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# Effect of heat stress and solar radiation on dry matter intake, biochemical indicators, production, and quality of Holstein and Jersey cows' milk

[*Efeito do estresse calórico e da insolação sobre consumo, indicadores bioquímicos, produção e qualidade do leite de vacas Holandês e Jersey*]

A. *Hauser*, M. *França*[1], R. *Hauser*[2], A. L. *Scheid*[1], L. *Perazzoli*[1], B. P. B. *Mendes*[1], I. P. *Telles*[1], L. C. *Miletti*[1], A. *Thaler Neto*[1]

[1]Centro de Ciências Agroveterinárias, Udesc, Lages, SC, Brasil
[2]Centro Universitário FAI, Uceff, Itapiranga, SC, Brasil

## ABSTRACT

The objective of this work was to compare the dry matter intake, milk yield and quality, physiological and biochemical parameters in Holstein (n=10) and Jersey (n=10) cows under heat stress and insolation, in two treatments: CL – cooling by ventilation and sprinkling and HS – heat stress and insolation. Data were submitted to ANOVA. There was an interaction between treatment and breed and day effect for dry matter intake. For consumption in % of body weight, CL and Jersey cows consumed more. CL cows produced more milk and 3.5% fat-corrected milk. Feed efficiency was similar between treatments and breeds. Fat, lactose, total solids, and somatic cell score did not differ. The concentration of milk urea nitrogen was higher for CL cows. Milk from Holstein cows had greater stability to alcohol, and from HT cows had a greater freezing point of milk. HT cows had higher respiratory rates in the morning and surface temperatures in the afternoon. There were no differences in beta-hydroxybutyrate and glucose concentrations. Heat stress, with insulation, reduces intake, especially in Holstein cows, as well as milk production and increases the freezing point of milk, respiratory rate, and surface temperature.

Keywords**:** thermotolerance, solar radiation, milk composition, milk physical and chemical properties, metabolism

## RESUMO

*O objetivo deste trabalho foi comparar o consumo de matéria seca, a produção e a qualidade do leite, os parâmetros fisiológicos e bioquímicos em vacas das raças Holandesa (n=10) e Jersey (n=10) sob estresse calórico e insolação, em dois tratamentos: CL – resfriamento por ventilação e aspersão; HS – estresse térmico e insolação. Os dados foram submetidos à análise de variância. Houve interação entre tratamento e raça e efeito de dia para consumo de matéria seca. Para consumo em % de peso vivo, vacas CL e Jersey consumiram mais. Vacas CL produziram mais leite e leite corrigido a 3,5% de gordura. A eficiência alimentar foi similar entre tratamentos e raças. Teores de gordura, lactose, sólidos totais e escore de células somáticas não diferiram. A concentração de nitrogênio ureico do leite foi maior para vacas CL. O leite das vacas Holandesas apresentou maior estabilidade ao álcool, e de vacas HT maior crioscopia. Vacas HT apresentaram maior frequência respiratória de manhã e temperatura superficial à tarde. Não houve diferenças para concentração de beta-hidroxibutirato e glicose. O estresse calórico, com insolação, reduz o consumo, especialmente em vacas Holandesas, bem como a produção de leite, com aumento da crioscopia, elevando a frequência respiratória e a temperatura superficial.*

*Palavras-chave: termotolerância, radiação solar, composição do leite, propriedades físico-químicas do leite, metabolismo*

Corresponding author: adrhauser@gmail.com
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## INTRODUCTION

Heat stress is a problem in most regions of the world and puts animal welfare, profitability, and food security at risk (Baumgard *et al.*, 2015). In dairy cows, air temperature and humidity (often combined as a Temperature and Humidity Index, THI), exceeding the species tolerated limits, reduce dry matter intake (DMI), milk yield and fertility, which are associated with physiological changes, including increased body temperature, respiratory rate, panting, salivation, sweating (Garner *et al.*, 2016), important in maintaining homeostasis.

In dairy cows under heat stress, there is a reduction in nutrient absorption, and energy requirements increase due to the activation of thermoregulatory mechanisms (Hill and Wall, 2017). In addition, metabolic and endocrine changes occur (Garner *et al*., 2016) that cause a decrease in milk yield and can change the composition and physicochemical properties (Bernabucci and Calamari, 1998; Bernabucci *et al*., 2002; Hill and Wall, 2017).

At high temperatures, evaporative cooling is the predominant mode of heat loss in dairy cows. This mechanism is affected by wind speed, air temperature, relative humidity, and solar radiation. The physical properties and color of the coat and skin also affect the effectiveness of evaporative cooling. These factors can even increase the absorption of solar radiation (Gebremedhin *et al.*, 2008), a climatic element that greatly impacts cattle breeding in tropical and subtropical regions. Characteristics inherent to the animal, such as high milk production, physiological state, stage of lactation, breed, weight, and age, also make dairy cows more susceptible to heat stress (Garner *et al*., 2016; Taraba and Bewley, 2016). Understanding which environmental stressors most affect dairy cows and thus identifying animals, breeds, and means that effectively mitigate the effects of heat, becomes fundamental in dairy production. Thus, the objective of this work was to determine and compare dry matter intake, milk yield, composition and physicochemical properties, feed efficiency (FE), biochemical and physiological parameters in Holstein and Jersey cows under ventilation and sprinkler cooling, or under solar radiation exposure and without cooling.

## MATERIAL AND METHODS

The experiment was carried out on a farm (Itapiranga, SC, Brazil, latitude 27° 7'2.12"S, longitude 53°47'42.57"W). The climate type is classified as Cfa, according to the Köppen-Geiger classification (Dubreuil *et al*., 2018). All procedures involving the experiment cows were approved by the Animal Ethics and Experimentation Committee of the Santa Catarina State University, under protocol number: 6172220518.

Twenty cows, (n = 10) Holstein and (n = 10) Jersey were enrolled in a two treatments continuous trial: **CL** – Cooled group, and **HS** – Heat stressed group. CL group was under evaporative cooling conditions cooled by sprinklers (each sprinkler 0.9 m apart) over the feed bunk, and fans (diameter = 2m, Turbo Max 2000, Ordemax®, Brazil) over the feed bunk and the resting area. Fans ran continuously, and sprinklers ran intermittently in cycles of 2 minutes spray and 15 minutes off, when the environmental temperature was ≥ 24°C. The water flow rate of each sprinkler was 0.75 L per minute. HS group was without access to ventilation and sprinklers and with solar radiation exposure 1 daily hour. Cows were housed in a Compost Bedded Pack Barn (bedding area = 11.62 $m^2$/cow). The experimental groups were homogeneous to body weight (BW), parity, days in milk (DIM), body condition score (BCS) and milk production, each of the groups with 5 Holstein cows and 5 Jersey cows: CL (BW= 496, 3 ± 123 kg; parity = 3.5±1.58; DIM=233.7±37.5; BCS=2.88±0.32 and milk production = 18.84±3.75kg/day) ; HT (BW= 510.6±112kg; parity = 3.4±1.42; DIM=223.1±88; BCS=3.02±0.21 and milk production = 18.75±5.4kg).

