www.igi-global.com

# Effect of Evaporative Cooling System on Behavior, Milk Yield, and Milk Quality of Holstein Cows in Tropical Climate

Cristiane Gonçalves Titto[1], Evaldo Antonio Lencioni Titto[1], Rafael Martins Titto[1], Alfredo Manuel Franco Pereira[2], Messy Hannear de Andrade Pantoja[1], João Alberto Negrão[3]

*[1]Departamento de Zootecnia, Faculdade de Zootecnia e Engenharia de Alimentos Universidade de São Paulo, Pirassununga, SP, Brazil.*

*[2]Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Évora, Portugal.*

*[3]Departamento de Ciências Básicas, Faculdade de Zootecnia e Engenharia de Alimentos Universidade de São Paulo, Pirassununga, SP, Brazil.*

## EXECUTIVE SUMMARY

*In a tropical climate, air temperature can be stressful for Holstein cows along the year. Our objective was to determine how the use of ventilation and fogging on a freestall barn can positively change the behavior, milk production and milk quality of Holstein cows in all seasons. Twenty-eight lactating cows were divided into two groups: cooling group (CG) and non-cooled group (NCG). For different weather patterns throughout the year, behaviors (position, posture and activity) were observed every 30 min during the day. Milk production was measured and milk samples were collected for analysis of protein, fat, lactose, total solids, and somatic cell count. All animals spent most of the day standing in the shade eating, ruminating and idling regardless of the season ($P<0.05$). Also, animals under cooling system showed greater milk production, fat content, and higher somatic cell count ($P<0.01$). Provision of cooling system during the warmer months of the year proved to be efficiently increasing the feeding time, milk production, and milk quality of lactating cows.*

Keywords: Air temperature, Eating, Drinking, Fat, Fogging, Freestall, Heat Stress, Idleness, Lactose, Protein, Somatic cells, Ruminating, Ventilation

## BACKGROUND

Milk production can be negatively affected by heat, especially in animals of high genetic merit (Ahmed et al., 2022), and heat stress can have negative effects on the welfare (Lacetera, 2019), which can be rated by physiological and behavioral responses. Climatic factors, such as temperature, relative humidity, and solar radiation, may cause increase in rectal temperature and respiratory rate (Li et al., 2020) and reduction in daily activities (Hut et al 2022) and dry matter intake (Chang-Fung-Martel et al., 2021).

Decrease in lying and eating behavior are the first changes on daily activities when the air temperature rises (; Chang-Fung-Martel et al., 2021; Tullo et al., 2019; DelCurto-Wyffels et al., 2021). Besides the decline in milk production, heat stress may also reduce milk quality: somatic cells increase (Zeinhom et al., 2016) and protein and fat contents become smaller (Bernabucci et al., 2015). Animals under heat stress reduced milk yield from 34.3 kg/day to 22.5 kg/day when compared to thermoneutral conditions (Fontoura et al., 2022), in addition, findings by Bertocchi et al. (2014) showed higher somatic cell scores in heat (4.613) compared to cold (4.287).

However, there is evidence that providing shade or other cooling methods is beneficial for cattle, based on changes in respiratory rate and body temperature ( Schütz et al., 2011; Brown-Brand, 2018), diurnal feeding (Portugal et al., 2000), and plasmatic cortisol and IGF-I (Chaiyabutr et al., 2008; Brown-Brandl et al 2017).In freestall barns, cows stay inside during the hottest hours of the day to obtain shelter from intense solar radiation; however, the barn itself can cause heat stress if not well ventilated or has high roofing. Environmental modification on dairy barn has demonstrated improvement in microclimate and a positive effect on cow's performance with forced ventilation (Marumo et al., 2022), ventilation with misting (Titto et al., 2013), and ventilation with sprinkling (Román et al., 2019). Studies that investigate animal behavior to show animal preferences is important to increase welfare (Arnott et al., 2017).

Within this context, this study aimed to evaluate the behavior in relation to the environmental condition system (fans with fogging), milk yield, and milk quality under different climatic conditions throughout the year.

## MATERIAL AND METHODS DESCRIPTION

### Animals and facilities

The experiment was conducted at the FZEA-USP Division of Dairy Cattle located in Pirassununga, SP, Brazil (21°80'00″'' S, 47°25'42''″ W, altitude 634 m) during summer, and was approved by the Ethics Committee of FZEA/USP. The study utilized 28 Holstein cows between the first and third lactations, with an average milk yield of 20 kg $cow^{-1}$. $day^{-1}$. The animals were divided into two groups of 14 homogeneous cows each, housed postpartum in two pens: one cooled group (CG) with an environment conditioning system (fans and fogging), and the other non-cooled group (NCG) with natural ventilation and shade. Cow selection was performed postpartum based on the calving number and milk yield to create homogeneous groups. Two side-by-side freestall barns were placed northwest-southeast, with a cement floor, covered with fiber cement roof panels, 3.5 m high with sand bedding. The feeding and water troughs were positioned under the roof. The cows had unlimited access to a 0.3 ha paddock of coast-cross grass without shade.

The cooled area was equipped with five axial flow fans (125 cm diameter; 300 $m^3 . min^{-1}$ maximum airflow rate) installed on the east side of the feed passage (Figure 1). The fans were spaced 3 m apart on either side of the drive-through feed alley and positioned to provide airflow in the direction of prevailing winds. The fans were mounted at a height of approximately 2.5 m and angled downward approximately 10° from the vertical. A set of two fogging lines was mounted above the feed alley and bedding. The evaporative cooling system was thermostatically controlled and was switched on at 26°C. A plastic wall separated the two experimental pens to avoid interference.

The evaporative cooling system was triggered automatically during all seasons when the air temperature exceeded 26°C, a value determined for the system call.

