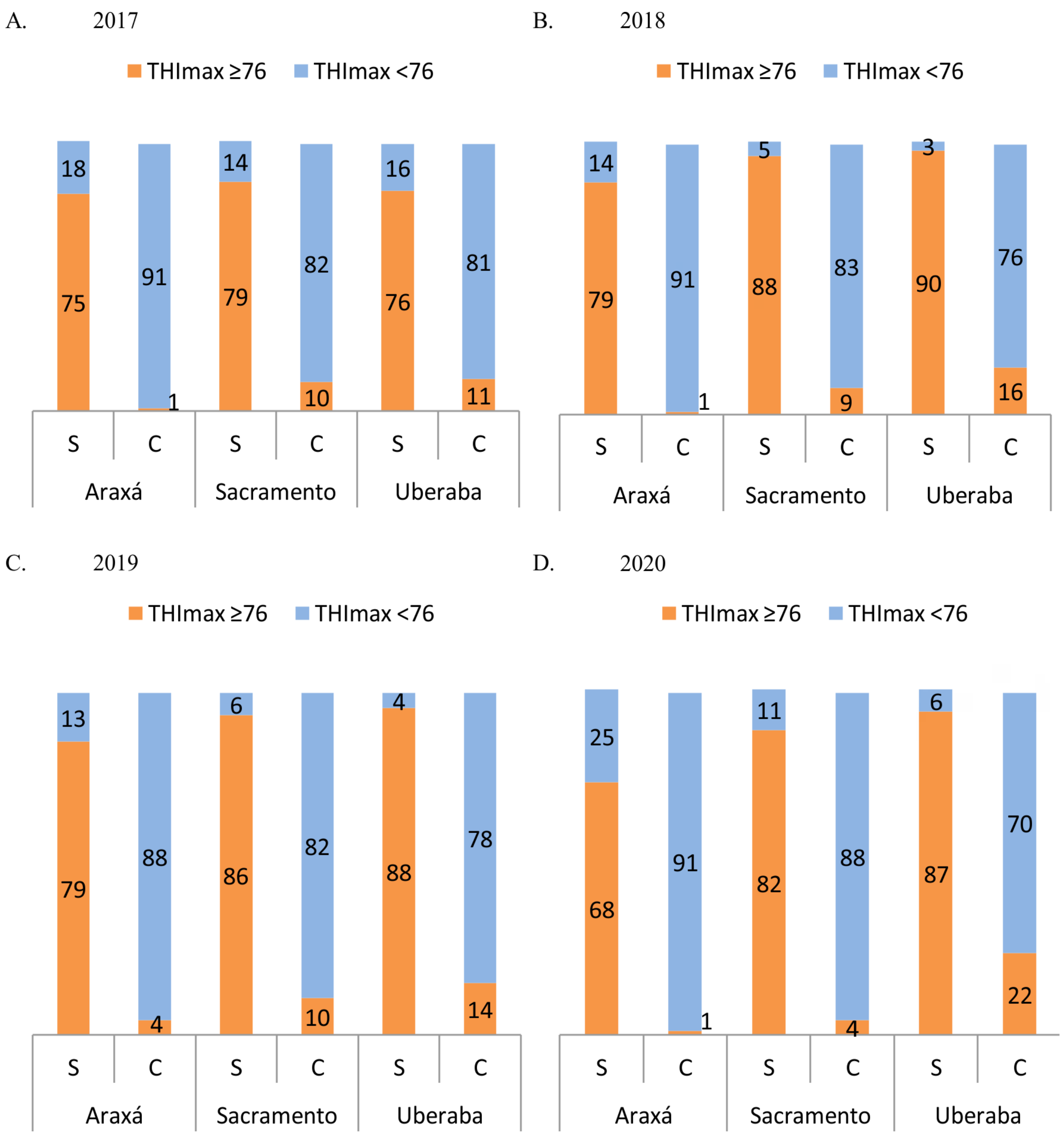
In the S months, the number of days characterized by heat stress, i.e., with THImax ≥ 76, ranged from 68 to 90 (Figure 1). In Uberaba, for example, there were 90 days of heat in the three S months of 2018 and 88 in 2019 (Figures 1B and C), but 25 to 3 days classified as thermal comfort (THImax < 76). In the C periods, the number of days of thermal comfort ranged from 70 to 91, with 22 to 1 day of heat stress.