The trial lasted 22 days, with 15 days of adaptation period (AP), and 7 days of experimental period (EP). During AP, cows were adapted to the management, and all the cows were cooled. The last day of the AP was considered day 0 (D0), with all cows being cooled. In EP, only cows in the CL group continued to be cooled. The HT group was not cooled and was exposed to solar radiation for 1 h daily, from 11 to noon, in a no-shaded paddock, with access to water, but without feed access, 20 meters away from the barn. Data collection,

blood, and milk samples were performed on days 0 (D0), 3 (D3), 5 (D5), and 7 (D7). The cows of the two treatments were separated in the barn with electric fences.

Air temperature (T, °C), humidity (RH, %), solar radiation (SR, W/m²) and wind speed conditions (WS, km/h), inside and outside the barn, were measured at 15-minute intervals, with the aid of two weather stations, ATMOS 41 (METER Group, Court Pullman, WA, USA), coupled with a Zentra ZL6 *data logger*. (METER Group, Court Pullman, WA, USA). From the temperature and humidity data, the temperature and humidity index (THI) was calculated according to the equation (Mader *et al.*, 2006): $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$. The black globe (BG) temperature was calculated by the equation: $BG = (1.33 \times T) - (2.65 \times T^{0.5}) + (3.21 \times \log10(SR+1) + 3.5$. If BG > 25, the Heat Load Index (HLI) was calculated according to the following equations: $HLI = 8.62 + (0.38 \times RH + (1.55 \times BG) - 0.5 \times WS + e^{(2.4 - WS)}$. If BG > 25, the Heat Load Index (HLI) was calculated according to the following equations: $HLI = 10.66 + (0.28 \times RH + (1.3 \times BG) - WS$ (Gaughan *et al*. 2008). BW was measured using a digital scale (Filizola Balanças Industriais, Brazil), and BCS (Ferguson *et al*., 1994), both at the beginning of the AP. Respiratory rate (RR) (movements/min) was measured by counting flank movements for 15 seconds and then multiplied by four to obtain the respiratory rate per minute (Pilatti, 2017). Heart rate (HR) (beats/min) was determined using a stethoscope. Surface temperature (ST) was determined in the afternoon, with an infrared thermometer 1 m away and measured at 5 points on the cow's body. The ST value considered was the arithmetic mean of the 5 points determined, according to the methodology described by Pilatti (2017). Rectal temperature (RT) was determined with the aid of a clinical thermometer. Ruminal movements (RM) were determined by 2 minutes observation. RR, HR, ST, RT, and RM were determined twice a day, in the morning and the afternoon, at 10 am and at 7 pm, on days 0, 3, 5, and 7. BW was measured using a digital scale (Filizola Balanças Industriais, Brazil), and BCS (Ferguson *et al*., 1994), both at the beginning of the AP. Respiratory rate (RR) (movements/min) was measured by counting flank movements for 15 seconds and then multiplied by four to obtain the respiratory rate per minute (Pilatti, 2017). Heart rate (HR) (beats/min) was determined using a stethoscope. Surface temperature (ST) was determined in the afternoon, with an infrared thermometer 1 m away and measured at 5 points on the cow's body. The ST value considered was the arithmetic mean of the 5 points determined, according to the methodology described by Pilatti (2017). Rectal temperature (RT) was determined with the aid of a clinical thermometer. Ruminal movements (RM) were determined by 2 minutes observation. RR, HR, ST, RT, and RM were determined twice a day, in the morning and the afternoon, at 10 am and at 7 pm, on days 0, 3, 5, and 7.

The diet was formulated to meet 100% of the nutritional requirements, according to the Nutrient (2001) (Table 1), being supplied as a total diet (TMR), individually to each cow. At feedings, cows restrained in headlocks by 1.5 hour, four times daily: 08:00, 12:30, 16:00, and 19:00. The cows received fresh feed at 08:00 and at other times, whenever necessary, it was replenished. The amount of feed offered to each animal was adjusted daily to provide between 5 and 10% leftovers. Leftovers were weighted after the last feeding time, to determine daily intake. Leftovers and Feed samples were collected on day 15 of the AP period and days 3, 5, and 7 of the EP. Samples composed of leftovers from each treatment (CL and HT), corn silage, Tifton 85 hay, commercial concentrate, and soybean meal were collected. The samples were sent to the 3RLab Laboratory (Chapecó, SC, Brazil) for chemical analysis by NIRS (Near infrared spectrophotometry). The DM content of feeds and diet samples was measured in forced air circulation equipment, Air Fryer type (Fryer Silver AFS-02, Agratto, China) at a temperature of 135°C for 30 minutes (Wallau and Vendramini, 2019).

Table 1. Composition and chemical composition of the diet based on dry matter (DM) fed to cows

| Item, % DM unless specified | Value |
|---|---|
| Corn silage | 45.55 |
| Tifton hay | 5.63 |
| Commercial concentrate [1] | 47.31 |
| Mineral mix [2] | 1.51 |
| NDF | 31.53 |
| ADF | 18.02 |
| CP | 16.73 |
| Ash | 9.38 |
| EE | 3.99 |
| NFC [3] | 39.15 |
| NEl (Mcal/kg DM) | 1.64 |

[1] Puro Milk Bypass 26 PB (Puro Trato®). Assurance Levels, NDF=18%, ADF=9%, Ash 9%, Ether Extract=5%, Moisture=13% Ca=12.5%, P=0.6%, S=0.13%, Mg=0.3%, K= 0.002%, Co=0.92mg/kg, Cu=27.37mg/kg, Zn=146mg/kg, Fe=40mg/kg, Mn=96mg/kg, I=2.12mg/kg, Se=1mg/kg kg, Vitamin A= 11800 IU/kg, Vitamin D= 2800 IU/kg, Vitamin E 50 IU/kg, Biotin 6 mg/kg and Monensin 40 mg/kg.
[2] Mineral mixture containing 40% sodium bicarbonate, 40% sodium chloride and 20% mineral vitamin (Miner A1 Pro Biotin, Ca=23%, P=6%, S=2%, Mg=1.2%, K =0.35%, Na=4.7%, Cl=7%, Co=95mg/kg, Cu=950mg/kg, Zn=6000mg/kg, Fe=700mg/kg, Mn=3000mg/kg, I = 80 mg/kg, Se = 30 mg/kg, Vitamin A= 600000 IU/kg, Vitamin D= 120000 IU/kg, Vitamin E 2500 IU/kg, Niacin 2500 mg/kg, Biotin 150 mg/kg.

Cows were milked twice a day, at 07:00 and 17:30. Milk production was measured daily during the AP and EP, using WAIKATO milk meters (WAIKATO Multi Meter®, New Zealand). On days 0, 3, 5, and 7, milk samples were collected from each cow, consisting of an aliquot of the afternoon and morning milk production. The samples were placed in flasks with bronopol as a preservative and sent to the Laboratory of the Associação Paranaense de Criadores de Bovinos da Raça Holandesa (APCBRH) in Curitiba, PR, for the analysis of fat, protein, lactose, total solids, and milk urea nitrogen (MUN), by the infrared method (Bentley Combisystem, Bentley Instruments Inc., USA). Also, somatic cell counts were analyzed by flow cytometry (Delta Combiscope, Advanced Instruments Inc., USA).