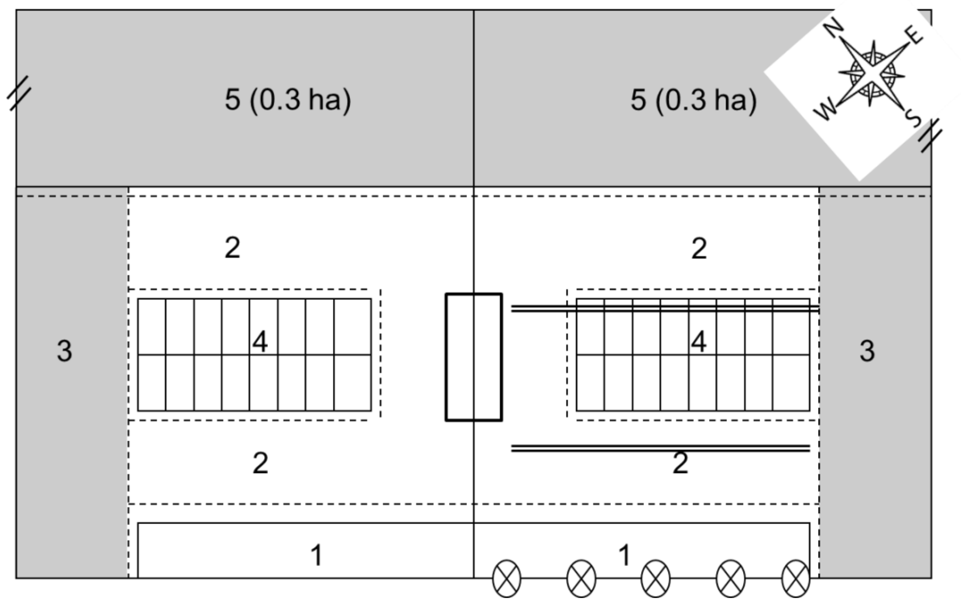


*Figure 1. Sketch of free stall and coast-cross area (not scaled). Dotted lines indicate each region. White area covered by fiber cement tiles, grey area not covered. Numbers 1 to 5 indicate the region of installation occupied by the animals. The circles represent the axial flow fans. The rectangle indicates the water trough. Double Solid lines are the fogging lines: 1) trough; 2) corridors; 3) unshaded cemented area inside freestall; 4) bed; 5) coast-cross area*

## Macroclimate and microclimate parameters

Weather variables including air temperature, relative humidity, and solar radiation were recorded by an electronic weather station (Table 1). Inside each barn, the microclimate was measured with a black globe thermometer with data logger located at cow height in the center area and Temperature-Humidity Index (THI) – a measure of the degree of discomfort or stress placed on living animals  was calculated (West, 2003). Each season of the year was characterized by temperature, relative humidity and solar radiation measured at the meteorological station of the Construction and Ambience Laboratory at FZEA/USP (see Table 1). These data were used to divide the seasons: Autumn (April, May, June), Winter (June, July, August), Spring (September, October, November), Dry Summer (December to January), Rainy Summer (January to February).

*Table 1. Climatic data for seasons were recorded by weather station electronics and a black globe thermometer.*

| | Autumn | Winter | Spring | Dry summer | Rainy summer |
|---|---|---|---|---|---|
| **Air temperature min (°C)** | 8.9 | 3.4 | 11.9 | 14.4 | 15.4 |
| **Air temperature max (°C)** | 29.6 | 27.7 | 33.3 | 34.8 | 35.3 |
| **THI min** | 42 | 21 | 50 | 57 | 57 |
| **THI mean** | 69 | 59 | 75 | 78 | 78 |
| **THI max** | 92 | 87 | 99 | 99 | 99 |
| **Relative humidity min (%)** | 42 | 30 | 26 | 24 | 35 |
| **Relative humidity max (%)** | 100 | 100 | 99 | 99 | 98 |
| **Solar radiation mean ($W/m^2$)** | 376.0 | 299.7 | 403.1 | 510.5 | 511.8 |
| **Accumulated rainfall** | 82.0 | 46.4 | 156.6 | 32.4 | 272.8 |
| **BGT cooled pen (°C)** | 24.0-32.0 | 20.0-28.5 | 24.0-29.0 | 25.3-28.3 | 25.3-32.3 |
| **BGT non-cooled pen (°C)** | 25.0-32.0 | 20.0-28.5 | 24.0-33.0 | 25.3-29.3 | 25.7-33.3 |

## Behavior

Cow behaviors were recorded using instantaneous scan sampling (Martin & Bateson, 1993) every 30 min during the hottest hours of the day (9:30 am-6:00 pm) on three consecutive days in each different weather pattern (Autumn: April, May, June; Winter: June, July, August; Spring: September, October, November; Dry summer: December until January 15; Rainy summer: January 16 until February 28) in a total of 15 days. The observations were interrupted by milking between 2:00 pm and 3:00 pm (milking lasted 68 ± 10 min, mean ± S.E.). It was recorded the location of the individual animal at the facility within the freestall or picket defined by the numbers from 1 to 5 delimited by dotted lines: 1) trough; 2) corridors; 3) cemented area inside the freestall; 4) bed; 5) coast-cross area (Figure 1), time spent standing in the shade, and their daily activities such as eating, drinking, ruminating, interactions (positive, negative, sexual, and external), and idleness (Table 2).

*Table 2. Description of behavior observed in experimental cows*

| Behavior | Description |
|---|---|
| **Position** | |
| **In the shade** | At least with the head in the shade |
| **In the barn** | Trough; corridors; discovery area (cemented inside the freestall); bed; coast-cross area (see Figure 1) |
| **Posture** | |
| **Standing** | 4 with ground anchors |
| **Lying down** | flank in contact with the ground |
| **Activity** | |
| **Eating** | Ingesting silage and/or feed and/or fodder and/or salt |
| **Ruminating** | Chewing movements without food intake |
| **Drinking** | Ingesting the drinking water, with the mouth in water or water dripping from the mouth shortly after the stay in the water trough |
| **Idling** | No apparent activity |
| **Interaction** | Licking/scratching, fighting, mounts, external interface |

## Production and quality of milk

Samples were collected during autumn, winter, spring, dry summer, and rainy summer seasons in bottles containing bronopol tablets for analysis of somatic cell count (SCC) and other main components of milk: protein, fat, lactose, total solids, and nonfat dry extract. SCC was determined by flow cytometry (Somacount, 300, Bentley Instruments Inc., Chaska, MN) and the other main components by infrared techniques (Bentley 2000, Bentley Instruments Inc., Chaska, MN). Total milk production was also measured for each collection.

