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Thermal comfort and productive responses of 7/8 holstein-gyr cows subjected to cooling system

Marcos Vinícius da Silva[1*], Gledson Luiz Pontes de Almeida[1], Héliton Pandorfi[1], Alex Souza Moraes[2], Gleidiana Amélia Pontes de Almeida Macêdo[3], Maria Eduarda Gonçalves de Oliveira[1], Maria Vitória Neves de Melo[1] and Taize Calvacante Santana[1]

[1]Departamento de Engenharia Agrícola, Universidade Federal Rural de Pernambuco, Rua Manuel de Medeiros, s/n, 52171-900, Dois Irmãos, Recife, Pernambuco, Brazil. [2]Departamento de Química, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil. [3]Departamento de Zootecnia, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil. *Author for correspondence. E-mail: marcolino_114@hotmail.com

ABSTRACT. The objective of this research was to identify the influence of the evaporative adiabatic cooling system (EACS) on the thermal comfort and productive responses of dairy cattle, through multivariate analysis by principal components (PC), in the summer and winter seasons of the semiarid region of Pernambuco, Brazil. The data came from an experiment that included 16 multiparous lactating cows (7/8 Holstein-Gyr), randomly distributed in 4 sets, with 4 experimental phases and 4 treatments (0, 10, 20, and 30 min.). The multivariate analysis was carried out through PC for the thermal comfort indices, physiological variables, and milk production and composition. The highest milk production in the summer season was obtained for animals exposed to the cooling system for 30 min. In the winter season in the morning period, the use of the EACS for 10 min. was sufficient for improvements in milk production. The times of exposure to EACS caused changes in the composition of milk, for both seasons. Principal component analysis made it possible to verify a positive correlation of evaporative cooling with thermal comfort, physiological responses, and production and composition of milk of lactating cows.

Keywords: multivariate analysis; dairy cattle; evaporative cooling system.

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Introduction

Tropical and subtropical regions generally have high air temperatures that affect the homeothermy of dairy cows, with negative consequences for food intake, milk production and milk composition (Garner et al., 2017; Tresoldi, Schütz, & Tucker, 2019; Souto et al., 2021).

High milk production cows succumb to heat stress before low production animals, due to higher nutritional requirements and metabolic heat production, which significantly alter the energy balance between the animal and the environment (Shu, Wang, Guo, & Bindelle, 2021; Burhans, Burhans, & Baumgard, 2022). Strategies that minimize this problem include managing the animal in the shade, using an evaporative adiabatic cooling system (EACS) and an automated cooling system (Silva & Passini, 2018; Tresoldi, Schütz, & Tucker, 2019).

The characterization of the thermal environment using comfort indices allows the integrated assessment of more than one meteorological variable associated with the effect of stress and/or comfort on farm animals, such as the temperature and humidity index (THI) commonly used for determining the thermal condition in the housing of dairy cattle. As for phenotypic plasticity, rectal temperature is one of the main physiological variables used to identify thermal stress in dairy cattle; under normal conditions they have a body core temperature of 38.5°C and the thresholds for environmental fever vary between 39.1 and 39.7°C (Sousa, Silva Rodrigues, Abreu, Tabile, & Martello, 2018; Oliveira et al., 2019; Yan, Zhao, & Shi, 2020).

From the large amount of data generated to characterize the cause and effect of environmental thermal stress, one of the statistical methods to evaluate the phenomena that influence milk production is multivariate statistics, which makes it possible to explain a set of two or more variables in time (Silva et al., 2020; Batista et al., 2021; Silva et al., 2021a; b; Silva et al., 2022). Recent research shows satisfactory results with the use of multivariate analysis, such as evaluation of influence of nutrients and experimental period on the composition and quality of bovine milk (Gabbi et al., 2018).

Given the above, this research was conducted to identify the influence of the EACS on the thermal comfort and productive responses of dairy cattle, through multivariate analysis by principal components, in the summer and winter seasons of the semiarid region of Pernambuco, Brazil.

Material and methods

The research was conducted in one database from an experiment carried out in a dairy unit (Figure 1), located in the Agreste Mesoregion, Ipojuca Valley Microregion, Pernambuco State dairy basin (8°36'34.82"S and 36°37'33.09"W; 755 m), in the summer (from February to March) and winter (from July to August), of the year 2019.