A second milk sample was collected from each cow, composed of an aliquot of milk production in the afternoon and the morning, placed in containers without preservatives, and kept refrigerated for 12 h between 3 and 8ºC, for the determination of pH, test of the alcohol, titratable acidity, and Freezing point of milk. The pH of the milk was measured using a pH meter (Kasvi K39, Kasvi, Brazil). The alcohol test was performed by mixing 2 mL of milk and 2 mL of ethanol in a Petri dish on a black background. The ethanol solutions used ranged from 56 to 90% (v/v) and were prepared with ethanol concentrations varying at 2% intervals. Milk samples analyzed by the alcohol test were considered unstable when clot formation occurred. Titratable acidity was determined in Dornic degrees ($^{o}$ D) according to the methodology described in IN 68 (Brasil, 2006) in which 4 to 5 drops of 1% phenolphthalein solution were added to a 10mL sample of milk. The resulting solution was titrated with a 0.1mol/L NaOH solution until a persistent pink color appeared for approximately 30 seconds.

The production of 3.5% fat corrected milk (3,5% FCM) was estimated from the equation: 3.5% FCM (kg) = (0.4324 x kg of milk) + (16.216 x kg of fat). Feed efficiency (FE) was calculated by dividing the milk yield by the DMI and the 3.5% FCM FE was calculated using the formula: FE = 3.5% FCM (kg)/DMI (kg) (Hutjens, 2010).

On days 0, 3, 5, and 7, after feeding at 08:00, blood samples were collected from the coccygeal vein with the aid of a 21 G needle. An amount of blood was collected to fill two vacutainer tubes of 10 mL with lytic heparin (BD, USA), and two 4 mL vacutainer tubes with $K_2$ EDTA (BD, USA) and two 10mL tubes with clot activator for serum collection (BD, USA). After collection,

the blood was centrifuged at 1328*g* for 10 minutes. The resulting plasma and serum samples were packed in 2mL plastic tubes and frozen until biochemical analysis.

Beta-hydroxybutyrate (BHB) and glucose concentrations in each blood sample were determined immediately after collection in blood packed in vacutainer tubes containing sodium EDTA. Analyzes were performed using a portable analyzer (Optium Xceed ®, Abbott, USA), with glucose and beta-hydroxybutyrate assay strips.

SCC data were transformed to somatic cell score (SCS), using the equation SCS = $\log_2$(SCC/100) + 3. Data were analyzed in a factorial arrangement 2 x2 (treatments x breeds) utilizing ANOVA and previously tested for normality of residues by the Shapiro-Wilk test, using the SAS statistical package (SAS, 2017). The statistical model was composed of the effects of treatment, breed, experiment days, interactions between treatment and breed, treatment and day, day and breed, and the covariate measured for each variable on the D0. The results were expressed as means of least squares ± standard error of the mean (SEM), being considered different if P≤0.05.

## RESULTS

The thermal conditions of the environments in which the cows were housed during the experimental period are characterized by the temperature and humidity index (THI) and the heat load index (HLI) of the experimental period (EP) throughout the days and are shown in the Figures 1 and 2, respectively.

There was interaction between treatment and breed (P = 0.014) and day effect for DMI (P < 0.0001). When DMI was calculated as % body weight (DMI as % BW), CL (P=0.011), and Jersey (P=0.041) cows consumed more, in addition to a day effect (P = 0.0001; Table 2 and 3).

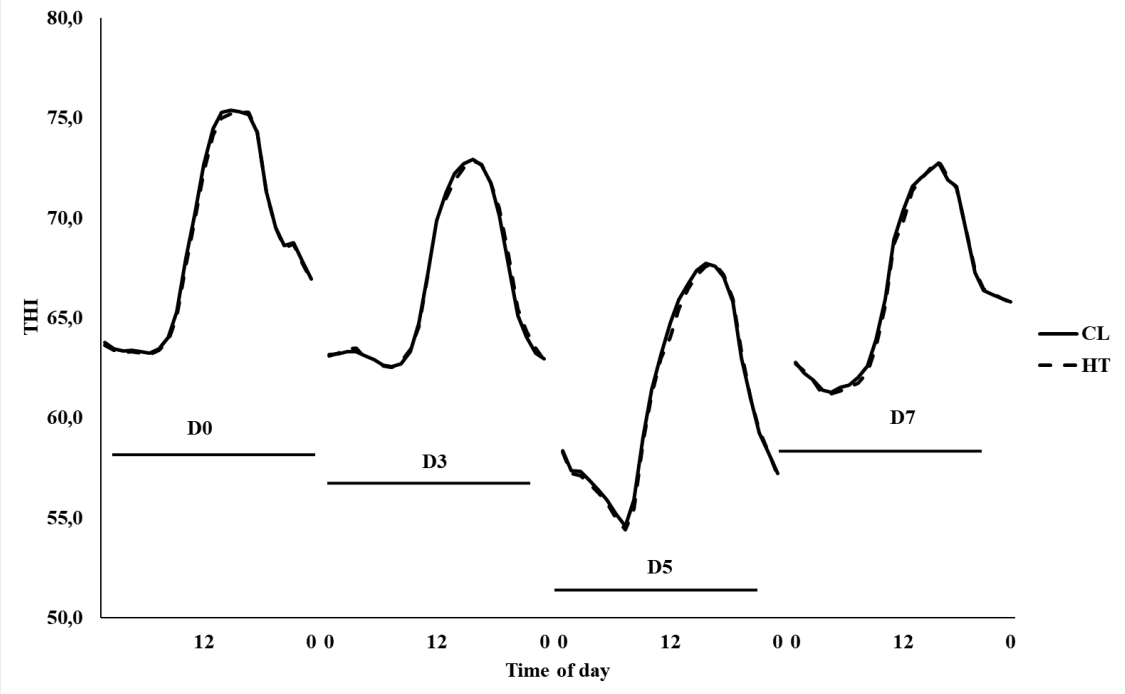


Figure 1. Temperature and humidity index (THI) during the experimental period (EP) in the environments of cooled group (CL), and heat stressed group (HT).