## Statistical Analysis

To analyze the behavioral data, a nonlinear, but linearizable model was adjusted by applying the Generalized Linear Model (Nelder & Wenderburn, 1972), using the GENMOD procedure of SAS software (version:9, Cary, NC, EUA). The model for behavioral variables, such as position in the shade, standing posture, and activities (eating, ruminating, drinking water, idling, and interaction), included the animal effect as the replicate measure to evaluate the fixed effect of treatment (CG or NCG) and time along the daylight and air temperature as covariates. The effect of the seasons on the average animal behavior with and without the availability of a cooling system was compared using ANOVA and means comparison by Tukey-Kramer at a significance level of 0.05. To compare variables between animals of the experimental groups (CG or NCG), a t-test was performed with Satterthwaite's approximation for degrees of freedom at the 0.05 significance level.

Milk production was analyzed in the seasons with a random effect of days in milk by variance analysis and mean comparison by Tukey-Kramer at a significance level of 0.05. A similar procedure was used for variable milk quality (fat, protein, lactose, non-fat dry extract, total solids, and SCC). Comparisons between treatments were performed by t-test with Satterthwaite's approximation for degrees of freedom at a significance level of 0.05. All values are presented as mean ± standard error of the mean

# RESULTS AND DISCUSSION

## Behavior

The animals remained standing in the shade for most of the day (84.2% of the observed time), regardless of the season and the availability of the evaporative cooling system ($P = 0.18$). In the non-cooled animals, the standing time decreased from 11 h and increased after 14 h (Figure 2), and it was linked with reduced feed and increased rumination. When lying down, the cows preferred to lie in bed (86% winter and an average of 64% in the other seasons), and in the remaining time, the animals preferred to lie on the grass and avoid lying on the concrete floor. Although rumination activity is associated with the lying position, a decrease in this behavior was observed when the days were hottest ($P < 0.05$), and there was a difference in the availability of cooling systems, especially in dry summer, when there was a greater number of animals that preferred ruminating while standing ($P < 0.01$) or remained in leisure at bay without cooling.

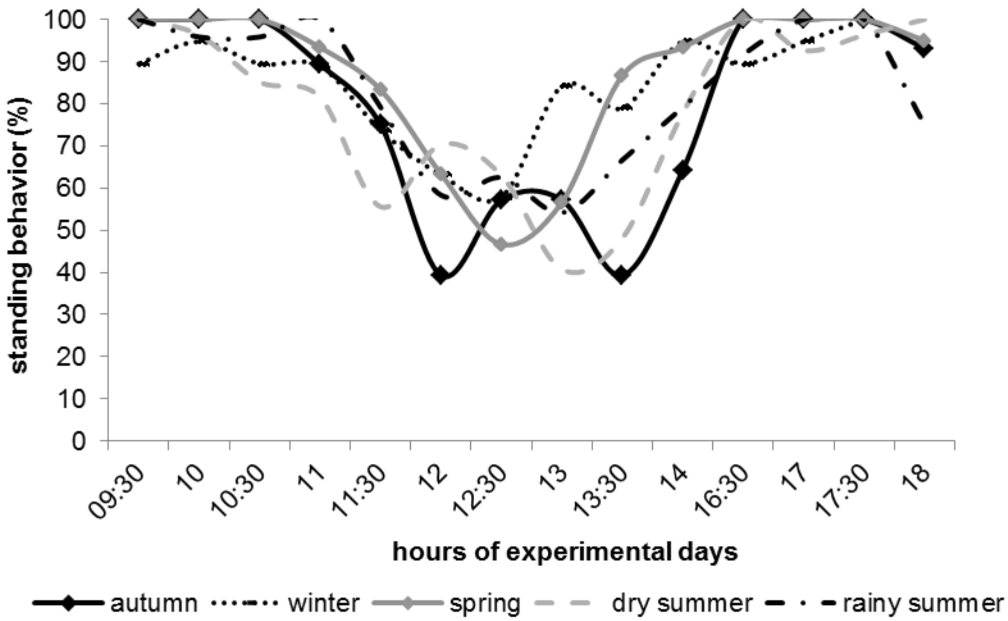


*Figure 2. Mean frequency of standing behavior observed from 9:30 to 18:00 h of Holstein cows without (NCG) the availability of evaporative cooling system during the year*

Rafiee et al. (2022) also noted a predominance of the standing position in cows, and longer time for eating was mainly with high milk producing animals. Cows in free stall with availability of fans and sprinkling over the trough spent more time standing rather than lie on the bed to promote heat loss (Calegari et al., 2014). With the increase in the temperature inside the shed, there was a decrease in the number of animals lying (Allen et al., 2015; Vieira, 2021). The standing posture maximizes the surface area exposed to the body environment and increases the airflow around the animal, facilitating heat loss by convection (Igono et al., 1987; Wang et al., 2018). After milking, food being available animals preferred eating and standing for the next 40-60 min (DeVries et al., 2011). Immediately after this period, most of the cows were observed in idling. In the period before milking, more than half of the animals were no longer lying. Longer standing may occur due to increased intramammary pressure and early start of milk ejection (DeVries et al., 2011; Melvin et al., 2019). It was found that in animals without the availability of a conditioned environment, there was a greater decrease in milk production between periods that are warm.