In the four comfort periods (quarters) established for Araxá, Sacramento and Uberaba (one quarter per year analyzed), May, June, July and August featured2, 4, 4 and 2 times, respectively, that, part of autumn and winter and the remaining months did not feature.

In the stress months in Araxá, October featured in all 4 years, January in 3, December in 2, and September in 1 year, representing spring/summer; in Sacramento, January was identified in 4 of the years studied, December in 3, March and October in 2, and November in year, (part summer and spring); in Uberaba, December was observed for all the years analyzed, March in 3 of the years, January and November in 2, and October in 1 year (spring and part of summer).

Based on the means of the maximum THImax values (Table 1), the highest results (except 2020 in Sacramento) indicative of more intense heat stress were recorded in Uberaba, followed by Sacramento and Araxá. Additionally, except 2017, the number of days with THImax ≥ 76 in the S group was higher in Uberaba (Figure 1), followed by Sacramento and Araxá. By contrast, for all the years analyzed, the number of days with THImax ≥ 76 in the C group was lower in Araxá, followed by Sacramento and Uberaba, indicating a milder thermal environment in Araxá when compared to Uberaba and an intermediate scenario in Sacramento. As a result, the lowest S:C value was recorded in Uberaba, associated with the fat content in 2017 and 2019 (Table 2).

The months classified as stress and comfort varied according to the municipality and year assessed. These results reinforce the suitability of the methodology adopted in the present study of selecting these months based on THImax as opposed to seasons. Guinn et al. (2019) compared the S:W ratio for several performance variables of dairy herds in different regions of the United States and found that intense heat stress does not necessarily occur during summer and as such, using the dates that delimit summer and winter as reference when calculating the S:W ratio can exclude important heat stress periods from other seasons, such as spring.



BLUE - Thermal comfort (THImax < 76); ORANGE - Heat stress (THImax ≥ 76)

Figure 1. Number of days with maximum daily temperature and humidity index (THImax) ≥ 76 and < 76 in the Stress group (S - Three months of the year with the greatest frequency of THImax ≥ 76) and Comfort group (C - Three months of the year with greatest frequency of THImax < 76) in the municipalities of Araxá, Sacramento and Uberaba (2017 to 2020), Minas Gerais State, Brazil

It is important to underscore that factors such as diet and lactation length are statistically considered as error terms. These factors increased the overall variability of the dataset, which could potentially obscure significant differences between periods S and C. However, the Student's t-test revealed significant differences between these periods, which were attributed to the effects of heat stress.

The fat, protein, lactose, total solids and SNF contents of raw milk were above 3.0, 2.9, 4.3, 11.4 and 8.4%, respectively (Tables 2, 3 and 4), the minimum concentrations required by Ordinance 76/2018 (BRASIL, 2018).

In all the municipalities and years analyzed, milk fat content was lower in the S than C period, with S:C > 1.0 (Tables 2, 3 and 4). The lowest (worst) S:C was for fat content (0.90) in Uberaba in 2017 and 2019 (Table 2), with a 10% reduction in the S period compared to the C months in 2017, and 9.6% in 2019. This corresponds to a loss of approximately 3.8 kg of fat per 1000 liters of milk with average density of 1031 g/ml, according to Ordinance 76 2018 (BRASIL, 2018).

In the present study, heat stress altered the milk composition of crossbred cows in a tropical environment, especially fat percentage. Higher fat levels are desirable from an economic perspective, since producers are paid a bonus for levels exceeding 3.0% (Lima et al., 2021), and also affect yields. For all the years analyzed in the three municipalities studied, the milk fat percentage was lower in the S than C

Table 2. Means and standard deviations of the composition of refrigerated raw milk from crossbred dairy cows in the three months with the greatest frequency of days with maximum daily temperature and humidity index (THImax) ≥ 76 (Stress - S) and the three months with a higher frequency of days with THImax < 76 (Comfort - C), and S:C ratio, in Uberaba (2017 to 2021), Minas Gerais State, Brazil

