Research Article

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Natural tree shade increases milk stability of lactating dairy cows during the summer in the subtropics

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Abstract

This research communication addresses the hypothesis that, during the summer in the subtropics, natural tree shade helps to improve milk functional characteristics such as stability and acidity. Sixteen Holstein lactating cows were enrolled. The study consisted of three periods (pre-stress, heat stress and post-stress) based on allocating grazing cows into two treatments (with and without access to shade during the Heat Stress period). Overall THI during the trial was (mean ± SE) 76.0 ± 3.4. Access to shade prevented the heat stress-related decrease in milk stability both in the ethanol and in the coagulation time test, as well as maintained milk acidity within an acceptable range (14 to 18°D).

Heat stress reduces milk yield (Silanikove *et al.*, 2009) and milk synthesis, decreasing its protein, fat and lactose contents (Bernabucci *et al.*, 2015). Raw milk suitability to industrial processing involving heating treatments is evaluated with stability tests, such as the ethanol test, which is still used in several countries such as Brazil, Russia, Taiwan, China, Argentina, Uruguay, South Africa (Chavez *et al.*, 2004; Horne, 2015; Machado *et al.*, 2017). Certain physiological and pathological conditions of cows may induce changes in milk's chemical composition (Fagnani *et al.*, 2014), resulting in abnormal alterations in the concentrations of protein, sodium, chloride and lactic acid that might impair milk's stability. There are few studies evaluating the effects of heat stress on functional characteristics of raw milk such as stability and acidity. In the cheese making process, milk coagulation properties, such as clotting time and curd firmness were negatively affected when the temperature–humidity index (THI) was higher than 75 (Bernabucci *et al.*, 2015). To our knowledge, there are no studies on the provision of shade to mitigate the adverse effects of heat stress on milk stability.

Our objective was to test the hypothesis that during the summer in the subtropics, access to shade helps to maintain functional properties of raw milk acceptable to industrial processing, this study aimed to investigate the effects of tree shade provision for dairy cows on stability and acidity of raw milk.

Materials and methods

Local description, animals and management

The trial was conducted on a commercial dairy farm in Brazil (latitude of −27°05′05″, longitude of −53°35′38″; altitude of 206 m above sea level) during the summer, from January to March and lasted 38 d. Handling of animals was performed following Brazilian National guidelines and was approved by the Ethics Committee of the Federal University of Rio Grande do Sul, under the number 21901.

Sixteen lactating Holstein cows were selected from the dairy herd. At the beginning of the experiment animals had 520 ± 74 kg body weight (BW), body condition score (BCS) of 3.0 ± 0.3, yielded 21.5 ± 4.2 kg/d milk (MY) and were in milk for 120 ± 61.2 d (days in milk – DIM). Cows were divided into two groups of eight cows each considering initial milk production, age and lactation period.

Each group was placed in its own specific but adjacent rectangular-shaped paddock (40 × 700 m each) with Tifton 85 (*Cynodon dactylon*) grass, and this separation lasted until the end of the trial. Cows within groups grazed a new strip of pasture of approximately 0.8 ha every day, moving the front and back fence lines inside the paddocks. The shaded area of each paddock, approximately 200 m², was located at the end of the paddock (shorter side of the

rectangle) and was not covered with pasture. Cows had permanent access to water in drinkers installed in the paddocks.

During the first 14 d (day 1 to 14 – pre-stress period) all cows had free access to the shaded area of its specific paddock, with at least 10 m^2 of shade per animal. On days 15 to 24 (stress period), one group of cows was deprived of accessing the shaded area by the use of a fence line behind the projection of the tree's shadow. On days 25 to 38 (post-stress period), fence line that prevented access to shade from one group was removed and all the two groups of cows had free access to their respective shaded areas. Since the shaded area was not covered with pasture, cows with access to shade in the stress period did not have increased pasture allowance when compared with cows without access to shade.