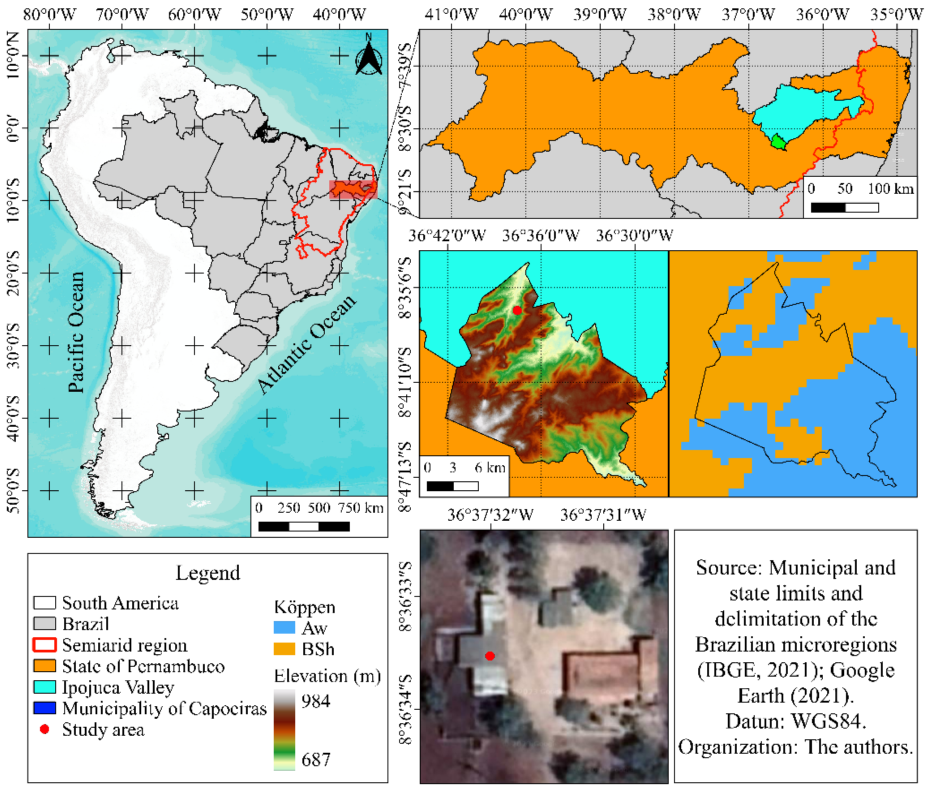

Figure 1. Location of the property in the municipality of Capoeiras, State of Pernambuco, Brazil (IBGE, 2021).

The average rainfall in the region is 620.3 mm (Agência Pernambucana de Águas e Climas [APAC], 2019), with an average annual temperature of 20.3°C (Instituto Nacional de Meteorologia [INMET], 2019). According to Köppen's classification, the region is characterized by the climate transition between "BSh" and "Aw" (Alvares, Stape, Sentelhas, Moraes Gonçalves, & Sparovek, 2013; Beck et al., 2018). In the summer of 2009, the average temperature based on a historical series of 30 years was 22.8°C and in the winter it was 20.3°C (INMET, 2019).

The experiment was conducted using 16 lactating multiparous Girolando cows (7/8 Holstein-Gyr), with an average weight of 500 kg and average milk production of 18 kg day^{-1}, randomly distributed in 4 sets (S1, S2, S3, and S4), with 4 experimental phases (P1, P2, P3, and P4) and 4 times of exposure of the animals to the evaporative adiabatic cooling - EACS (0, 10, 20 and 30 min.).

The experimental period was 56 days for the summer and 56 days for the winter, totaling 112 days for the entire experiment. Each season was divided into 4 phases of 14 days, with the first seven days of each phase intended for the animals to adapt to the pre-milking acclimation times of 10, 20 and 30 min. under EACS, plus the control (0 min.). The other seven subsequent days were used to record the meteorological variables in the waiting pen, physiological responses, and production of lactating cows, with subsequent determination of the composition of the milk.

To determine the comfort indices, the meteorological variables air temperature (T, °C), relative air humidity (RH, %) and the temperature of the black globe (BGT, °C) were recorded through dataloggers HOBO Pro Dataloggers HB8 model, with a temperature measurement range between -20 and 70 (± 0.35°C) and relative humidity between 5 and 100 (± 2.5%). The wind speed (m s^{-1}) was recorded by a propeller anemometer. The sensors were positioned in the geometric center of the waiting room, at 2.5 m from the floor.

The thermal efficiency of the installation was determined by calculating the globe temperature and humidity index (GTHI) proposed by Buffington et al. (1981), the temperature and humidity index (THI) proposed by Thom (1959), the radiant thermal load (RTL; W m^{-2}) proposed by Esmay (1982) and the enthalpy (h; KJ kg^{-1}) proposed by Albright (1990).

The physiological variables recorded were rectal temperature (RT; °C), respiratory rate (RR; mov $min.^{-1}$) and skin temperature (ST; °C), twice a week in pre-milking, after acclimatization, at times from 0500h (morning period) and 1400h (afternoon period).

The RR variable was checked from the count of the number of movements of the flank region performed by the animal, in the interval of 1 min. After registration, RT measurements were performed, with the aid of a digital veterinary thermometer (scale between 20 and 50°C), introduced into the rectum of the animals, for 1 min. for stabilization and obtaining the temperature value; the recording of RR and RT follows the recommendations of Almeida, Pandorfi, Guiselini, Henrique and Almeida (2011) and Almeida et al. (2013). The recording of ST was performed using an infrared thermometer, based on the temperature records of the head, back, shin and udder of each animal studied, for later determination of the average temperature of the skin according to the methodology established by Batista et al. (2021).

Milk production (Prod) was determined individually, in the evaluated seasons, for the two daily milking periods. The chemical composition (fat - Fat, protein - Pro, lactose - Lac and total solids - Sol) was determined in two collections for each phase, with individual samples of the milk of each animal, in their respective treatments and analyzed in the *Programa de Gerenciamento de Rebanhos Leiteiros do Nordeste* (PROGENE), of the Animal Sciences Department at UFRPE.