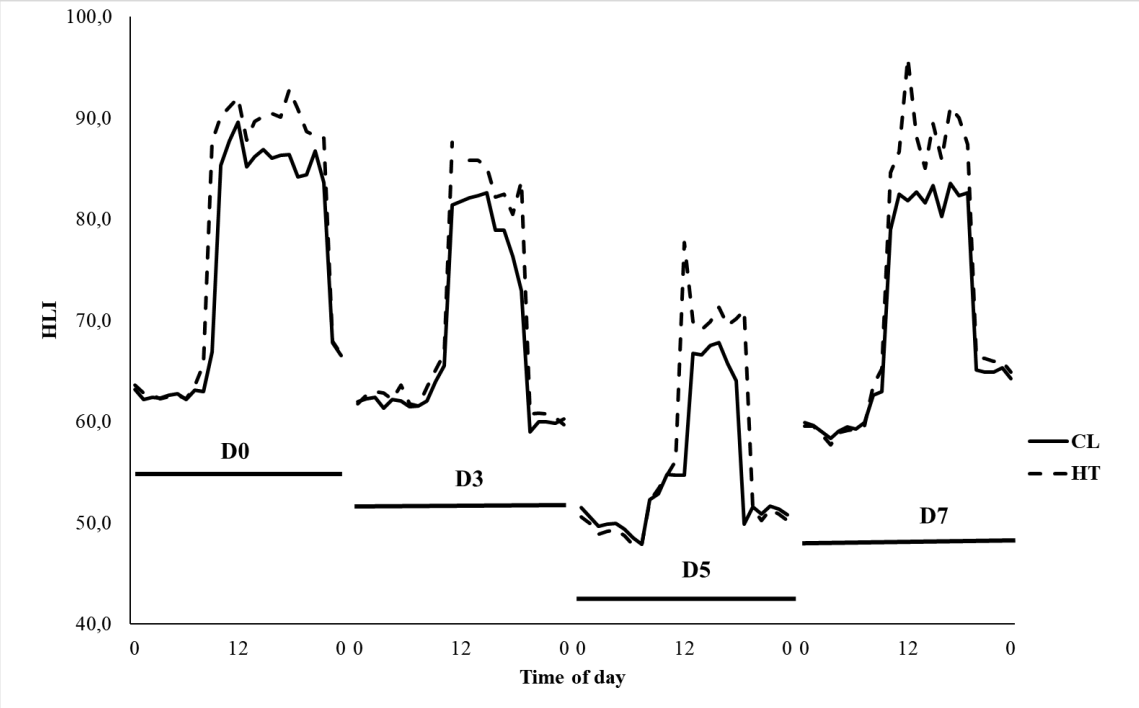


Figure 2. The thermal load index (HLI) during the experimental period (EP) in the environments of cooled group (CL), and heat stressed group (HT).

Table 2. Least squares mean, standard error of the mean (SEM) and P value for dry matter intake (DMI), feed efficiency (FE), milk yield, milk composition, milk urea nitrogen (MUN), and somatic cell score (SCS) of cooled group (CL), and heat stressed group (HT)

| Variables | Treatment | | SEM | Breed | | SEM | P-value | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL | HT | | H | J | | Treatment | Breed | Day | Treatment x Breed | Treatment x Day | Day x breed | Covariate |
| DMI (kg) | 17.05 | 15.23 | 0.436 | 17.56 | 14.72 | 0.627 | 0.006 | 0.014 | <0.0001 | 0.014 | 0.826 | 0.205 | 0.174 |
| DMI as % BW (%) | 3.36 | 3.03 | 0.09 | 3.04 | 3.35 | 0.10 | 0.011 | 0.041 | 0.0001 | 0.107 | 0.798 | 0.647 | 0.046 |
| Milk (kg/day) | 21.92 | 19.58 | 0.498 | 20.82 | 20.68 | 0.505 | 0.002 | 0.850 | 0.053 | 0.848 | 0.963 | 0.241 | <0.0001 |
| Milk corrected to 3.5% fat (kg/day) | 23.21 | 20.80 | 0.404 | 22.14 | 21.86 | 0.403 | 0.0002 | 0.627 | 0.294 | 0.317 | 0.475 | 0.023 | <0.0001 |
| Feed efficiency | 1.30 | 1.29 | 0.044 | 1.27 | 1.32 | 0.043 | 0.816 | 0.368 | 0.0001 | 0.199 | 0.614 | 0.984 | <0.0001 |
| FE corrected at 3.5% fat | 1.41 | 1.36 | 0.047 | 1.33 | 1.46 | 0.053 | 0.738 | 0.107 | 0.0003 | 0.190 | 0.991 | 0.389 | <0.0001 |
| Fat (%) | 3.99 | 3.88 | 0.125 | 3.87 | 4.00 | 0.152 | 0.527 | 0.615 | 0.013 | 0.932 | 0.939 | 0.014 | 0.002 |
| Protein (%) | 3.51 | 3.35 | 0.039 | 3.44 | 3.42 | 0.043 | 0.009 | 0.735 | 0.025 | 0.635 | 0.017 | 0.073 | <0.0001 |
| Lactose (%) | 4.33 | 4.30 | 0.040 | 4.35 | 4.28 | 0.039 | 0.559 | 0.267 | <0.0001 | 0.914 | 0.904 | 0.900 | <0.0001 |
| Total solids (%) | 12.75 | 12.43 | 0.138 | 12.51 | 12.67 | 0.166 | 0.106 | 0.571 | 0.013 | 0.870 | 0.594 | 0.007 | 0.001 |
| MUN (mg/dL) | 21.92 | 19.58 | 0.498 | 20.82 | 20.68 | 0.505 | 0.002 | 0.850 | 0.053 | 0.848 | 0.963 | 0.241 | <0.0001 |
| SCS | 3.57 | 3.85 | 0.428 | 4.47 | 3.63 | 0.321 | 0.372 | 0.068 | 0.983 | 0.513 | 0.236 | 0.834 | <0.0001 |

The treatments affected milk yield (P = 0.002) and 3.5% fat corrected milk (P = 0.0002), which were higher in cows in the CL treatment. There was an interaction between day and breed (P=0.023) for 3.5% FCM. Feed efficiency (FE) and FE 3.5% FCM were similar between treatments and breeds, with only a day effect. For protein content, there was an interaction between

treatment and day (P = 0.017). Fat, lactose, total solids, and SCS values did not differ between treatments. There was a day effect for lactose (P < 0.0001) and interaction between day and breed for fat (P = 0.014) and total solids contents (P = 0.007). The concentration of MUN (P=0.002) was higher in milk from CL cows. Detailed results are shown in Table 2, Table 3, and Figures 3 (A, B, C, and D), Figures 4 (A, B, C, and D), and Figures 5 (A, B, C, and D).

Regarding the physicochemical properties of the milk, there was no difference between the treatments regarding the mean values of alcohol test and titratable acidity (Table 4), but Holstein cows showed greater stability to ethanol (P=0.029). There was an interaction between treatment and day for pH values (P = 0.0001). The freezing point of milk was higher in HT cows (P = 0.007), in addition to day effect (P = 0.0003). The other results are shown in Table 4 and Figure 7 (A, B, C, and D).

Table 3. Total dry matter intake (DMI), and as % of body weight (DMI as % of BW) of cooled (CL) or heat stressed group (HT), and by breed of the cows

| | DMI (kg/DAY) | | | DMI as % BW | | |
|---|---|---|---|---|---|---|
| | Holstein | Jersey | Mean | Holstein | Jersey | Mean |
| CL | 19.27 Aa | 14.89 Ab | 17.05 A | 3.30 | 3.41 | 3.36 A |
| HT | 15.85 Bb | 14.59 Ab | 15.23 B | 2.76 | 3.28 | 3.02 B |
| Mean | 17.56 to | 14.72 b | | 3.03 b | 3.35 to | |

*Same capital letters in columns and lowercase letters in rows do not differ from each other at the level of 5%.