Production and milk composition traits

Milk yield (kg) was recorded at the morning and evening milking using Westfalia milk meter. Individual milk samples were taken daily at the morning and evening milking. Proportional volumes of milk from morning and evening milking from each cow were mixed and composed 200-ml samples. Concentrations of fat, total protein and lactose in milk were determined by an infrared analyzer (Bentley 2000® equipment). Acidity and density were determined by titration with 0.1 N NaOH solution and using a thermolactodensimeter, respectively (Tronco, 1997). Ethanol stability was determined according to Machado et al. (2017); results were expressed as the minimal ethanol concentration in the alcoholic solution that induced milk coagulation. Heat stability was estimated using the coagulation time test (CTT) described by Negri et al. (2001) and Machado et al. (2017). Somatic cell count (SCC) was determined by flow cytometry with Somacount 300®. Detailed materials and methods are provided in the online Supplementary File.

Statistical analysis

Variables with continuous values were submitted to normality test using Shapiro–Wilk test. Somatic cell count was transformed using log 10 (log$_{10}$ SCC). The power analysis of the sample size was calculated using the POWER procedure

Data were analyzed by univariate variance analysis with cows as the experimental units according to a completely randomized design with a factorial arrangement ($n = 2$: treatments – shade and no shade) and period ($n = 2$, Stress and Post-stress) and repeated measurements over time (days). PROC MIXED (SAS®) was used to evaluate a model: $Y_{ij} = m + T_i + P_j + TP_{ij} + \beta(X_{ij} - X_m) + \epsilon_{ij}$, where Y_{ij} is the observed response, m = constant, T_i is the effect access to shade ($n = 2$, with or without shade), β is the linear regression coefficient between the covariate and the response, X_{ij} is the observed value of the characteristics measured on pre-stress period (used as covariate), X_m is the mean of the covariate, P_j is the effect of period ($n = 2$, stress and post-stress periods), TP_{ij} is the effect of the interaction between treatment and period and ϵ_{ij} is the error term; using the following parameters: method = REML, covariance matrix = AR(1), repeated = day. Mean separation was performed by using PDIFF option. The significance criterion was taken as $P < 0.05$. Data measured on pre-stress period, when all cows had access to shade were used as a covariate. Additionally, long term effect of shade on milk ethanol stability was analyzed with PROC MIXED on all days of measurement, covariance matrix = AR(1).

Results

The sample sufficiency was confirmed by a power analysis with the result of 0.99 for all variables tested.

During the stress period, overall means (±SE) of air temperature (AT – °C) were 25.9 ± 2.7 and 28.4 ± 3.1, relative humidity means (RH – %) were 72.9 ± 8.3 and 60.0 ± 12.7 and Temperature-Humidity Index means (THI) were 75.4 ± 3.7 and 77.4 ± 3.5 at the shaded and unshaded areas of the paddocks, respectively (online Supplementary Fig. S1).

There were significant interactions between treatment and period ($P < 0.05$) for milk yield, milk crude protein, density and acidity. During the stress period, cows without access to shade showed lower values ($P < 0.05$) for milk protein concentration (2.8 vs. 3.0 g/100 g, SE = 0.04) and density (1028.3 vs. 1029.6, SE = 0.38) but with higher acidity (18.7 vs. 15.4 °D, SE = 1.0) compared with cows with access to shade. The stressed cows also exhibited a numerical but non-significant decrease in milk yield (17.7 vs. 19.4 kg/d, SE = 1.0). The largest effects of shade unavailability in the stress period were increased milk acidity and reduced stability both in the ethanol and in the coagulation time test (Table 1). In unshaded cows, milk ethanol stability dropped below the minimal values required by the dairy industries (e.g. 70 to 72 °GL, depending on the country's legislation) on days 22 and 24 (stress period), while ethanol stability in shaded cows showed much less variation, remaining above the minimal values required. Recovery to stability values similar to those registered during the pre-stress period took approximately 14 d (online Supplementary Fig. S2). Values for coagulation time were lowest for the cows without access to shade both in stress and post-stress periods. Values for milk urea during the pre-stress were lowest, increasing for both groups, but the highest values were noticed for the cows with access to shade during the stress period. During the post-stress period, there were no significant differences ($P > 0.05$) for milk yield, milk crude protein, acidity and SCC between groups of cows. There were no effects ($P > 0.05$) of treatment (access to shade) or interaction between treatment and period for fat and lactose concentrations in milk (Table 1).