The data were subjected to descriptive statistical analysis to obtain the mean, median and coefficient of variation (CV), classified as low when the CV < 12%; medium when 12% < CV < 24% and high when CV > 24% (Warrick & Nielsen, 1980). The Kolmogorov-Smirnov normality test ($p \leq 0.01$) was also applied.

For the use of principal component analysis, 12 variables (h, THI, GTHI, RTL, RR, RT, ST, Prod, Fat, Pro, Lac, Sol) were considered for each season of the year (summer/winter), totaling 24 variables. From the principal components extracted from the data sets in the summer and winter seasons, the covariance matrix was obtained, in which the eigenvalues that originated the eigenvectors were extracted (Kaiser, 1958).

This analysis was performed to identify parameters that explained most of the influence of the variables. For this, the Kaiser criterion was used, which considers eigenvalues above 1, because they generate components with a relevant amount of information contained in the original data, disregarding components that showed eigenvalues below 1 (Kaiser, 1958).

Results and discussion

Morning period

Descriptive statistics for the morning period, in both seasons, are described in Table 1 and Table 2. The coefficient of variation (CV) was low for all variables studied, except milk fat (Fat) in winter, from animals subjected to 10 min. of cooling in pre-milking. The enthalpy (h) in winter, for the cooling times of 10 and 20 min. and, the respiratory rate (RR) in the summer, for the animals subjected to the control (0 min.), showed medium CV (12% < CV < 24 %).

The low values of the coefficient of variation indicate that the use of the evaporative adiabatic cooling system (EACS) proved to be efficient in homogenizing the environment in which the animals were. The THI values in both seasons, in the morning period, were lower than 72, characterized as a condition of comfort for the animals, as established by Armstrong (1994).

Table 3 shows the principal components (PC) obtained through multivariate analysis, for comfort indices, physiological variables, production, and composition of milk of cows in the morning period (summer/winter). Components 1 (PC1) and 2 (PC2) showed an eigenvalue greater than 1, according to the criterion established by Kaiser (1958), with eigenvalues on the order of 16.732 and 5.590, respectively. PC1 and PC2 had a total variance on the order of 93.00% for PC2.

Corroborating the results of the present study, Silva et al. (2021a) evaluated the influence of abiotic variables on the behavioral patterns of dairy cows subjected to an adiabatic evaporative cooling system in a semiarid region of northeastern Brazil, for the dry and rainfall season, through the analysis of PCs, and observed a total variance in the correlation of the variables of 88.10 and 90.00% for the morning and afternoon periods, respectively.

Table 1. Descriptive statistics of milk production (Prod, liters), fat (Fat, %), protein (Pro, %), lactose (Lac, %), total solids (Sol, %) in the morning period in both seasons.

[1]Var	[2]Tim	Summer					Winter				
		[3]m	[4]SD	[5]CV	[6]Min	[7]Max	m	SD	CV	Min	Max
Prod	0	10.84	0.63	5.80	9.91	11.30	11.69	0.73	6.26	10.84	12.62
	10	11.08	0.37	3.34	10.75	11.59	11.77	0.38	3.23	11.42	12.26
	20	11.36	0.16	1.41	11.21	11.58	11.77	0.96	8.18	11.07	13.18
	30	11.54	0.48	4.20	11.04	12.20	11.74	0.96	8.20	10.45	12.62
Fat	0	3.08	0.22	7.19	2.85	3.35	3.01	0.26	8.72	2.63	3.19
	10	2.64	0.23	8.77	2.39	2.86	3.00	0.40	13.24	2.62	3.55
	20	2.88	0.34	11.80	2.41	3.19	2.99	0.35	11.53	2.59	3.34
	30	2.74	0.31	11.36	2.37	3.02	3.10	0.25	8.04	2.73	3.27
Pro	0	2.71	0.14	5.13	2.52	2.84	2.75	0.09	3.17	2.63	2.83
	10	2.69	0.10	3.57	2.60	2.82	2.74	0.09	3.40	2.61	2.81
	20	2.71	0.05	1.83	2.64	2.76	2.76	0.06	2.20	2.67	2.80
	30	2.66	0.06	2.14	2.58	2.71	2.79	0.06	1.98	2.72	2.85
Lac	0	4.42	0.11	2.48	4.34	4.58	4.56	0.11	2.32	4.43	4.65
	10	4.50	0.15	3.28	4.36	4.70	4.61	0.06	1.40	4.54	4.67
	20	4.51	0.12	2.69	4.41	4.69	4.60	0.05	1.08	4.53	4.65
	30	4.52	0.12	2.58	4.39	4.67	4.61	0.11	2.43	4.50	4.76
Sol	0	11.11	0.19	1.68	10.91	11.27	11.25	0.14	1.28	11.06	11.39
	10	10.72	0.10	0.93	10.61	10.81	11.28	0.41	3.60	10.97	11.88
	20	10.96	0.32	2.93	10.56	11.32	11.28	0.34	3.03	10.83	11.64
	30	10.82	0.35	3.25	10.36	11.16	11.43	0.18	1.58	11.19	11.62

[1]Var: variables; [2]Tim: adiabatic evaporative cooling time (0, 10, 20 and 30 min.); [3]m: mean; [4]SD: standard deviation; [5]CV: coefficient of variation; [6]Min: minimum; [7]Max: maximum.