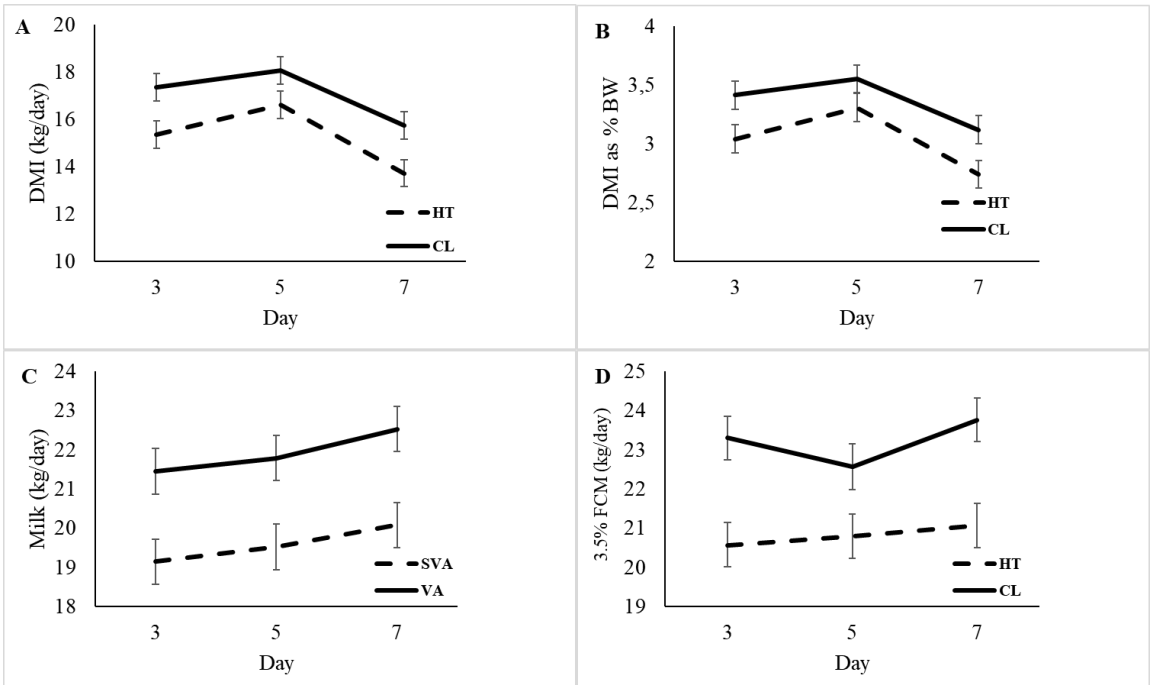


Figure 4. Dry matter intake (DMI) and milk production of cooled (CL) or heat stressed group (HT), as a function of treatment days: A) DMI, B) dry matter consumption as % body weight (DMI as % BW), C) milk production, D) milk production corrected to 3.5% fat (3.5% FCM).

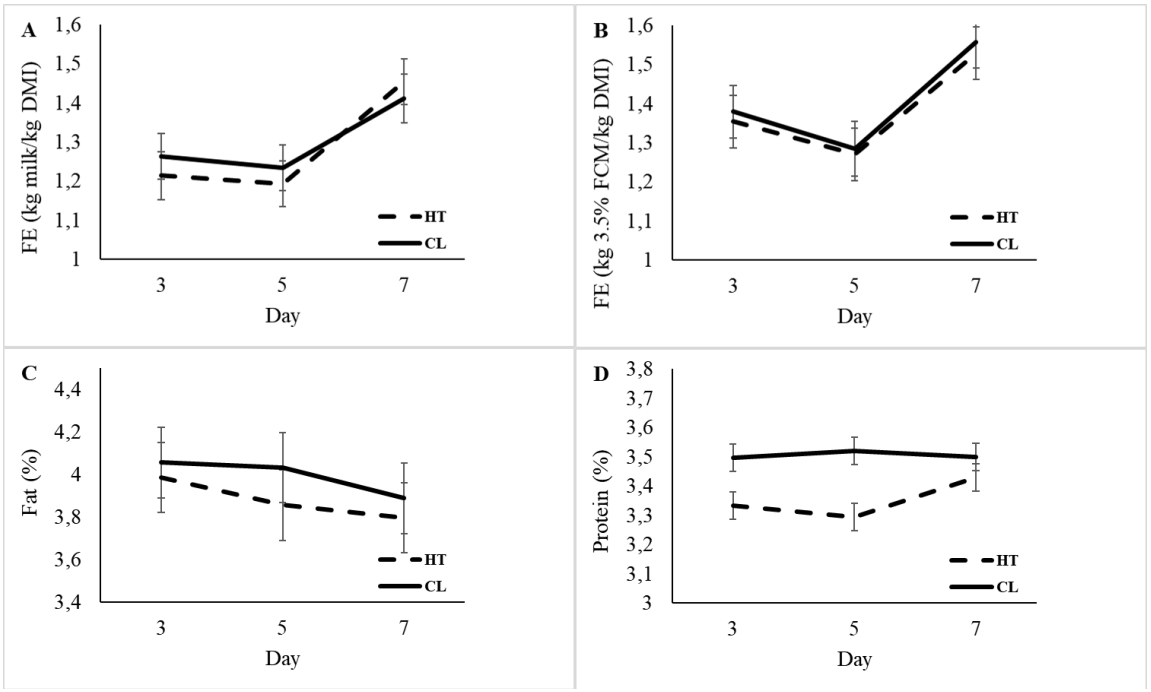


Figure 5. Feed efficiency (FE), and milk composition of cooled (CL) or heat stressed group (HT), as a function of treatment days: A) feed efficiency, B) feed efficiency corrected to 3.5% fat, C) fat content, D) protein content.

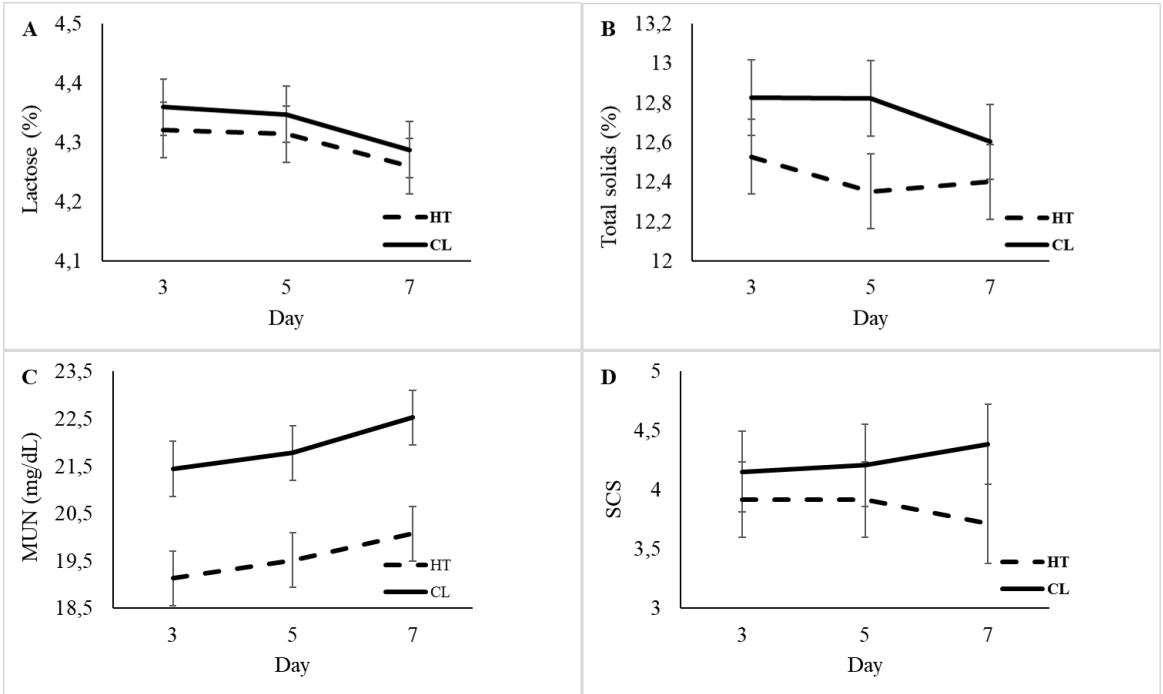


Figure 6. Composition of milk from of cooled (CL) or heat stressed group (HT), as a function of treatment days: A) lactose content, B) total solids content, C) milk urea nitrogen concentration, D) somatic cell score.