Discussion

The main contribution of the present study is to highlight the positive effects of providing natural tree shade to grazing cows on milk stability and acidity during the summer in the subtropics. Functional characteristics of milk such as stability are important during milk thermal processing in the dairy industry. Ethanol stability is a cheap and fast test run at farm and at industry reception to check raw milk suitability for industrial purposes (Horne, 2015), and usually the minimal acceptable value varies from 70 °GL ethanol, as in Argentina (Chavez et al., 2004) to 72 °GL ethanol, as in Brazil (Machado et al., 2017). However, a minimal value of 74 °GL or even higher is recommended for UHT milk (Shew, 1981). Milk stability may also be measured by the time needed for milk to coagulate at high temperature, e.g. 144°C (Chavez et al., 2004). It should be taken into consideration that one of the limitations of this last test is the lack of a clear definition of the value for the low threshold for milk stability, although it might be assumed that, the longer milk resists treatment without the formation of clots, the greater is its thermal stability (Negri et al., 2001; Machado et al., 2017).

Cows with access to shade produced milk with ethanol stability (76.5 °GL) suitable for UHT process. In cows with shade access throughout the study, milk stability presented small and no

Table 1. LSmean values for milk yield and composition of cows under alert THI conditions with or without access to shade at Stress and Post-stress periods

Trait	Pre-stress		Stress		Post-stress		SEM
	No shade	Shade	No shade	Shade	No shade	Shade	
Daily milk yield (l)	21.4	21.0	17.7	19.4	21.4	20.2	1.0
Milk crude protein (g/100 g)	2.9	2.9	2.8[b]	3.0[a]	3.1	3.1	0.04
Milk fat (g/100 g)	3.2	3.4	3.5	3.3	3.4	3.4	0.4
Lactose (g/100 g)	4.5	4.3	4.3	4.5	4.4	4.4	0.1
Milk urea (mg/dl)	16.1	15.7	25.9[b]	30.6[a]	27.4[b]	29.0[a]	2.7
Log_{10} SCC[1]	5.5	5.6	5.6[b]	5.8[a]	5.8	5.7	0.08
Density (g/l)	1031.8[a]	1035.5[b]	1028.3[a]	1029.6[b]	1031.7	1030.5	0.38
Titratable acidity[2] (°D)	15.0	15.2	18.7[a]	15.2[b]	16.2	16.1	0.8
Ethanol stability[3] (°GL)	77.0	76.8	71.9[b]	76.5[a]	73.6	74.8	4.9
Coagulation time test (seconds)	213.3	244.6	138.6[b]	196.4[a]	146.1[b] b	188.9[a]	16.1

[a–b]LSmeans within a row and within periods (pre-stress, stress and post-stress) with different superscripts are statistically different ($P < 0.05$).
[1]Log_{10} SCC = Somatic cell count logarithmically transformed (log_{10}).
[2]Dornic degrees (°D), 1 Dornic degree (1°D) is equal to 0.1 g of lactic acid per liter of milk.
[3]The volume of ethanol (ml) in 100 ml of an alcoholic solution, in Gay-Lussac (GL) degrees.

significant variation between periods, which was expected. When cows without shade access regained shade in post-stress, partial recovery of ethanol stability to pre-stress levels took approximately 14 d, showing long term effects (online Supplementary Fig. S2). Stability decrease in cows without shade might be partially related to the high acidity in milk, which could have increased the ionic calcium concentration in milk, acknowledged as important factor down-regulating milk stability (Chavez *et al.*, 2004; Horne, 2015). It is important to emphasize that the lower ethanol concentration used in the study was 68 °GL, so it is possible that milk stability in unshaded cows was even lower, but out methodology did not enable us to detect it.