Table 2. Descriptive statistics of enthalpy (h, KJ kg^{-1}), black globe temperature and humidity index (GTHI), temperature and humidity index (THI), radiant thermal load (RTL, W m^{-2}), rectal temperature (RT, °C), respiratory rate (RR, mov $min.^{-1}$) and skin temperature (ST, °C) in the morning period in both seasons.

[1]Var	[2]Tim	Summer					Winter				
		[3]m	[4]SD	[5]CV	[6]Min	[7]Max	m	SD	CV	Min	Max
h	0	60.30	1.54	2.55	58.10	61.50	54.42	2.03	3.73	51.90	56.80
	10	58.33	1.16	1.98	57.10	59.70	50.55	6.58	13.02	41.10	55.20
	20	57.93	1.41	2.43	56.70	59.90	49.73	6.43	12.94	40.40	54.10
	30	57.45	1.02	1.78	56.20	58.70	52.33	1.54	2.94	50.20	53.60
GTHI	0	69.25	0.50	0.72	69.00	70.00	64.75	0.96	1.48	64.00	66.00
	10	68.00	0.00	0.00	68.00	68.00	64.00	0.82	1.28	63.00	65.00
	20	67.50	0.58	0.86	67.00	68.00	63.50	0.58	0.91	63.00	64.00
	30	67.50	0.58	0.86	67.00	68.00	63.50	0.58	0.91	63.00	64.00
THI	0	70.00	0.00	0.00	70.00	70.00	67.00	0.82	1.22	66.00	68.00
	10	69.00	0.00	0.00	69.00	69.00	66.00	1.41	2.14	64.00	67.00
	20	68.75	0.50	0.73	68.00	69.00	65.75	1.50	2.28	64.00	67.00
	30	68.00	0.00	0.00	68.00	68.00	65.75	0.50	0.76	65.00	66.00
RTL	0	420.35	6.16	1.47	411.80	426.50	381.32	11.26	2.95	367.70	392.30
	10	401.90	14.83	3.69	385.60	420.30	352.60	26.80	7.60	317.50	376.70
	20	396.60	23.50	5.94	364.60	419.40	359.80	30.20	8.39	319.50	387.80
	30	407.60	10.57	2.59	394.30	420.10	348.90	23.40	6.70	323.10	376.50
RT	0	38.18	0.13	0.33	38.00	38.30	38.03	0.22	0.58	37.80	38.30
	10	38.28	0.05	0.13	38.20	38.30	38.15	0.10	0.26	38.00	38.20
	20	38.15	0.17	0.45	38.00	38.30	38.13	0.19	0.50	38.00	38.40
	30	38.03	0.15	0.39	37.90	38.20	37.95	0.10	0.26	37.90	38.10
RR	0	36.00	4.76	13.22	33.00	43.00	28.00	1.41	5.05	26.00	29.00
	10	27.75	1.26	4.53	26.00	29.00	26.50	2.65	9.98	24.00	30.00
	20	28.00	2.71	9.67	26.00	32.00	26.75	2.22	8.29	24.00	29.00
	30	25.75	1.26	4.89	24.00	27.00	26.00	2.83	10.88	22.00	28.00
ST	0	30.08	0.94	3.12	29.20	31.30	26.70	1.51	5.65	25.10	28.60
	10	27.70	0.92	3.32	26.60	28.60	26.58	1.14	4.31	25.60	28.20
	20	26.68	0.85	3.17	25.60	27.40	25.70	0.08	0.32	25.60	25.80
	30	26.45	0.79	2.97	25.40	27.10	24.95	2.25	9.01	22.60	28.00

[1]Var: variables; [2]Tim: adiabatic evaporative cooling time (0, 10, 20 and 30 min.); [3]m: mean; [4]SD: standard deviation; [5]CV: coefficient of variation; [6]Min: minimum; [7]Max: maximum.

Table 3. Principal components of comfort indices, physiological variables, behavioral parameters, and production and composition of milk of dairy cows in the morning period in summer and winter.

Variable	Principal Component in Summer		Principal Component in Winter	
	*PC1	PC2	PC1	PC2
[1]h	0.244	-0.017	0.165	-0.298
[2]GTHI	0.239	-0.077	0.242	-0.032
[3]THI	0.239	0.026	0.225	-0.112
[4]RTL	0.184	-0.259	0.234	-0.043
[5]RT	0.121	0.338	0.033	0.417
[6]RR	0.242	-0.041	0.240	0.013
[7]ST	0.239	-0.027	0.196	0.220
[8]Prod	-0.227	-0.111	-0.196	0.251
[9]Fat	0.192	-0.125	-0.118	-0.355
[10]Pro	0.191	0.208	-0.134	-0.342
[11]Lac	-0.242	0.053	-0.234	0.094
[12]Sol	0.182	-0.125	-0.178	-0.281
Eigenvalue	16.732	5.590	16.732	5.590
Proportion	0.697	0.233	0.697	0.233
Accumulated	0.697	0.930	0.697	0.930

*PC: principal component; [1]h: enthalpy (h; KJ kg^{-1}); [2]GTHI: globe temperature and humidity index; [3]THI: temperature and humidity index; [4]RTL: radiant thermal load (W m^{-2}); [5]RT: rectal temperature (°C); [6]RR: respiratory rate (mov $min.^{-1}$); [7]ST: skin temperature (°C); [8]Prod: milk production (liters); [9]Fat: fat (%); [10]Pro: protein (%); [11]Lac: lactose (%); [12]Sol: soluble solids (%).