Eating activity was most frequent during daylight hours ($P < 0.05$). There was no difference between the experimental seasons, except during the dry summer and rainy summer seasons, when the animals in the non-cooled group spent less time eating ($P = 0.03$, Figure 3). Throughout the year, animals in the non-cooled group had lower ingestion during summer ($P<0.05$); however, the cooled group did not reduce their ingestion in summer ($P = 0.26$, Table 3). The frequency of water consumption was higher during winter in the non-air-conditioned barn ($P = 0.03$) despite the low values observed throughout the day. There were no differences between seasons for the cooled group, and there was no effect on this activity ($P > 0.05$).

*Table 3. Mean frequency of daily activities observed from 9:30 am to 6:00 pm of Holstein cows with (CG) and without (NCG) the availability of evaporative cooling system during the year*

| | Autumn | Winter | Spring | Dry Summer | Rainy Summer | P season |
|---|---|---|---|---|---|---|
| **Eating** | | | | | | |
| **CG** | 49.75$^{A}$ | 53.51$^{A}$ | 50.98$^{A}$ | 45.09$^{A}$ | 43.15$^{A}$ | 0.26 |
| **NCG** | 51.23$^{Aa}$ | 52.56$^{Aa}$ | 44.12$^{Aa}$ | 37.20$^{Bb}$ | 36.11$^{Bb}$ | 0.05 |
| **Ruminating** | | | | | | |
| **CG** | 13.80$^{Ac}$ | 15.81$^{Ac}$ | 18.40$^{Abc}$ | 26.49$^{Aab}$ | 27.97$^{Aa}$ | 0.017 |
| **NCG** | 11.25$^{Ac}$ | 14.57$^{Abc}$ | 18.78$^{Aabc}$ | 20.99$^{Bab}$ | 24.70$^{Aa}$ | 0.05 |
| **Drinking water** | | | | | | |
| **CG** | 3.99 | 5.56 | 3.33 | 5.38 | 3.17 | 0.53 |
| **NCG** | 4.50$^{ab}$ | 6.88$^{a}$ | 2.68$^{bc}$ | 2.91$^{bc}$ | 1.49$^{c}$ | 0.03 |
| **Interaction** | | | | | | |
| **CG** | 6.13 | 5.03 | 7.23 | 4.17 | 3.56 | 0.31 |
| **NCG** | 8.50 | 2.83 | 7.32 | 8.97 | 8.55 | 0.38 |
| **Idling** | | | | | | |
| **CG** | 26.33 | 20.09 | 20.06 | 28.87 | 22.19 | 0.47 |
| **NCG** | 24.52 | 23.14 | 27.10 | 29.93 | 29.16 | 0.42 |

Means within a row with different lower-case letters and means within a column with different upper-case letters differs significantly by Tukey-Kramer test <0.05.

Higher feeding frequencies were observed shortly after the start of milking activity (9:30 am and 4:30 pm) when a new diet was offered (Figure 3). One hour after these peaks, there was a decrease in the number of animals engaged in eating activity. The lowest frequencies occurred during the hottest hours of the day, between 11:30 am and 2:00 pm, when the frequency of rumination increased ($P < 0.05$). The same pattern was observed in animals from the two experimental groups ($P > 0.05$).

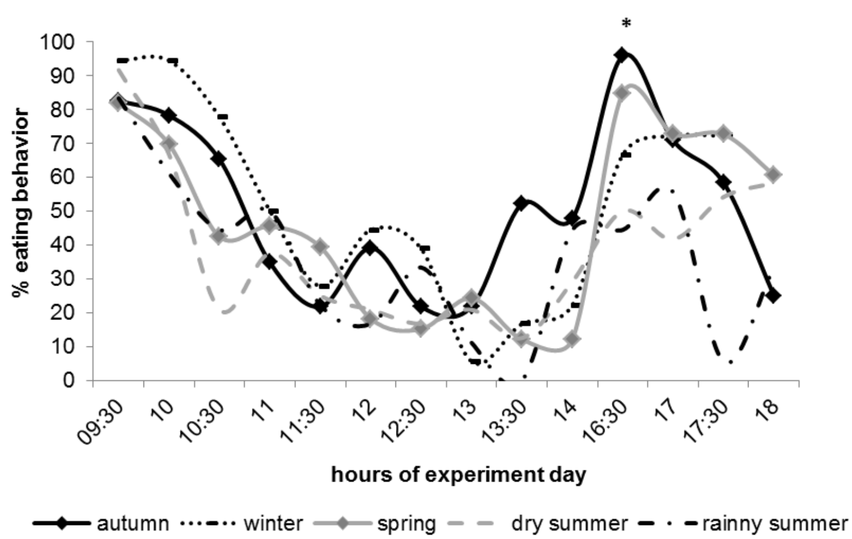


*Figure 3. Eating behavior of Holstein cows without (NCG) availability of an evaporative cooling system during the year. * (P=0.03)*

The animals remained in the covered corridors (area 2) near the water trough for most of the observed hours, with higher frequencies found during winter (61.3% and 58.5% for CG and NCG, respectively, $P > 0.05$) and lower frequencies during summer in the non-cooled barn (45.6%, $P < 0.01$). The animals oscillated between the bed (Area 4) and feeder (Area 1) during the remaining period. The uncovered area that was cemented was used less (less than 2% of the time in both groups at all times of the year), following the picket, which was most used at the end of the day, when the sun was setting.

In summer, cows change their eating patterns during daytime and nighttime to avoid the heat increment caused by dry matter intake during the hottest hours of the day (Portugal et al., 2000). The shorter feeding time of the non-cooled animals during the summer may have been influenced by higher exposure of animals to high air temperature and THI values, and this may have affected thermoregulation, dry matter intake, and probably the induction of metabolic changes as part of the physiological adaptations (Ferrazza et al., 2017). Hillman et al. (2005) found a greater decrease in the body temperature of cows in free stalls with fans, misting, and standing, when compared with animals lying. Even with access to artificial shade and cooling systems, cows in the present study spent more time standing under the structure, probably as a way of losing more heat.