Shade provision during stress period had only a small (non-significant) but positive effect on milk production but did significantly increase milk crude protein concentration. The negative effects of severe heat stress on milk production (Silanikove *et al.*, 2009) can be greater than those provoked by lower feed intake and are related to metabolism disturbances (Gao *et al.*, 2017). Chronic heat stress is known to reduce milk component concentration such as protein and lipids due to lower synthesis in the mammary gland (Cowley *et al.*, 2015).

The increase in SCC observed for the cows with access to shade in stress period might be related to higher humidity at the ground with accumulated mud in the shaded area, conditions usually associated with high SCC (Sandrucci *et al.*, 2014). Also, a behavioral study (Vizzotto *et al.*, 2015) showed that cows with access to shade spent more time lying, increasing the udder's exposure to environmental pathogens, especially after milking. However SCC is not usually related to milk stability (Machado *et al.*, 2017) as increases in pH reduce ionic calcium (Horne, 2015), probably counteracting the changes in minerals (e.g. increases in chlorides and sodium) (Chavez *et al.*, 2004) and serum proteins (Horne, 2015) that usually lower milk stability.

In conclusion, under the prevailing environmental conditions in the study when cows faced THI that could be classified as in the alert range, access to shade maintained milk acidity and stability within an acceptable range, increasing its suitability for dairy processing. Shade provision should be considered in dairy production in the tropics.

References

Bernabucci U, Basiricò L, Morera P, Dipasquale D, Vitali A, Piccioli Cappelli F and Calamari L (2015) Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science* **98**, 1815–1827.

Chavez MS, Negri LM, Taverna MA and Cuatrín A (2004) Bovine milk composition parameters affecting the ethanol stability. *Journal of Dairy Research* **71**, 201–206.

Cowley FC, Barber DG, Houlihan AV and Poppi DP (2015) Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science* **98**, 2356–2368.

Fagnani R, Beloti V and Battaglini APP (2014) Acid-base balance of dairy cows and its relationship with alcoholic stability and mineral composition of milk. *Pesquisa Veterinária Brasileira* **34**, 398–402.

Gao ST, Guo J, Quan SY, Nan XM, Fernandez MVS, Baumgard LH and Bu DP (2017) The effects of heat stress on protein metabolism in lactating Holstein cows. *Journal of Dairy Science* **100**, 5040–5049.

Horne DS (2015) Ethanol stability and milk composition. In McSweeney PLH and O'Mahony JA (eds), *Advanced Dairy Chemistry. Volume 1B: Proteins: Applied Aspects*, pp. 225–246. 4th Edn. Cork: Springer.

Machado SC, Fischer V, Stumpf MT and Stivanin SCB (2017) Seasonal variation, method of determination of bovine milk stability, and its relation with physical, chemical, and sanitary characteristics of raw milk. *Revista Brasileira de Zootecnia* **46**, 340–347.

Negri L, Chavez M, Taverna M, Roberts L and Speranza J (2001) Fatores que afectan la estabilidad térmica y la prueba de alcohol en leche cruda de calidad higiénica adecuada. Informe técnico final del proyecto. INTAEEA/Rafaela – INTI CITIL Rafaela, 27

Sandrucci A, Bava L, Zucali M and Tamburini A (2014) Management factors and cow traits influencing milk somatic cell counts and teat

hyperkeratosis during different seasons. *Revista Brasileira de Zootecnia* **43**, 505–511.

Shew DI (1981) IDF Bulletin, New monograph on UHT milk, document *133*, Brussels, pp. 115–121.

Silanikove N, Shapiro F and Shinder D (2009) Acute heat stress brings down milk secretion in dairy cows by up-regulating the activity of the milk-borne negative feedback regulatory system. *BMC Physiology* **9**, 1–9.

Tronco VM (1997) Manual para inspeção e qualidade do leite. Santa Maria: Ed. Da UFSM, 166p.

Vizzotto EF, Fischer V, Thaler Neto A, Abreu AS, Stumpf MT, Werncke D, Schmidt FA and McManus CM (2015) Access to shade changes behavioral and physiological attributes of dairy cows during the hot season in the subtropics. *Animal: An International Journal of Animal Bioscience* **9**, 1559–1566.