The thermal comfort indices and physiological variables showed a positive correlation for the morning period in summer and winter, except for the rectal temperature (RT), which was neutral, that is, without the influence of comfort indices (Table 3). This was due to the lower temperatures in the morning period, which were within the comfort range of the animals.

The negative association between the levels of protein, fat and total solids with milk production (Table 3) occurred due to greater dilution of protein, fat and total solids in milk, given the higher volume produced in the morning period (Table 1). Gabbi et al. (2018) also found the same negative relationship between protein, fat, and total solids. The relationship between fat and protein was already expected, as these variables have a positive relationship with each other (Macciotta, Cecchinato, Mele, & Bittante, 2012; Mele et al., 2016).

Figure 2 presents the PCs of the physiological variables, animal comfort indices, and production and composition of milk of cows subjected to EACS (0, 10, 20 and 30 min.) in the morning period (summer/winter).

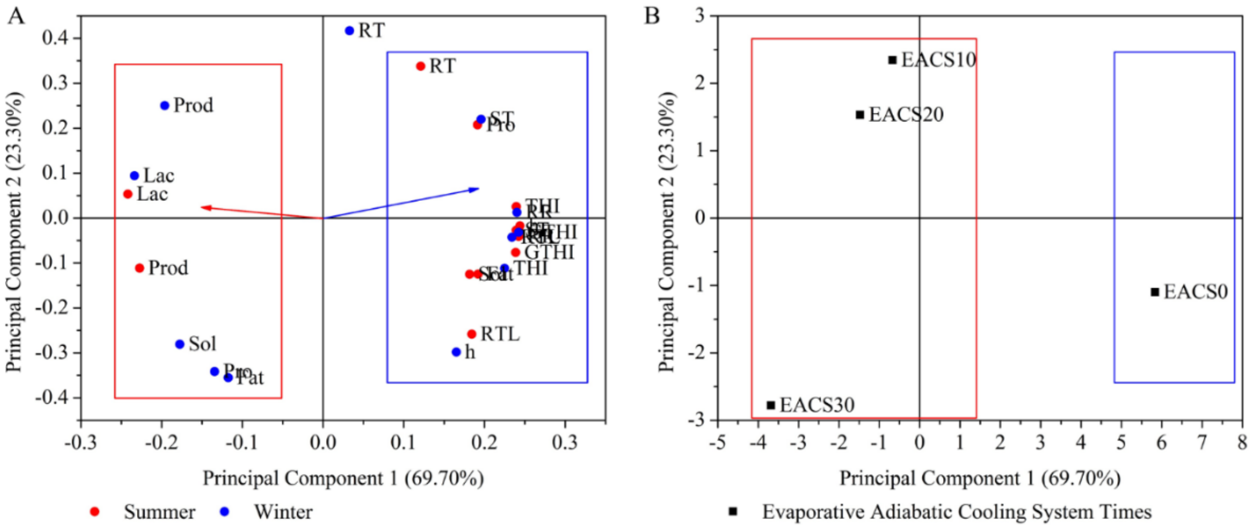

Figure 2. Principal components of the physiological variables, animal comfort indices, and production and composition of milk of cows in the morning period in summer and winter (A); PCs of the operating time of the EACS in the morning (B). Note: h: enthalpy (h; KJ kg^{-1}); GTHI: globe temperature and humidity index; THI: temperature and humidity index; RTL: radiant thermal load (W m^{-2}); RT: rectal temperature (°C); RR: respiratory rate (mov min^{-1}); ST: skin temperature (°C); Prod: milk production (liters); Fat: fat (%); Pro: protein (%); Lac: lactose (%); Solid: soluble solids (%); EACS: adiabatic evaporative cooling system operating for times of 0, 10, 20 and 30 minutes.

The highest milk production (Prod) in the summer season was obtained for animals exposed to EACS for 30 min. in pre-milking. The longer exposure to EACS promoted better comfort, with improvements in thermal comfort indices and physiological variables (Table 1). It is noted in Figure 2A that the time of 30 min. is opposite to comfort indices and physiological variables, which occurs because the EACS time of 30 min. allows significant reductions in these variables, promoting thermal comfort for the animals, as observed by Silva et al. (2021a). Silva and Passini (2018) evaluated different cooling systems in the waiting room for crossbred cows (⅞ Holstein x ⅛ Dairy Gyr), using environmental variables, milk production, and economic indices in the summer in a tropical climate region, and observed similar results to those obtained in the present study, in which the evaporative adiabatic cooling time of 30 minutes provided the best comfort conditions for the animals.

In the winter season, in the morning period, the time of 10 min. of exposure of the animals to the EACS was sufficient for thermal conditioning, with a positive effect on milk production (Figure 2A and 2B).

PC1 was the one that best explained milk production due to the other variables (Figure 2A). Крамаренко et al. (2017) evaluated the total yield of lactating cows through Principal Component Analysis (PCA) and observed that PC1 was sufficient to determine the potential level of yield of dairy cows.