Animals seek shade in response to an increase in the absorbed thermal load (Van Laer et al., 2015). In the present study, the roof of the shed provided shade, and there was even absorption of heat from the radiation facility. An evaporative cooling system would be a way to reduce the increase in radiant heat load of the structure by cooling the ambient air, providing greater comfort for cows. And that greatest comfort does not seem to have been evident when observing frequencies in standing; however, despite eating activity during the dry summer and rainy summer being decreased from 1 to 1.5 hours when compared to winter, where there was the greatest time of eating, animals kept in a cooled environment spent more time in this activity during summer. Therefore, the frequency of rumination during the two phases of summer was higher in the cooled group. This result shows that thermal comfort should have been higher to allow the animal to ruminate, an activity preferably carried out when the animal is under comfort.

In contrast, animal under heat stress may exhibit reduced of pH, passage rates and consequently affects the production of volatile fatty acids (Nonaka et al., 2008; King et al., 2011). Which are important modifiers of rumen fermentation and influence on microbial synthesis (Wang et al., 2021; Wang et al., 2022). This impairs the digestion process that needs microbial population decreasing the dry matter intake and rumination, and it can result in later metabolic disorders and reduce in milk yield (Beauchemin et al., 2018).

## Milk Quality and Production

Season had no effect on milk quality ($P > 0.05$). The lack of a cooling system resulted in a lower content of dry extract, with no significant difference ($P > 0.05$), and there was no difference between protein and lactose content ($P > 0.05$). The mean values of fat were lower (2.56%) than those of the animals kept in the cooling barns (3.05%, $P < 0.05$, Table 4).

*Table 4. Milk composition in Holstein cows with and without the availability of evaporative cooling system during the year (mean ± S.E.)*

| | **Fat (%)** | **Protein (%)** | **Lactose (%)** | **Total solids (%)** | **Dry extract (%)** |
|---|---|---|---|---|---|
| **Non-cooled animals** | 2.56 ± 0.12b | 2.97 ± 0.03 | 4.42 ± 0.02 | 11.27 ± 0.14 | 8.32 ± 0.03 |
| **Cooled animals** | 3.05 ± 0.14a | 3.08 ± 0.07 | 4.30 ± 0.06 | 11.35 ± 0.14 | 8.30 ± 0.08 |

Means within a column with different upper-case letters differs significantly by Tukey-Kramer test <0.05

On evaluating the milk composition of Holstein subjected to heat stress, during summer a lower concentration of milk protein and lactose were found, but milk fat content was not changed (Garcia et al., 2015). In the present study, we did not find changes for protein and lactose in non-cooled group animals, but found lower percentage of fat. In non-cooled group animals a decline in fat percentage of 15.2% was observed. The effect of increasing temperature was also seen by Toghdory et al. (2022) found that with increasing temperature of 6.2 to 31.3 °C, the fat and protein decreased 4.09% and 5.75%, respectively.

The fat reduction in NCG occur because animals were not able to overcome the thermal challenge and probably suffered thermal stress due to high air temperature and THI recorded in spring and summer, according to Gorniak et al. (2014) elevation THI may contribute with decreased amount of fat in milk. The increase of the temperature can cause lower dry matter intake (Pereira et al., 2019), besides alterations in the ruminal fermentation parameters, reducing pH values and consequently acetate and butyrate concentration (Zhao et al., 2019). Therefore, directly influencing amount of milk fat.

Difference was found between the experimental groups in relation to the SCC, with lowest mean for the cooled animals in autumn and winter ($P < 0.01$, Figure 4) and higher values during the warmer season ($P < 0.01$). As the SCC data had no normal distribution, it was transformed to log base 10. There was effect of the seasons, with higher SCC found in winter for non-cooled group, in the summer dry and rainy for the cooled group ($P < 0.01$). Milk production decreased during warmer months in non-cooled animals ($P < 0.01$; Figure 5).

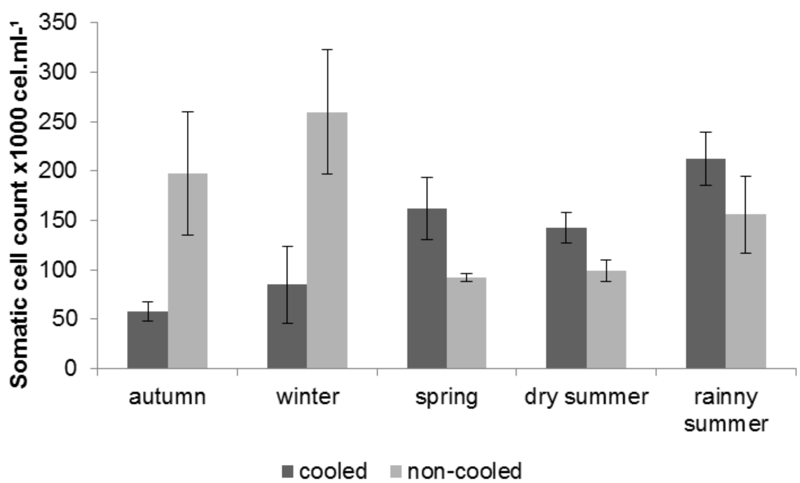


*Figure 4. Somatic cell count (x1000 cel.ml$^{-1}$) in milk of Holstein cows with and without availability of evaporative cooling system during the year*

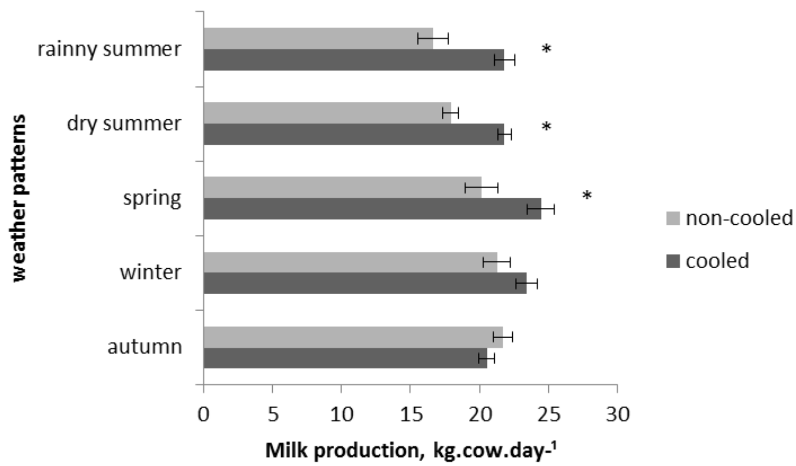


*Figure 5. Milk production (kg.cow$^{-1}$. day$^{-1}$) in cooled and non-cooled Holstein cows with and without the availability of an evaporative cooling system during the year. * (P<0.01)*