Thermal environment	Fat	Protein	Lactose	Total solids	Solids-not-fat
			$(g\ 100\ g^{-1})$		
2017					
S (n = 629)	$3.42^{b} \pm 0.47$	$3.19^{b} \pm 0.18$	$4.50^{a} \pm 0.19$	$12.09^{b} \pm 0.60$	$8.67^{b} \pm 0.27$
C (n = 652)	$3.80^{a} \pm 0.47$	$3.32^{a} \pm 0.22$	$4.44^{b} \pm 0.20$	$12.55^{a} \pm 0.62$	$8.75^{a} \pm 0.31$
S/C	0.90	0.96	1.01	0.96	0.99
2018					
S (n = 832)	$3.51^{b} \pm 0.64$	$3.30^{b} \pm 0.21$	4.40 ± 0.26	$12.20^{b} \pm 0.74$	$8.68^{b} \pm 0.31$
C (n = 574)	$3.66^{a} \pm 0.68$	$3.34^{a} \pm 0.25$	4.42 ± 0.23	$12.38^{a} \pm 0.80$	$8.72^{a} \pm 0.34$
S/C	0.96	0.99	1.00	0.98	0.99
2019					
S (n=479)	$3.47^{b} \pm 0.55$	$3.29^{b} \pm 0.21$	$4.45^{a} \pm 0.24$	$12.14^{b} \pm 0.73$	8.68 ± 0.38
C (n=652)	$3.84^{a} \pm 0.59$	$3.35^{a} \pm 0.27$	$4.38^{b} \pm 0.27$	$12.53^{a} \pm 0.85$	8.69 ± 0.46
S/C	0.90	0.98	1.02	0.97	1.00
2020					
S (n=414)	$3.47^{b} \pm 0.56$	3.30 ± 0.19	$4.45^{a} \pm 0.19$	$12.15^{b} \pm 0.65$	8.68 ± 0.29
C (n=410)	$3.79^{a} \pm 0.54$	3.30 ± 0.23	$4.41^{b} \pm 0.21$	$12.43^{a} \pm 0.74$	8.63 ± 0.38
S/C	0.92	1.00	1.01	0.98	1.01*

(a, b) Within each year, means followed by different letters in the same column differ according to the t test ($p \leq 0.05$); n - Number of samples analyzed; *S:C considered 1.0 since there was no statistical difference between the means of the stress (S) and comfort (C) periods

Table 3. Means and standard deviations of the composition of refrigerated raw milk from crossbred dairy cows in the three months with the greatest frequency of days with maximum daily temperature and humidity index (THImax) ≥ 76 (stress - S), the three months with the highest frequency of days with THImax < 76 (comfort - C), and S:C ratio in the municipality of Araxá (2017 to 2021), Minas Gerais State, Brazil

Thermal environment	Fat	Protein	Lactose	Total solids	Solids-not-fat
			$(g\ 100\ g^{-1})$		
2017					
S (n=394)	$3.52^{b} \pm 0.36$	$3.22^{b} \pm 0.18$	$4.47^{a} \pm 0.16$	$12.19^{b} \pm 0.51$	$8.67^{b} \pm 0.26$
C (n=391)	$3.72^{a} \pm 0.43$	$3.32^{a} \pm 0.31$	$4.42^{b} \pm 0.39$	$12.46^{a} \pm 0.72$	$8.74^{a} \pm 0.63$
S/C	0.95	0.97	1.01	0.98	0.99
2018					
S (n=362)	$3.51^{b} \pm 0.41$	$3.26^{a} \pm 0.17$	$4.44^{a} \pm 0.15$	12.18 ± 0.49	$8.68^{a} \pm 0.22$
C (n=222)	$3.65^{a} \pm 0.38$	$3.23^{b} \pm 0.14$	$4.39^{b} \pm 0.17$	12.25 ± 0.48	$8.60^{b} \pm 0.20$
S/C	0.96	1.01	1.01	0.99*	1.01
2019					
S (n=305)	$3.41^{b} \pm 0.38$	$3.19^{b} \pm 0.18$	$4.43^{a} \pm 0.15$	$12.00^{b} \pm 0.51$	$8.58^{b} \pm 0.25$
C (n=191)	$3.72^{a} \pm 0.39$	$3.34^{a} \pm 0.19$	$4.40^{b} \pm 0.16$	$12.42^{a} \pm 0.52$	$8.71^{a} \pm 0.27$
S/C	0.92	0.96	1.01	0.97	0.99
2020					
S (n=383)	$3.41^{b} \pm 0.44$	$3.24^{b} \pm 0.17$	$4.44^{a} \pm 0.16$	$12.00^{b} \pm 0.56$	$8.59^{b} \pm 0.26$
C (n=359)	$3.71^{a} \pm 0.49$	$3.36^{a} \pm 0.20$	$4.40^{b} \pm 0.18$	$12.38^{a} \pm 0.60$	$8.68^{a} \pm 0.28$
S/C	0.92	0.96	1.01	0.97	0.99