Table 4. Mean of least squares, standard error of the mean (SEM), and P-value for alcohol test, titratable acidity (TA), pH, and freezing point of milk (FPM) of cooled (CL) or heat stressed group (HT), and by breed of the cows (H = Holstein, and J = Jersey)

| Variables | Treatment | | | Breed | | | P-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL | HT | SEM | H | J | SEM | Treatment | Breed | Day | Treatment x Breed | Treatment x Day | Day x Breed | Covariate |
| Alcohol (%) | 78.18 | 80.08 | 1.275 | 81.22 | 77.05 | 1.275 | 0.305 | 0.029 | 0.113 | 0.725 | 0.979 | 0.091 | 0.175 |
| TA ($^0$D) | 16.72 | 15.84 | 0.480 | 16.27 | 16.30 | 0.485 | 0.203 | 0.967 | 0.047 | 0.245 | 0.850 | 0.110 | 0.207 |
| pH | 6.77 | 6.83 | 0.009 | 6.80 | 6.79 | 0.010 | 0.0003 | 0.516 | <0.0001 | 0.252 | <0.0001 | 0.178 | 0.053 |
| FPM ($^0$H) | -0.5422 | -0.5365 | 0.001 | -0.5406 | -0.5382 | 0.001 | 0.007 | 0.248 | 0.0003 | 0.881 | 0.979 | 0.900 | 0.232 |

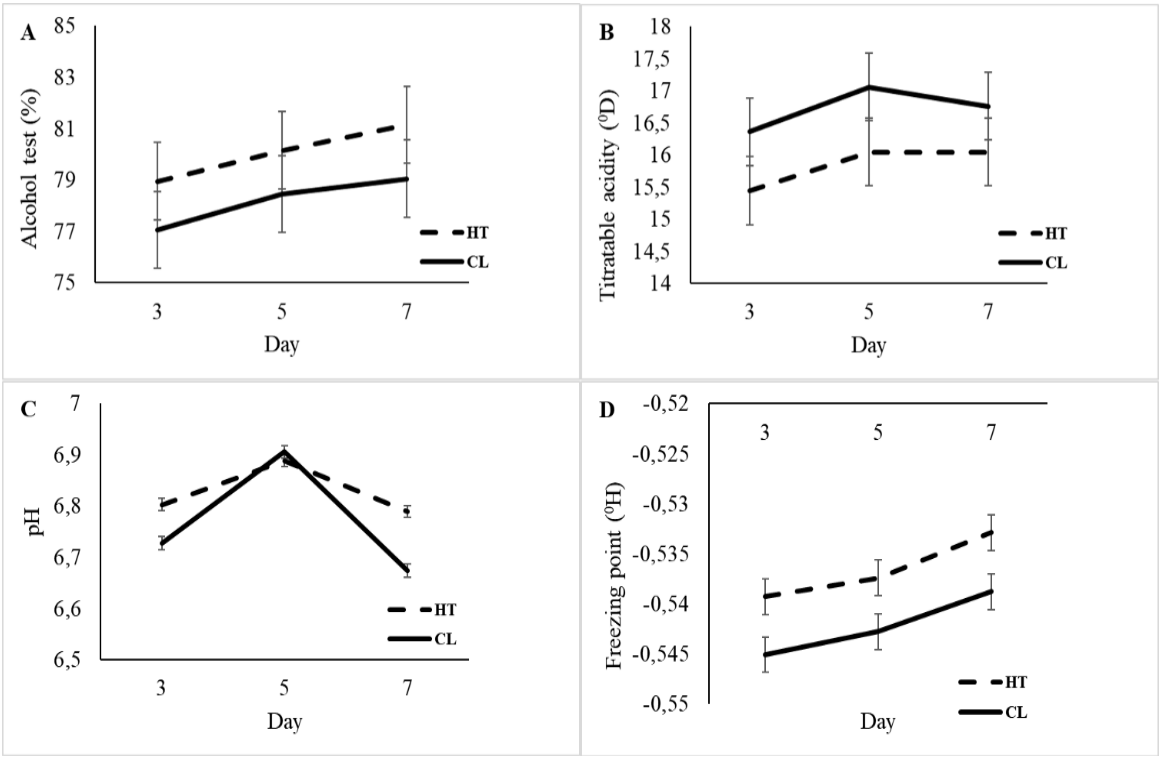


Figure 7. Milk physical-chemical properties of cooled (CL) or heat stressed group (HT), as a function of the days of treatment: A) Alcohol test, B) titratable acidity, C) pH and D) Freezing point of Milk.

In the evaluation of physiological parameters, treatment differences were found in the morning RR parameters (higher in the HT treatment; P= 0.047), and afternoon ST (higher in the HT treatment; P=0.015), as shown in Table 4. Detailed results are shown in Table 4 and Figures 8 (A, B, C, and D) and 9 (A, B, and C). There was a day effect (P < 0.0001) for morning RR, morning HR, morning RT, afternoon RR, and afternoon ST. There was an interaction between treatment and day for afternoon RT (P = 0.0001) and afternoon RM (P = 0.002). There was an interaction between day and breed for afternoon HR (P = 0.001). Detailed results are shown in Table 5 and Figures 8 (A, B, C, and D) and 9 (A, B, and C).

In the biochemical parameters evaluated, there was no difference between treatments and breeds for the concentration of beta-hydroxybutyrate and glucose (Table 6 and Figure 10).

Table 5. Mean of least squares, standard error of the mean (SEM), and P value for the physiological parameters of cooled (CL) or heat stressed group (HT), and by breed of the cows (H = Holstein, and J = Jersey)

| Variables | Treatment | | SEM | Breed | | SEM | P-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CL | HT | | H | J | | Treatment | Breed | Day | Treatment x Breed | Treatment x Day | Day x Breed | Covariate |
| Morning | | | | | | | | | | | | | |
| FR (resp./min) | 51.51 | 59.02 | 2,456 | 54.08 | 56.45 | 2,381 | 0.047 | 0.493 | <0.0001 | 0.926 | 0.120 | 0.458 | 0.734 |
| HR (bat./min) | 76.73 | 78.57 | 2,474 | 77.90 | 77.40 | 2,152 | 0.653 | 0.870 | <0.0001 | 0.723 | 0.521 | 0.212 | 0.498 |
| TR ( $^0$C) | 38.22 | 38.20 | 0.080 | 38.21 | 38.21 | 0.080 | 0.810 | 0.965 | <0.0001 | 0.466 | 0.066 | 0.541 | 0.007 |
| Afternoon | | | | | | | | | | | | | |
| FR (resp./min) | 52.23 | 59.37 | 2,647 | 54.24 | 57.36 | 2,556 | 0.076 | 0.399 | <0.0001 | 0.982 | 0.217 | 0.234 | 0.463 |
| HR (beats/min) | 89.40 | 90.13 | 2,379 | 86.28 | 93.25 | 2,046 | 0.848 | 0.024 | <0.0001 | 0.686 | 0.226 | 0.001 | 0.409 |
| TS ( $^0$C) | 36.46 | 36.77 | 0.082 | 36.51 | 36.72 | 0.077 | 0.015 | 0.059 | <0.0001 | 0.469 | 0.530 | 0.910 | 0.643 |
| TR ( $^0$C) | 38.40 | 39.46 | 0.082 | 38.88 | 38.97 | 0.071 | <0.0001 | 0.353 | <0.0001 | 0.763 | 0.0001 | 0.533 | 0.001 |
| MR (mov./min) | 3.76 | 2.77 | 0.195 | 2.99 | 3.54 | 0.199 | 0.001 | 0.064 | <0.0001 | 0.724 | 0.002 | 0.752 | 0.856 |