The times of exposure of the animals to the EACS caused changes in the composition of the milk, according to the averages shown in Table 1. In contrast, Almeida et al. (2013) concluded through analysis of variance and Tukey test that the use of different cooling times in the waiting pen in the summer season did not cause changes in the chemical composition of milk in the morning period. The same can be observed for the winter season (Figure 2A and 2B), in which the EACS influenced the composition of milk (Table 1). However, Almeida Neto, Pandorfi, ,Almeida, and Guiselini (2014), using classical statistics, found that different times of evaporative adiabatic cooling in winter, in a semi-arid region, did not lead to changes in milk composition. In view of these findings, it is observed that the application of multivariate analysis by PCs made it possible to extract results not previously observed based on classical/conventional statistics.

Garner et al. (2017) observed that THI > 72 implied less milk production for Holstein cows. The results found by these authors corroborate that of the present study, in Figure 2A and 2B, which show that in the two seasons, milk production was inversely proportional to THI. However, it is noteworthy that in winter the THI values, even for the EACS 0 min. (control), were less than 68.00, and for the summer season the THI did not exceed 70.00 units, therefore, with little influence on milk production (Table 1).

Afternoon period

The coefficient of variation (CV) was low for all variables studied, except for milk fat (Fat) of the animals subjected to EACS for 30 min., for the respiratory rate (RR) of the animals at all cooling times, and for the skin temperature (ST) of the control animals in the summer season, which showed medium CV (12% < CV < 24%). In the winter season, the CV was medium for Fat among animals exposed to EACS for 30 min. and for ST at 0 and 10 min. (Table 4 and 5).

Table 4. Descriptive statistics of milk production (Prod, liters), fat (Fat, %), protein (Pro, %), lactose (Lac, %), total solids (Sol, %) in the afternoon period in both seasons.

[1]Var	[2]Tim	Summer					Winter				
		[3]m	[4]SD	[5]CV	[6]Min	[7]Max	m	SD	CV	Min	Max
Prod	0	6.67	0.29	4.40	6.35	6.98	7.67	0.62	8.06	6.83	8.21
	10	6.89	0.26	3.72	6.67	7.20	7.61	0.37	4.81	7.20	7.98
	20	6.75	0.29	4.30	6.39	7.04	7.61	0.35	4.63	7.20	7.94
	30	6.87	0.19	2.78	6.63	7.09	7.70	0.54	7.07	7.08	8.18
Fat	0	4.15	0.42	10.21	3.55	4.49	4.34	0.27	6.10	4.14	4.71
	10	4.69	0.55	11.66	4.11	5.32	4.31	0.44	10.30	3.74	4.74
	20	4.14	0.31	7.51	3.79	4.53	4.46	0.15	3.33	4.29	4.59
	30	4.40	0.61	13.95	3.72	5.11	4.44	0.59	13.26	4.03	5.29
Pro	0	2.71	0.17	6.32	2.45	2.82	2.79	0.08	2.91	2.67	2.84
	10	2.67	0.13	4.77	2.55	2.85	2.79	0.09	3.14	2.66	2.87
	20	2.72	0.07	2.44	2.64	2.80	2.79	0.04	1.34	2.74	2.82
	30	2.68	0.10	3.60	2.55	2.77	2.82	0.09	3.06	2.70	2.88
Lac	0	4.41	0.12	2.79	4.33	4.59	4.47	0.03	0.76	4.43	4.51
	10	4.42	0.13	2.87	4.28	4.59	4.53	0.03	0.77	4.49	4.57
	20	4.44	0.06	1.42	4.38	4.53	4.52	0.05	1.01	4.47	4.56
	30	4.43	0.14	3.09	4.30	4.60	4.51	0.07	1.65	4.46	4.62
Sol	0	12.19	0.40	3.26	11.61	12.50	12.54	0.33	2.65	12.23	13.01
	10	12.70	0.59	4.63	11.97	13.39	12.57	0.52	4.14	11.90	13.10
	20	12.22	0.38	3.12	11.81	12.68	12.72	0.23	1.80	12.44	12.97
	30	12.45	0.59	4.73	11.68	12.98	12.71	0.68	5.36	12.15	13.69

[1]Var: variables; [2]Tim: adiabatic evaporative cooling time (0, 10, 20 and 30 min.); [3]m: mean; [4]SD: standard deviation; [5]CV: coefficient of variation; [6]Min: minimum; [7]Max: maximum.

Table 5. Descriptive statistics of enthalpy (h, KJ kg^{-1}), black globe temperature and humidity index (GTHI), temperature and humidity index (THI), radiant thermal load (RTL, W m^{-2}), rectal temperature (RT, °C), respiratory rate (RR, mov min.$^{-1}$) and skin temperature (ST, °C) in the afternoon period in both seasons.