(a, b) Within each year, means followed by different letters in the same column differ according to the t test ($p \leq 0.05$); n - Number of samples analyzed; *S:C ratio considered 1.0 since there was no statistical difference between means of the stress (S) and comfort (C) periods

months, resulting in S:C < 1.0 (Tables 2, 3 and 4). The lowest (0.90) S:C value was for fat content in Uberaba in 2017 and 2019 (Table 2), representing a 10% decline (0.38 g 100g^{-1}) in the S compared to C period in 2017, and 9.6% (0.37 g 100g^{-1}) in 2019.

Despite the different study site, Guinn et al. (2019) reported a lower fat content in milk produced by Holstein cows during summer in several regions of the United States, ranging from 3.53 to 3.63% in summer and 3.72 to 3.83% in winter, with S:W values between 0.94 and 0.96. Bernabucci et al. (2015) also reported a lower fat percentage in summer (15.8% lower than in winter) in milk from Holstein cows in Italy, and Ouellet et al. (2019) observed that milk fat content declined by up to 6% in Holstein cows under heat stress in Canada when compared to those under thermal comfort. However, Cowley et al. (2015) found no changes in milk fat content in Holstein cows exposed to heat for seven days (THI = 78).

A reduction in milk fat content is expected in hot weather due to several contributing factors. During heat stress, dry matter consumption declines and changes occur in the rumen microbiota, affecting rumen fermentation and pH and resulting in less nutrient availability and absorption by the mammary gland, which compromises the synthesis of milk components, including fat (Kim et al., 2022). In addition, lower food intake leads to shorter rumination time with less saliva directed to the rumen and, consequently, a lower rumen bicarbonate concentration. This compromises the buffering effect of saliva and results in ruminal acidosis. Low rumen pH alters its microbiota and hampers the synthesis of acetate, the main precursor for fat synthesis in the mammary gland (Kim et al., 2022).

Table 4. Means and standard deviations of the composition of refrigerated raw milk from crossbred dairy cows in the three months with the greatest frequency of days with maximum daily temperature and humidity index (THImax) ≥ 76 (Stress - S) and the three months with a higher frequency of days with THImax < 76 (Comfort - C), and S:C ratio in the municipality of Sacramento (2017 to 2021), Minas Gerais State, Brazil

Thermal environment	Fat	Protein	Lactose	Total solids	Solids-not-fat
			(g 100 g^{-1})		
2017					
S (n=409)	3.48^b ± 0.42	3.23^b ± 0.16	4.45^a ± 0.14	12.14^b ± 0.50	8.66^b ± 0.22
C (n=448)	3.72^a ± 0.43	3.35^a ± 0.19	4.42^b ± 0.15	12.48^a ± 0.54	8.76^a ± 0.24
S/C	0.94	0.96	1.01	0.97	0.99
2018					
S (n=376)	3.54^b ± 0.46	3.29 ± 0.16	4.42 ± 0.17	12.25^b ± 0.55	8.71 ± 0.21
C (n=361)	3.71^a ± 0.47	3.28 ± 0.17	4.42 ± 0.18	12.40^a ± 0.59	8.69 ± 0.26
S/C	0.95	1.00	1.00	0.99	1.00
2019					
S (n=329)	3.51^b ± 0.36	3.28 ± 0.15	4.49 ± 0.13	12.23^b ± 0.46	8.72 ± 0.21
C (n=181)	3.86^a ± 0.50	3.30 ± 0.22	4.42 ± 0.17	12.59^a ± 0.67	8.73 ± 0.28
S/C	0.91	0.99*	1.02*	0.97	1.00
2020					
S (n=353)	3.49^b ± 0.40	3.25^b ± 0.17	4.49^a ± 0.14	12.13^b ± 0.50	8.65^b ± 0.22
C (n=357)	3.81^a ± 0.42	3.40^a ± 0.20	4.46^b ± 0.15	12.58^a ± 0.56	8.77^a ± 0.26
S/C	0.92	0.96	1.01	0.96	0.99