RR: respiratory rate, HR: heart rate, RT: rectal temperature, ST: surface temperature

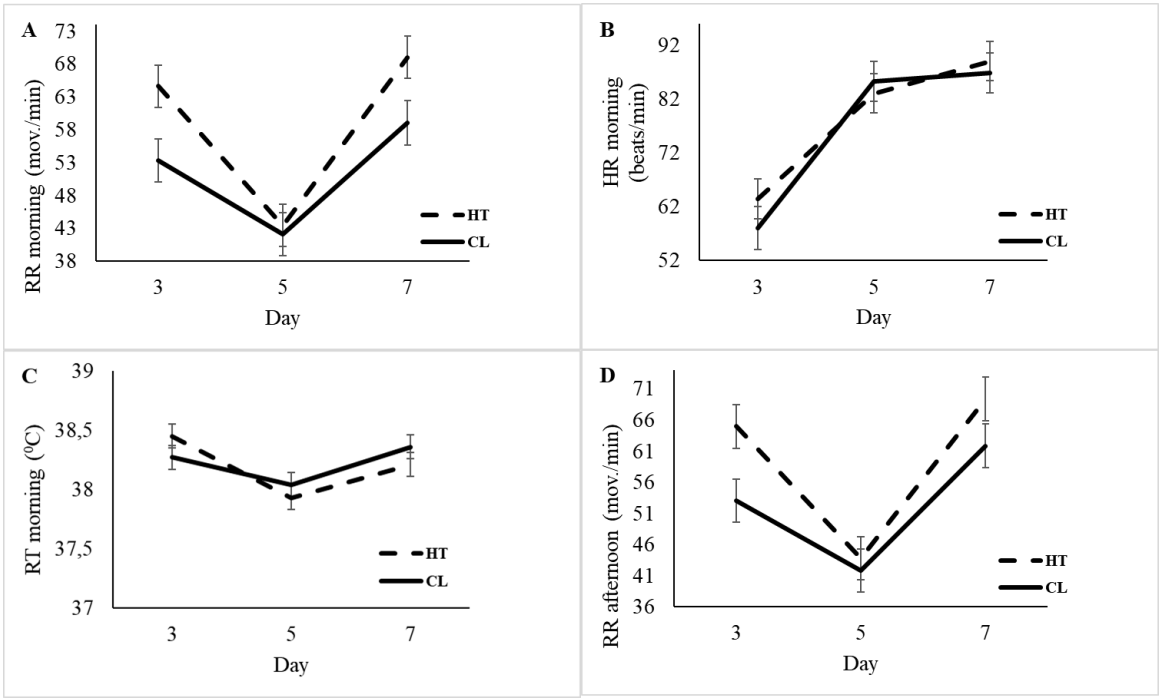


Figure 8. Physiological parameters of cooled (CL) or heat stressed group (HT) as a function of the days of treatment: A) Morning respiratory rate, B) Morning heart rate, C) Morning rectal temperature, D) Afternoon respiratory rate.

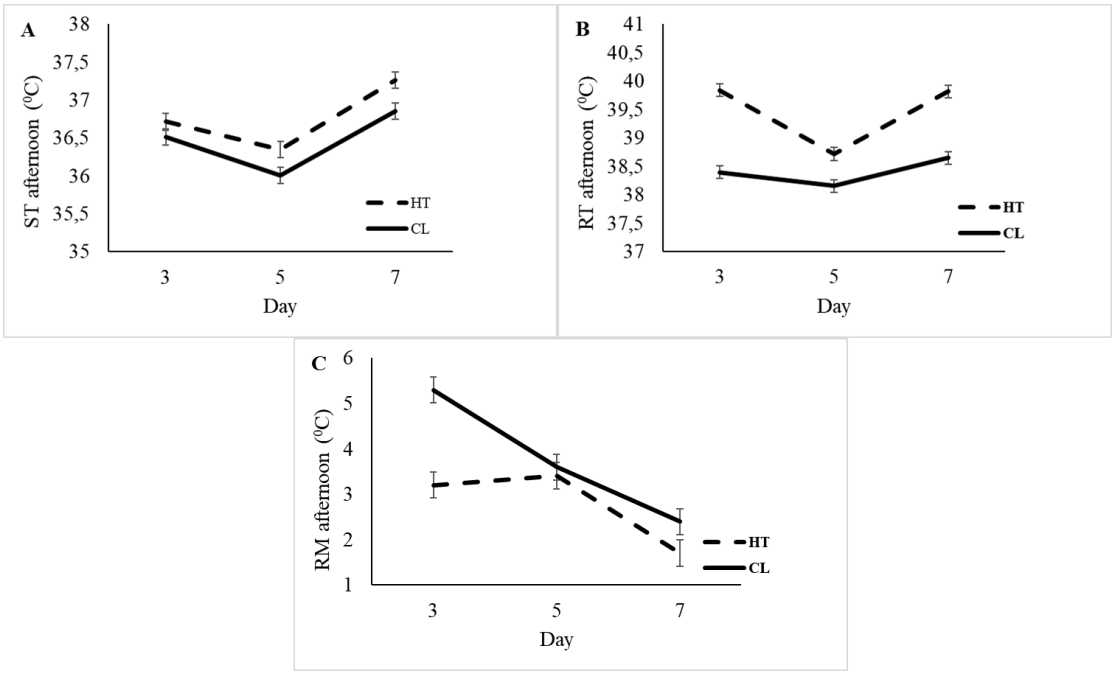


Figure 9. Physiological parameters of cooled (CL) or heat stressed group (HT), as a function of the days of treatment: A) Afternoon surface temperature, B) Afternoon rectal temperature, C) Afternoon ruminal movements.