[1]Var	[2]Tim	Summer					Winter				
		[3]m	[4]SD	[5]CV	[6]Min	[7]Max	m	SD	CV	Min	Max
h	0	70.65	2.23	3.16	68.40	72.90	65.93	1.73	2.63	63.50	67.60
	10	69.03	1.04	1.51	68.10	70.20	64.78	2.38	3.67	61.60	66.70
	20	67.63	0.96	1.42	66.80	69.00	62.72	2.50	3.99	60.40	65.80
	30	66.38	1.20	1.81	64.70	67.30	64.00	2.34	3.65	60.70	65.80
GTHI	0	82.25	2.50	3.04	79.00	85.00	75.75	1.89	2.50	73.00	77.00
	10	72.25	0.50	0.69	72.00	73.00	69.50	1.92	2.76	67.00	71.00
	20	71.50	0.58	0.81	71.00	72.00	69.00	1.41	2.05	67.00	70.00
	30	71.25	0.50	0.70	71.00	72.00	68.75	1.26	1.83	67.00	70.00
THI	0	77.75	1.26	1.62	76.00	79.00	74.50	0.58	0.77	74.00	75.00
	10	75.25	0.96	1.27	74.00	76.00	73.00	1.16	1.58	72.00	74.00
	20	73.50	1.00	1.36	72.00	74.00	72.25	0.96	1.33	71.00	73.00
	30	73.00	0.82	1.12	72.00	74.00	72.00	0.82	1.13	71.00	73.00
RTL	0	534.40	31.00	5.80	503.20	573.20	482.60	28.80	5.98	443.40	508.20
	10	388.32	8.85	2.28	379.30	399.30	353.70	40.10	11.34	313.80	396.70
	20	390.32	7.15	1.83	380.10	395.30	348.10	33.30	9.55	315.20	391.30
	30	397.45	5.48	1.38	390.40	403.10	347.40	32.50	9.36	317.10	389.30
RT	0	39.05	0.24	0.61	38.80	39.30	38.95	0.24	0.61	38.70	39.20
	10	38.93	0.21	0.53	38.70	39.10	38.73	0.21	0.53	38.50	39.00
	20	38.90	0.28	0.73	38.70	39.30	38.75	0.30	0.77	38.60	39.20
	30	38.73	0.15	0.39	38.50	38.80	38.63	0.17	0.44	38.40	38.80
RR	0	61.50	12.12	19.71	51.00	79.00	51.75	9.74	18.83	41.00	60.00
	10	38.75	7.41	19.12	32.00	47.00	38.75	7.14	18.41	30.00	47.00
	20	39.75	9.18	23.09	29.00	50.00	33.75	3.10	9.17	31.00	38.00
	30	35.25	7.14	20.24	27.00	44.00	31.50	3.11	9.87	28.00	35.00
ST	0	38.50	5.09	13.22	33.20	45.30	34.23	2.18	6.38	31.70	36.80
	10	31.93	1.27	3.98	30.10	33.00	30.15	0.70	2.31	29.50	31.10
	20	29.83	1.90	6.38	27.60	32.10	28.43	0.64	2.25	27.60	29.10
	30	30.78	2.07	6.73	27.80	32.60	29.48	0.91	3.08	28.60	30.60

[1]Var: variables; [2]Tim: adiabatic evaporative cooling time (0, 10, 20 and 30 min.); [3]m: mean; [4]SD: standard deviation; [5]CV: coefficient of variation; [6]Min: minimum; [7]Max: maximum.

The components PC1 and PC2 had eigenvalues greater than 1 (16.616 and 4.806), respectively. The PCs used in the discussion of variables showed a total variance of around 89.30% for PC2 (Table 6). Gabbi et al. (2018) related levels of total digestible nutrients and experimental period to milk production, composition, and quality, with Jersey, Jersey × Holstein, and Holstein cows, through the analysis of PCs and obtained results of the total variance of 87.24%, hence similar to those found in the present study.

Table 6. Principal components of comfort indices, physiological variables, behavioral parameters, and production and composition of milk of dairy cows in the afternoon period in the summer and winter seasons.

Variable	Principal Component in Summer		Principal Component in Winter	
	*PC1	PC2	PC1	*PC1
[1]h	0.229	0.113	0.203	0.191
[2]GTHI	0.242	-0.060	0.243	-0.034
[3]THI	0.241	0.070	0.241	0.083
[4]RTL	0.233	-0.103	0.239	-0.075
[5]RT	0.204	0.078	0.240	-0.061
[6]RR	0.241	-0.089	0.244	0.036
[7]ST	0.240	0.004	0.236	0.025
[8]Prod	-0.181	0.282	0.027	-0.132
[9]Fat	-0.086	0.423	-0.150	-0.360
[10]Pro	0.059	-0.413	-0.160	-0.125
[11]Lac	-0.218	-0.181	-0.197	0.201
[12]Sol	-0.101	0.413	-0.204	-0.251
Eigenvalue	16.616	4.806	16.616	4.806
Proportion	0.692	0.200	0.692	0.200
Accumulated	0.692	0.893	0.692	0.893

*PC: principal component; [1]h: enthalpy (h; KJ kg^{-1}); [2]GTHI: globe temperature and humidity index; [3]THI: temperature and humidity index; [4]RTL: radiant thermal load (W m^{-2}); [5]RT: rectal temperature (ºC); [6]RR: respiratory rate (mov min.$^{-1}$); [7]ST: skin temperature (ºC); [8]Prod: milk production (liters); [9]Fat: fat (%); [10]Pro: protein (%); [11]Lac: lactose (%); [12]Sol: soluble solids (%).