(a, b) Within each year, means followed by different letters in the same column differ according to the t test ($p \leq 0.05$); n - Number of samples analyzed; *S:C ratio considered 1.0 since there was no statistical difference between means of the stress (S) and comfort (C) periods

These changes in the rumen are intensified by the increased respiratory rate in dairy cows exposed to heat conditions, causing respiratory alkalosis. Before this occurs, metabolic acidosis acts as a compensatory mechanism through greater renal excretion of bicarbonate. The decline in bicarbonate levels in the blood and saliva intensifies rumen acidosis and fat synthesis losses in milk (Garcia et al., 2015).

Another possible explanation for milk fat content reduction under heat stress is the preferred use of blood glucose by extra mammary tissue due to the need for increased thermoregulation (Bernabucci et al., 2015). This results in low glucose availability for the mammary gland, which is particularly detrimental to milk fat synthesis, since about 70% of the glycerol present in milk triglycerides comes from blood glucose and only 30% from free glycerol phosphorylation by glycerol kinase in the mammary gland (González et al., 2001; Wheelock et al., 2010; Garner et al., 2017).

In regard to protein, S:C values in Araxá were < 1 for all the years analyzed except 2018, with the largest decline (4.5%) in the S compared to C group recorded in 2019. In Sacramento, S:C was < 1 in 2017 and 2020, declining by 3.6 and 4.4%, respectively. In Uberaba, S:C was < 1 in 2017, 2018 and 2019, with reductions of 3.9, 1.2 and 1.8%, respectively, and an S:C of 1 in 2020.

The protein and fat contents of milk are key criteria in payment programs for milk quality (Medeiros et al., 2023). Protein content is especially important for cheese production, with casein, the primary milk protein, playing an important part in coagulation, a crucial step in cheese making (Warncke et al., 2022). In the present study, 66.67% of S:C values (Tables 2, 3 and 4) were below 1.0, indicating a decrease in milk protein synthesis as a function of heat stress. Guinn et al. (2019) also reported an S:W ratio < 1.0 (0.96 to 0.98) for milk protein in Holstein cows in different regions of the United States in summer (2.95 to 2.99%) when compared to winter (3.06 to 3.13%). Similarly, Bernabucci et al. (2015) reported that milk protein content was 6% lower in summer than in winter.

Cowley et al. (2015) also reported lower milk protein synthesis in Holstein cows exposed to heat for seven days (THI = 78) when compared to cows under thermal comfort with paired feeding. The authors concluded that this decline was not only caused by lower food intake, but the specific negative regulation of protein synthesis in the mammary gland caused by heat stress.

Similarly, Wheelock et al. (2010) observed post-absorptive metabolic changes in cows subjected to heat stress that are independent of reduced dry matter intake. This corroborates the findings of Bernabucci et al. (2010), who also reported that lower dry matter consumption during heat stress is not the main cause of losses in milk quality, but a consequence of factors that alter rumen function, resulting in lower nutrient absorption and hormonal imbalance. Factors that limit the supply of amino acids to the mammary gland for milk protein synthesis include a decline in ruminal microbial protein synthesis and increased systemic amino acid use during heat stress (Kim et al., 2022; Giannone et al., 2023).

Lactose content was higher in stressed than thermally comfortable cows in all the years studied in Araxá, with S:C >1.0, while in Sacramento and Uberaba, lactose content in the S group was greater than or equal to that of the C group, with S:C ≥ 1.0.

The synthesis of lactose, the most abundant milk component after water, appears not to be affected by heat stress (Summer et al., 2019). González et al. (2001) reported that lactose concentration varied least because it is the main osmotic regulator in milk (attracting water to the mammary epithelial cells).

In the present study, all the S:C values for lactose were equal to or slightly greater than 1 (Tables 2, 3 and 4). Cowley et al. (2015) also reported no changes in the lactose content of milk from Holstein cows under heat stress. In Italy, Bernabucci et al. (2015) found that lactose was the only component that remained unchanged across the seasons.