Table 6. Mean of least squares, standard error of the mean (SEM), and P value for the glucose and beta-hydroxybutyrate of cooled (CL) or heat stressed group (HT)

| | Treatment | | | Breed | | | P-value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | CL | HT | SEM | H | J | SEM | Treatment | Breed | Day | Treatment x Breed | Treatment x Day | Day x Breed | Cov. |
| Glucose (mg/L) | 57.45 | 53.45 | 1,670 | 56.91 | 53.99 | 1,710 | 0.101 | 0.247 | 0.300 | 0.992 | 0.465 | 0.625 | 0.019 |
| BHB (mmol/L) | 1.08 | 1.22 | 0.058 | 1.08 | 1.22 | 0.059 | 0.099 | 0.118 | 0.365 | 0.079 | 0.239 | 0.980 | 0.004 |

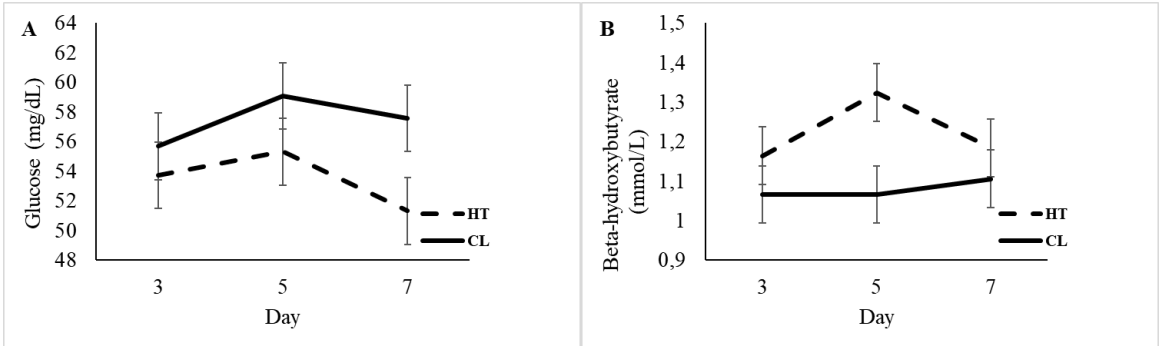


Figure 10. Biochemical parameters evaluated in cows from treatments CL (ventilation and sprinkling) and HT (with solar radiation and without ventilation and sprinkling) as a function of days of treatment: A) glucose; B) beta-hydroxybutyrate.

## DISCUSSION

The HLI and THI values determined in the experimental period show environmental conditions that can cause heat stress in dairy cows (Figures 1 and 2). Zimbelman *et al*. (2009), based on physiological and productive data, mentioned that THI values up to 68 provide comfortable conditions for high-producing dairy cows. However, this index does not take into account the heat gained by the animal due to the microenvironment, the effect of wind speed and solar radiation, and environmental elements that considerably affect the heat exchange mechanisms (Lees *et al.*, 2018). For Gaughan *et al*. (2008), HLI values indicate thermal comfort conditions for cattle when HLI < 70; warm, HLI > 70 < 77; hot, HLI > 77 < 86; very hot, HLI > 86. Considering these data together with physiological parameters, the cows in the HT group presented higher RR in the morning and the afternoon, possibly indicating physiological changes to dissipate accumulated heat.

The absence of ventilation and sprinkler cooling and the short daily period of solar radiation decreased the DMI as a % of BW. Heat stress decreases the DMI in kg of DM per day of Holstein cows. In tropical and subtropical climate regions, solar radiation is one of the climatic elements that most impact cattle breeding (Brettas *et al.*, 2017). The immediate effect of heat in dairy cows is a reduction in DMI and with it, a reduction in metabolic rate and endogenous heat production (Hill and Wall, 2017). However, the decrease in DMI explains only part of the reduction in milk production in cows under heat stress (Rhoads *et al*., 2009; Wheelock *et al*., 2010). Animals under heat stress initiate metabolic changes that do not reflect their nutritional plan, indicating changes in metabolism and productivity regardless of reduced feed consumption (Baumgard *et al.*, 2015). For Tao *et al*. (2018a), the effect of heat stress depends on the lactation stage, observing that cows in the middle of the lactation immediately decrease milk production, while cows at the beginning and end of lactation reduce milk production one week after the beginning of heat stress, when compared to cows under cooling conditions. For Wheelock *et al*. (2010) and Tao *et al*. (2018b), in dairy cows in the middle of the lactation, the effects of heat cause an increase in glucose uptake by other tissues to the detriment of the mammary gland, thus limiting glucose availability for lactose synthesis. It may explain in part the reduction in milk production and changes in milk composition, commonly observed in cows under heat stress.

Cows in the CL and HT groups presented similar FE. In general, environmental conditions of intense cold or heat affect the FE. Heat stress increases energy requirements for maintenance and can reduce FE as more nutrients are needed for functions such as the thermoregulation process (Hutjens, 2005). The short experimental period and the mild to moderate heat stress conditions of this experiment may explain the similar feed efficiencies of the two treatments. In addition, the western region of Santa Catarina, where the experiment was carried out, records high temperatures, mainly from November to March (Climatologia, 2021). Adverse environmental conditions trigger the acclimatization process, coordinated response to improving the fitness of dairy cows to various environmental stressors such as high temperature, humidity, and solar radiation (Collier *et al.*, 2018). Thus, similar feed efficiencies may also reflect the state of acclimatization of cows due to environmental conditions before the experiment.

Heat stress not only affects milk production but also its composition and the results of this experiment show that HT cows produced less milk, less milk corrected to 3.5% fat, and lower concentration of MUN. Gao *et al*. (2017), observed a 4.1% reduction in milk protein content in heat-stressed (HS) cows when compared to *pair-fed* cows under thermoneutral conditions (PFTN), (HS = 2.57% versus PFTN = 2.68%; P= 0.0076). They considered that heat stress not only reduces milk production but also affects protein synthesis in the mammary gland and linked this to an increase in protein turnover in the body and competition for amino acids between caseins and structural proteins. For Tao *et al*. (2018a), in cows under heat stress, changes in metabolism and nutrient absorption can affect the mammary gland, compromising the capacity of synthesis and mammary growth. For Bernabucci and Calamari (1998), in cows under heat stress, the reduction in milk protein content may be related to lower microbial protein synthesis due to lower protein and energy intake resulting from the decrease in DMI and

consequent decrease of the duodenal amino acid flow. They also consider that in cows under heat stress there may be a reduction in milk protein content as more amino acids are used as precursors of glucose and energy via gluconeogenesis. The concentration of MUN, in general, reflects the balance of energy and protein in the rumen and, consequently, the lower total nitrogen intake, by a decrease in DMI, may decreased the MUN (Green, 2012).

In this experiment, it was observed that the average test values of alcohol and titratable acidity were similar. HT cows, however, produced milk with greater freezing point compared to CL cows. For Bernabucci and Calamari (1998), freezing point of milk changes are dependent on physiological changes that occur in cows under heat stress. Lactose and dissolved ions such as chlorides, and calcium, account for about 80% of the decrease in the freezing point of milk, while urea, short-chain fatty acids, and $CO_2$ account for about 20% (Töpel, 2016). Variations in the soluble components of milk and the degree of hydration of cows directly reflect on milk freezing point of milk and may explain the results of this experiment.

Regarding the biochemical parameters evaluated, the concentrations of beta-hydroxybutyrate and glucose were similar between treatments and possibly reflect the acclimatization process or the stage of lactation of the cows.

## CONCLUSION

Heat stress and solar radiation exposure reduce the dry matter intake of dairy cows. Holstein cows subjected to heat stress with solar radiation exposure have lower total dry matter intake than Jersey cows. Holstein and Jersey cows, under heat stress, without access to cooling and under a short period of solar radiation in the hottest hours of the day, reduce milk yield and milk urea nitrogen concentration, but the freezing point of milk increases. Heat stress affects physiological parameters and increases respiratory rate and surface temperature. Heat stress does not affect the blood concentration of beta-hydroxybutyrate and glucose.

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