The lower CCS in non-cooled group during winter and autumn can be explained by the lower milk production since milk production has a negative correlation with CCS, so the occurrence of infections and the presence of pathogenic microorganisms in dairy cows are generally associated with a reduction in the volume of milk produced (Picinin et al. 2019). On the other hand, the greater SCC in animals cooled group in the summer and spring seasons may be a result of warmer and wetter periods that present the greatest challenges in relation to the hygiene and sanitary quality of milk, due to increased accumulation of mud on dairy facilities and a higher incidence of dust on the ceilings and roofs during milking. Such factors associated with failures in the milking routine may be responsible for high initial contamination and increased prevalence of mastitis in the herd evidenced by the greater tendency toward higher SCC and cause major risk for the decline in the quality and volume of milk produced during unfavorable weather conditions, such as rainfall and higher temperatures (Sant'Anna & Paranhos da Costa, 2011). However, despite the fluctuation in the values throughout the year, in most samples the SCC remained below 200.000 cells/mL, a threshold value for the absence of infection (Alves et al. 2014; Moroni et al., 2018).

The thermal environment is one of the main factors that can affect milk production in dairy cattle (Nardone et al., 2010). During spring and summer, were observed temperature peaks reached 35.3 °C. The THI reached mean values of 75 and 78, respectively, indicating an environmental warning situation for dairy cows, under these conditions, the productivity of the animal can be seriously impaired (M'Hamdi et al., 2021). The NCG animals presented lower milk yield in warmer periods, this may have occurred due of stressful environmental conditions within the experimental area that represented the greatest thermal challenge for this group, this may have caused changes in the biological functions of these animals, which include decrease in food intake (Marai et al., 2008). In a heat stress situation, the animal increases its effort to dissipate the excess of accumulated thermal energy, in an attempt to avoid detrimental changes to the body (Rashamol et al., 2018). However, if the thermolytic capacity is compromised, the energy deviation occurs that would be used in other metabolic and productive processes, to maintain homeothermy resulting in less milk synthesis (Das et al., 2016).

The use of an evaporative cooling system is not necessary in the cooler months of the year (autumn, winter, and spring); thus, the spending on energy and water can be reduced. The evaporative cooling environment for lactating cows proved effective during the hottest months of the year in increasing the daytime feeding time and milk yield, and better milk quality was observed with a higher percentage of fat.

## REFERENCES


Ahmed, H., Tamminen, L.M., & Emanuelson, U. (2022). Temperature, productivity, and heat tolerance: Evidence from Swedish dairy production. *Climatic Change*, 175, 10. https://doi.org/10.1007/s10584-022-03461-5

Allen, J.D., Hall, L.W., Collier, R.J., & Smith, J.F. (2015). Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. *Journal of Dairy Science*, 98, 1–10.

Alves, E.C., Dahmer, A.M., & Borges, A.F. (2014). Total bacterial count and somatic cell count in refrigerated raw milk stored in communal tanks. *Brazilian Journal of Food Technology*,17, 221-225. http://dx.doi.org/10.1590/1981-6723.1114.

Arnott, G., Ferris, C.P., & O'Connell, N.E. (2017). Review: Welfare of dairy cows in continuously housed and pasture-based production systems. *Animal*, 11, 261-273. https://doi.org/10.1017/S1751731116001336.

Beauchemin, K.A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of Dairy Science*, 101, 4762-4784. https://doi.org/10.3168/jds.2017-13706.

Bernabucci, U., Basiricò, L., Morera, P., Dipasquale, D., Vitali, A., Cappelli, F.P., & Calamari, L. (2015). Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science*, 98, 1–13.

Brown-Brandl, T. M., Chitko-McKown, C. G. , Eigenberg, R. A., Mayer, J. J., Welsh Jr., T. H., Davis, J. D., Purswell, J. L. (2017). Physiological responses of feedlot heifers provided access to different levels of shade. *Animal*, 11(8), 1344–1353. https://doi.org/10.1017/S1751731116002664.

Brown-Brandl, T.M. (2018). Understanding heat stress in beef cattle. Revista Brasileira de Zootecnia, 47, e20160414. https://doi.org/10.1590/rbz4720160414.

Calegari, F., Calamari, L., & Frazzi, E. (2014). Fan cooling of the resting area in a free stalls dairy barn. *International Journal of Biometeorology*, 58,1225-1236. https://doi.org/10.1007/s00484-013-0716-1.

Chaiyabutr, N., Chanpongsang, S., & Suadsong, S. (2008). Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. *International Journal of Biometeorology*, 52, 575-585.

Chang-Fung-Martel, J., Harrison, M. T., Brown, J. N., Rawnsley, R., Smith, A. P., & Meinke, H. (2021). Negative relationship between dry matter intake and the temperature-humidity index with increasing heat stress in cattle: A global meta-analysis. *International Journal of Biometeorology*, 65, 2099–2109. https://doi.org/10.1007/s00484-021-02167-0

Das, R., Sailo, L., Verma, N., Bharti, P., Saikia, J., Imtiwati, & Kumar, R. (2016). Impact of heat stress on health and performance of dairy animals: A review. *Veterinary World*, 9, 260-268.