According to the results observed in PC1, there was a relationship between protein and milk production and composition, as well as a positive correlation with thermal comfort index and physiological variables; however, no influence on the percentage of protein was observed in the afternoon period for the summer season (Table 6). Corroborating the results of the present study, Lambertz, Sanker, and Gauly (2014), who evaluated the climatic effects on milk yield traits and somatic cell score in lactating Holstein cows in different housing systems, observed that the percentage of milk protein decreased under conditions of thermal stress, as the animals under conditions of discomfort reduce food consumption, a source that is fundamental for the concentration of protein in their milk.

Wildridge et al. (2018) reported the existence of a delay in the animal's response from one to two days due to external weather conditions, identified by the THI. Thus, the non-influence on milk protein by comfort indices and physiological variables probably occurred in response to the better comfort conditions that the animals received in the morning period during pre-milking.

In Figure 3, the PCs of the physiological variables, animal comfort indices, milk production, and milk composition of animals subjected to EACS in the afternoon (summer/winter) are presented.

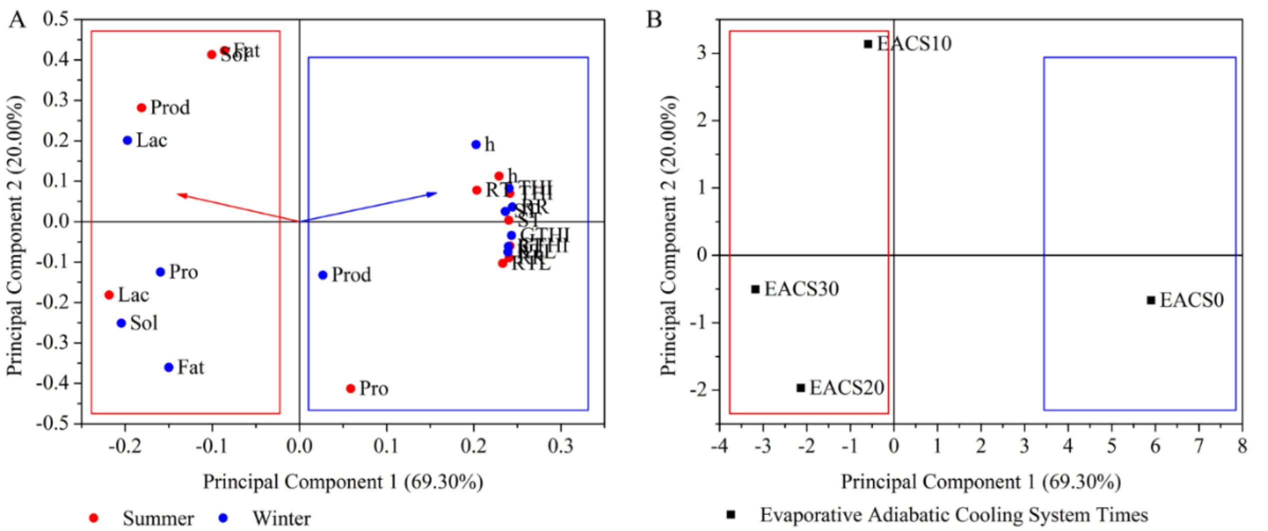

Figure 3. Principal components of the physiological variables, animal comfort indices, and production and composition of milk of cows in the afternoon period in summer and winter (A); PCs of the operating time of the EACS in the afternoon (B).

Note: h: enthalpy (h; KJ kg^{-1}); GTHI: globe temperature and humidity index; THI: temperature and humidity index; RTL: radiant thermal load (W m^{-2}); RT: rectal temperature (°C); RR: respiratory rate (mov min.$^{-1}$); ST: skin temperature (°C); Prod: milk production (liters); Fat: fat (%); Pro: protein (%); Lac: lactose (%); Solid: soluble solids (%); EACS: adiabatic evaporative cooling system operating for times of 0, 10, 20 and 30 minutes.

Unlike the morning period, in which the highest milk production in the summer was observed for animals exposed to EACS for 30 min., in the afternoon period it was observed that animals exposed to EACS for 10 min. had positive responses in milk production (Figure 2A and 2B and Figure 3A and 3B). In the winter season in the afternoon, the use of the EACS did not influence milk production (Table 4).

For the THI in both seasons (summer and winter), its values were higher in the afternoon period, exceeding the threshold of 72 (Table 5). Based on the findings by Wildridge et al. (2018), who observed that the animal's response to conditions of comfort or heat stress is not immediate, with time spent for the animal to respond to that condition to which it was subjected. Therefore, it is safe to say that the response to the effect of air conditioning in the morning in both seasons may be related to improvements in milk production and composition in the afternoon. Thus, the effect of the air-conditioning in the afternoon period contributes to improvements in milk production and composition in the morning of the following day. As for animal comfort indices and physiological variables, the effect of EACS is immediate.

As for the composition of the milk, the cooling times promoted better results, with reductions in comfort indices and physiological variables (Figure 3).

Conclusion

The principal component analysis allowed us to identify the positive influence of evaporative cooling on thermal comfort, physiological responses, and production and composition of milk of lactating cows.

The winter season provided the best thermal comfort conditions for the animals. In the summer season, the 30-minute evaporative cooling time in the morning period increased milk production in the afternoon period. From the time of exposure of 10 minutes to evaporative cooling in the afternoon, there was an improvement in the productive performance of the animals in the morning milking of the following day.

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