However, Wheelock et al. (2010) reported a decline in milk lactose in Holstein cows raised under heat stress. Wheelock et al. (2010) and Garner et al. (2017) attributed this to the fact that, during heat stress, glucose is preferentially used by extra mammary tissue. A higher serum glucose concentration reduces blood glucose and the supply of glucose to the mammary gland, which can ultimately compromise lactose synthesis, since it is composed of glucose and galactose.

For all the municipalities and years studied, the S:C for total solids was < 1.0, except 2018 in Araxá (Tables 2, 3 and 4), indicating a decline in total solids content in milk during periods of heat stress. Total solids, or total dry extract, is the sum of all milk components minus water. All the S:C values for total solids were less < 1.0 (Tables 2, 3 and 4), except in 2018 in Araxá. The decline in this component reflects the reduced milk fat and protein synthesis observed in the stressed cows. In Italy, Bernabucci et al. (2015) also recorded a 5.3% lower total solids content in summer than in winter. The total solids content did not differ between the S and C groups in 2018 in Araxá (Table 3), possibly because only fat content declined during the S period in this year, whereas there was no decline in the other milk components.

In Araxá, the S:C for SNF decreased in heat stressed cows when compared to their thermally comfortable counterparts, except in 2018 (Table 3), with declines also observed in Sacramento in 2017 and 2020 and in Uberaba in 2017 and 2018 (Tables 3 and 4). SNF or defatted dry extract represents the solid elements of milk other than fat, and as such, changes in its content are mainly correlated with variations in protein and lactose concentrations (Brito et al., 2020). Thus, since the greatest losses in the present study were for milk fat content and these are not reflected in the S:C value for SNF, values for the latter component ranged from 0.99 to 1.01 (Tables 2, 3 and 4). In addition, lactose, which is included in solids-not-fat, was not reduced by heat stress.

Bernabucci et al. (2015) recorded a 2.8% lower SNF fat content in summer than in winter. Declines in fat, protein and total solids were more significant at 15.8, 6 and 5.3%, respectively, with no difference for lactose. Similar results were obtained in the present study, since lactose synthesis was unaffected by heat stress and fat, protein, total solids and SNF synthesis was worse under stress, evident in the S:C values < 1.0 of 0.90, 0.96, 0.96 and 0.99, respectively.

There was a high to moderate negative correlation between THImax and milk fat ($r = -0.84$ and $p < 0.0001$), protein ($r = -0.56$ and $p = 0.009$) and total solids content ($r = -0.79$ and $p = 0.0001$), a moderate positive correlation between THImax and lactose ($r = +0.55$ and $p = 0.008$), and no correlation between THImax and SNF ($p = 0.049$).

The smaller decline in milk protein synthesis under stress when compared to fat was also reported by Guinn et al. (2019), with an S:W ratio for fat of 0.94 to 0.96 and 0.96 to 0.98 for protein, indicating greater losses in fat than protein content in summer.

These results are confirmed by the strong negative correlation between THImax and fat, demonstrating that this milk component declined most due to heat stress, followed by total solids and protein. By contrast, lactose exhibited a moderate positive correlation, indicating a possible increase in its content at higher THImax values. There was no association between THImax and SNF.

Mylostyvyi & Chernenko (2019) observed a negative correlation between THI and the production of fat ($r = -0.447$) and protein ($r = -0.354$) in milk from Holstein cows. In the Brazilian state of Goiás, Silva & Antunes (2018) also reported a negative correlation between THI and fat ($r = -0.52$) and total solids ($r = -0.48$), and a positive correlation with lactose content ($r = 0.54$), but no correlation between THI and protein content.

Recognizing and predicting heat stress is essential in adopting management practices to mitigate its negative effects. This is increasingly challenging due to the growing number of herds with high producing cows and, consequently, greater metabolic activity (Habimana et al., 2024; Rohleder et al., 2022). There are also growing concerns about global climate change, which has increased the frequency, duration and severity of heat stress (Carvajal et al., 2021).

CONCLUSIONS

1. The ratio between average milk composition in the three hottest (stress) and three mildest months of the year (comfort), calculated based on the daily THImax, can be used as an indicator to monitor heat stress. Ratios below 1.0 suggest reductions in the synthesis of milk components, highlighting the adverse effects of thermal discomfort in tropical regions.

2. The ratio can be used by dairy farmers to monitor heat stress and identify periods when mitigating measures are needed to ensure thermal comfort and animal welfare.

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