DelCurto-Wyffels, H.M., Dafoe, J.M., Parsons, C.T., Boss, D.L., DelCurto, T., Wyffels, S.A., Van Emon, M.L., & Bowman, J.G.P. (2021). The Influence of Environmental Conditions on Intake Behavior and Activity by Feedlot Steers Fed Corn or Barley-Based Diets. *Animals (Basel)*, 11, 1261. https://doi.org/10.3390/ani11051261.

DeVries, T.J., Deming, J.A., Rodenburg, J., Seguin, G., Leslie, K.E., & Barkema, H.W. (2011). Association of standing and lying behavior patterns and incidence of intramammary infection in dairy cows milked with an automatic milking system. *Journal of Dairy Science*, 94, 3845-3855. https://doi.org/10.3168/jds.2010-4032.

Ferrazza, R.A., Garcia, H.D.M., Aristizábal, V.H.V., Nogueira, C.H., Veríssimo, C.J., Sartori, J.R., Sartori, R., & Ferreira, J.C.P. (2017). Thermoregulatory responses of Holstein cows exposed to experimentally induced heat stress. *Journal of Thermal Biology*, 66, 68-80.

Fontoura, A.B.P., Javaid, A., Sáinz de la Maza-Escolà, V., Salandy, N.S., Fubini, S.L., Grilli, E., McFadden, J.W. (2022). Heat stress develops with increased total-tract gut permeability, and dietary organic acid and pure botanical supplementation partly restores lactation performance in Holstein dairy cows. *Journal of Dairy Science*, 105, 7842–7860. https://doi.org/10.3168/jds.2022-21820.

Garcia, A.B., Angeli, N., Machado, L., Cardoso, F.C., & Gonzalez, F. (2015). Relationships between heat stress and metabolic and milk parameter in dairy cows in southern Brazil. *Tropical Animal Heath and Production*, 47, 889-894.

Gorniak, T., Meyer, U., Südekum, K., & Dänicke, S. (2014). Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate. *Archives of Animal Nutrition*, 68, 358-369.

Hillman, P.E., Lee, C.N., & Willard, S.T. (2005). Thermoregulatory responses associated with lying and standing in heat-stressed dairy cows. *American Society of agricultural and Biological Engineers*, 48, 795-801.

Hut, P.R., Scheurwater, J., Nielen, M., van den Broek, J., & Hostens, M.M. (2022). Heat stress in a temperate climate leads to adapted sensor-based behavioral patterns of dairy cows. *Journal of Dairy Science*, 105, 6909-6922. https://doi.org/10.3168/jds.2021-21756.

Igono, M.O., Johnson, H.D., Steevens, B.J., Krause, G.F., & Shanklin, MD. (1987). Physiological, productive, and economic benefits of shade, spray, and fan systems versus shade for Holstein cows during summer heat. *Journal of Dairy Science*, 70, 1069-1079.

King, C.C., Dschaak, C.M., Eun, J.-S., Fellner, V., Young, A.J. (2011). Quantitative analysis of microbial fermentation under normal or high ruminal temperature in continuous cultures.*The Professional Animal Scientist*, 27 (4), 319-327.https://doi.org/10.15232/S1080-7446(15)30495-2.

Lacetera, N. (2019). Impact of climate change on animal health and welfare, *Animal Frontiers*, 9, 26–31. https://doi.org/10.1093/af/vfy030

Li, G., Chen, S., Chen, J., Peng, D., & Gu, X. (2020). Predicting rectal temperature and respiration rate responses in lactating dairy cows exposed to heat stress. *Journal of Dairy Science* 103:5466–5484 https://doi.org/10.3168/jds.2019-16411.

Marai, I.F.M., El-Darawanya, A.A., Fadielc, A., & Abdel-Hafezb, M.A.M. (2008). Reproductive performance traits as affected by heat stress and its alleviation in sheep. *Tropical and Subtropical Agroecosystems*. 8, 209-234.

Martin, P., & Bateson, P. (1993). *Measuring behavior: an introductory guide*. Cambridge University Press.

Marumo, J. L., Lusseau, D., Speakman, J. R., Mackie, M., & Hambly, C. (2022). Influence of environmental factors and parity on milk yield dynamics in barn-housed dairy cattle. *Journal of Dairy Science*, 105:1225–1241 https://doi.org/10.3168/jds.2021-20698

Melvin, J. M., Heuwieser, W., Virkler, P. D., Nydam, D. V., & Wieland, M. (2019). Machine milking–induced changes in teat canal dimensions as assessed by ultrasonography. *Journal of Dairy Science*, 102:2657–2669. https://doi.org/10.3168/jds.2018-14968 2019

M'Hamdi, N., Darej, C., Attia, K., Znaidi, I.L., Khattab, R., Djelailia, H., Bouraoui, R., Taboubi, R., Marzouki, L., & Ayadi, M. (2021). Modelling THI effects on milk production and lactation curve parameters of Holstein dairy cows. *Journal of Thermal Biology*, 99, 102917, https://doi.org/10.1016/j.jtherbio.2021.102917.

Moroni, P., Nydam, D.V., Ospina, P.A., Scillieri-Smith, J.C., Virkler, P.D., Watters, R.D., Welcome, F.L., Zurakowski, J., Ducharme, N.G., & Yeager, A.E. (2018). 8 diseases of the Teats and Udder. In Peek, S.F., & Thomas J. Divers (Eds), *Rebhun's Diseases of Dairy Cattle* (389-465). Elsevier. https://doi.org/10.1016/B978-0-323-39055-2.00008-5.

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S., & Bernabucci, U. (2010). Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science*, 130, 57-69.

Nelder, J.A., & Wedderburn, R.W.M. (1972). Generalized Linear Model. *Journal of the Royal Statistical Society: Series A (General)*, 135, 370-384.

Nonaka I., Takusari N., Tajima K., Suzuki T., Higuchi K., Kurihara M. (2008). Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. *Livestock Science* 113, 14–23. 10.1016/j.livsci.2007.02.010

Pereira, A.M.F., Titto, E.A.L., & Almeida, J.A.A. (2019). *Adaptação dos ruminantes aos climas quentes*. Appris Editora.

Picinin, L.C.A., Bordignon-Luiz, M.T., Cerqueira, M.M.O.P., Toaldo, I.M., Souza, F.N., Leite, M.O., Fonseca, L.M., & Lana, A.M.Q. (2019). Effect of seasonal conditions and milk management practices on bulk milk quality in Minas Gerais State – Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 71(4), 1355-1363.

Portugal, J.A.B., Pires, M.F.A., & Durães, M.C. (2000). Effect of air temperature and humidity on frequency of feeding, water ingestion and rumination in Holstein cows. *Arquivo. Brasileiro de Medicina Veterinaria e Zootecnia*, 52, 154-159.

Rafiee, H., Alikhani, M., & Ghorbani, G.R. (2022). Effect of dietary protein level and corn processing on behavior activity of high producing dairy cows. *Acta Scientiarum. Animal Sciences*, 44, e54603. https://doi.org/10.4025/actascianimsci.v44i1.54603.

Rashamol, V.P., Sejian, V., Bagath, M., Krishnan, G., Archana. P.R., & Bhatta, R. (2018). Physiological adaptability of livestock to heat stress: an updated review. *Journal of Animal Behaviour and Biometeorology*, 6, 62-71.

Román, L., Saravia, C., Astigarraga, L., Bentancur, O., & La Manna, A. (2019). Shade access in combination with sprinkling and ventilation effects performance of Holstein cows in early and late lactation. *Animal Production Science*, 59(2), 347. https://doi.org/10.1071/an16571

Sant'Anna, A.C., & Paranhos da Costa, M.J. (2011). The relationship between dairy cow hygiene and somatic cell count in milk. *Journal of Dairy Science* 94, 3835-3844.

Schütz, K.E., Rogers, A.R., Cox, N.R., Webster, J.R., & Tucker, C.B. (2011) Dairy cattle prefer shade over sprinklers: Effects on behavior and physiology, *Journal of Dairy Science*, 94, 273-283. https://doi.org/10.3168/jds.2010-3608.

Silva, M.V., Almeida, G.L.P., Pandorfi, H., Moraes, A.S., Macêdo, G.A.P.A., Batista, P.H.D., Silva, R.A.B., & Guiselini, C. (2021). Influence of meteorological elements on behavioral responses of gir cows and effects on milk quality. *Acta Scientiarum. Animal Sciences*, 43, e52604. https://doi.org/ 10.4025/actascianimsci.v43i1.52604

Titto, C.G., Negrão, J.A., Titto, E.A., Canaes, T.S., Titto, R.M., & Pereira, A.M. (2013). Effects of an evaporative cooling system on plasma cortisol, IGF-I, and milk production in dairy cows in a tropical environment. *International Journal of Biometeorology*, 57, 299-306.

Toghdory, A.; Ghoorchi, T.; Asadi, M.; Bokharaeian, M.; Najafi, M.; & Ghassemi Nejad, J. (2022). Effects of environmental temperature and humidity on milk composition, microbial load, and somatic cells in milk of Holstein dairy cows in northeast Iran. *Animals (Basel)*, 12, 2484. https://doi.org/10.3390/ani12182484.

Tullo E., Mattachini, G., Riva, E., Finzi, A., Provolo, G., & Guarino, M. (2019). Effects of Climatic Conditions on the Lying Behavior of a Group of Primiparous Dairy Cows. *Animals (Basel)*, 9, 869. https://doi.org/10.3390/ani9110869.

van Laer, E., Moons, C.P.H., Ampe1, B., Sonck, B., Vandaele, L., Campeneere, S., & Tuyttens, F.A.M. (2015). Effect of summer conditions and shade on behavioural indicators of thermal discomfort in Holstein dairy and Belgian Blue beef cattle on pasture. *Animal*, 9, 1536-1546.

Vieira, F.M.C., Soares, A.A., Herbut, P., Vismara, E.S., Godyń, D., Dos Santos, AC.Z., Lambertes, T.D.S., Caetano, & W.F. (2021). Spatio-thermal variability and behavior as bio-thermal indicators of heat stress in dairy cows in a compost barn: A case study. *Animals (Basel)*, 11,1197. https://doi.org/10.3390/ani11051197.

Wang, X., Zhang, G., & Choi, C.Y. (2018). Effect of airflow speed and direction on convective heat transfer of standing and reclining cows. *Biosystems Engeneering*,167, 87-98.

Wang, Z., Niu, K., Rushdi, H.E., Zhang, M., Fu, T., Gao, T., Yang, L., Liu, S., & Lin, F. (2022). Heat Stress Induces Shifts in the Rumen Bacteria and Metabolome of Buffalo. *Animals (Basel).* 12, 1300. http://dx.doi.org/10.3390/ani12101300.

Wang, Z., Yang, D.S., Li, X.Y., Yu, Y.N., Yong, L.Y., Zhang, P.H., He, J.H., Shen, W.J., Wan, F.C., Feng, B.L., Tan, Z.L., & Tang, S.X. (2021), Modulation of rumen fermentation and microbial community through increasing dietary cation-anion difference in Chinese Holstein dairy cows under heat stress conditions. *Journal of Applied Microbiology*, 130, 722-735. https://doi.org/10.1111/jam.14812.

West, J., Mullinix, B.G., & Bernard, J.K. (2003). Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. *Journal of Dairy Science*, 86, 232-242.

Zeinhom, M.M., Aziz, R.L.A., Mohammed, A.N., & Bernabucci, U. (2016). Impact of seasonal conditions on quality and pathogens content of milk in Friesian cows. *Asian-Australasian Journal of Animal Sciences*, 29(8), 1207.

Zhao, S., Min, L., Zheng, N., & Wang, J. (2019). Effect of Heat Stress on Bacterial Composition and Metabolism in the Rumen of Lactating Dairy Cows. *Animals (Basel)*, 9,925. http://dx.doi.org/10.3390/ani9110925.