Relationships between heat stress and metabolic and milk parameters in dairy cows in southern Brazil

Alejandra Barrera Garcia[1] · Natalia Angeli[1] ·
Letícia Machado[1] · Felipe Cardoso de Cardoso[2] · Félix Gonzalez[1]

Abstract This study approached the relationships between heat stress and metabolic and milk parameters in a commercial herd of Holstein cows located in southern Brazil. A total of 50 multiparous cows at different lactations and lactation stages were selected in order to obtain 450 samples during two consecutive years (2011 and 2012). The animals were fed a partial mixed ration along with ryegrass pasture in a semi-confinement system. Blood, milk, and urine samples were taken during the summer and winter for a total of eight samples. Three intervals of temperature-humidity index (THI) were established during the summer months (January and February) as follows: low group (LOW), THI between 75 and 81 ($N=100$); moderate group (MOD), THI between 81 and 82 ($N=150$); and severe group (SEV), THI between 83 and 90 ($N=150$). The group of cows sampled during winter (July) constituted the control group (CON; THI=59, $N=50$). Increased total protein, albumin, glucose, and cholesterol occurred in heat-stressed cows. Increased AST activity was also observed in heat-stressed cows, but triglycerides and beta-OH-butyrate did not show any difference among groups. Lower lactate and higher pO_2 were seen in cows with heat stress than CON. Cows in SEV had a 21 % milk yield decrease, while lactose and protein decreased with fat not being affected. Heat stress had strong effects on metabolic, clinical, and performance parameters in Holstein cows.

Keywords THI index · Metabolism · Milk quality · Acid-base balance

Introduction

In agriculturally relevant species, heat stress (a heat load) increases health care costs and, depending on the severity, animals can die of severe thermal stress, especially lactating cows without shade (Rhoads et al. 2013). The southern region of Brazil (latitude 28.21, longitude 52.4, altitude 684 m) is characterized by a humid subtropical weather with defined seasons of hot summer and cold winter. The temperature and relative humidity have maximum values in January and February, when Holstein cattle experience heat stress. Heat stress reduces reproductive performance (Samal 2013), reduction in milk yield, and changes in milk composition, in part as a consequence of reduced feed intake but also due to a direct metabolic effect on the mammary gland (Rhoads et al. 2009; Smith et al. 2013). Heat stress has a direct effect on cow's metabolism, but this approach has been less studied than the impact on milk yield and milk composition. The metabolic approach of this problem is necessary to identify strategies leading to improved well-being of animals under heat stress (Abeni et al. 2007; Honig et al. 2012).

Climatic, clinic, and metabolic parameters can be used to define the severity of the heat stress. The most accurate climatic parameter is the temperature-humidity index (THI), considering that THI values between 72 and 75 indicate mild stress, THI values between 75 and 80 indicate moderate stress, and THI higher than 88 indicate severe stress (Armstrong 1994). The most important clinical parameters on heat stress effects are rectal temperature (reference values: 38.3 to 38.9 °C) and respiratory frequency (reference values: 20 to 30 breaths/min), considering that heat stress occurs when

✉ Felipe Cardoso de Cardoso
  cardoso2@illinois.edu

1 School of Veterinary Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

2 Department of Animal Sciences, University of Illinois, 1207 W. Gregory Drive, Urbana, IL 61801, USA

rectal temperature is above 39.2 °C and respiratory frequency is above 60 breaths/min.

The aim of this work was to study the relationships among the environmental temperature-humidity index (THI) and the clinical, metabolic, and productive parameters in a dairy herd of high-yielding cows in southern Brazil.

Material and methods

Data collection

Fifty multiparous cows in different stages of lactation were randomly selected from the herd. Samples collected were blood, milk, and urine, during the summer months (January and February) for two consecutive years (2011 and 2012) and during the winter period (July). Blood, urine, and milk samples were collected right after the first morning milking (0800 hours). Cows were blocked by lactation number (3.8± 1.1). A total of 450 samples were analyzed. Another sample was taken during the winter of the second year. Therefore, a final total of 9 samples per cow × 50 = 450 observations. Cows were fed a partial mixed ration along with ryegrass pasture in a semi-confinement system. Cows stayed in the barn during the milking procedure and on pasture the remainder of the day. Cows were fed according to NRC (2001) recommendations ad libitum. Body condition score was assigned independently by more than 1 individual weekly using a 5-point scale (Ferguson et al. 1994) and the median score was used for each cow.

Blood samples were harvested through coccygeal vein venipunction in plain and heparinized tubes. Samples with lithium heparin were used for blood gas analysis (Radiometer ABL835). Within up to 1 h after harvest, samples were analyzed for sodium, potassium, lactate, pH, pCO_2, pO_2, bicarbonate, total CO_2, and base excess. Coagulated blood samples remained 1 h at room temperature and were centrifuged (2500 rpm for 15 min) and stored at −20 °C until biochemical analyses. The following metabolites were analyzed: total proteins, albumin, urea, creatinine, glucose, cholesterol, triglycerides, beta-OH-butyrate (BHB), and the enzymes AST, GGT, and ALP, using semi-automatic equipment (Metrolab D-1600, Argentine) and commercial diagnostic kits (Randox, Ireland, for BHB and Labtest, Brazil, for the rest). Globulin values were calculated by difference between total protein and albumin. Urine samples were obtained in sterile plastic glasses by inducing natural micturition by perineal massage, discarding the first streams for measuring pH (digital portable pHmeter Hanna model HI98127). Milk samples were sampled individually in conditioned glasses with preservative (bronopol) and sent to the laboratory for determination of fat, protein, lactose, urea N (MUN), and total solids by near infrared spectrometry (Bentley 2000, USA) and somatic cells

counting by flow cytometry (Somacount, Bentley, USA). Daily milk yield was registered individually on the day of blood sampling.

Measurements of environmental temperature and relative humidity were taken at the field where the cows were grazing using a portable meteorological station (Instrutherm, Brazil). The temperature-humidity index (THI) was calculated according to the following equation (McDowell et al. 1979):

$$THI = 0.8 \times AT + [RH \times (AT–14.4)] + 46.4$$
where AT = air temperature(¨C) and RH
= relative humidity(decimals).

Treatment groups were categorized at the tertile values of temperature-humidity index (THI) during summer months as follows: low group (LOW), THI between 75 and 81 (N=100); moderate group (MOD), THI between 81 and 82 (N=150); and severe group (SEV), THI between 83 and 90 (N=150). The group of cows sampled in winter constituted the control group (CON; THI=59, N=50).

Animals and housing

All experimental procedures were conducted according to protocols approved by the Federal University of Rio Grande do Sul Animal Care and Use Committee. This experiment was performed in a commercial dairy farm with 120 lactating cows. The mean milk yield of the herd 1 year before the experiment was 6.961 L/cow/lactation. All cows received the same diet within the collection period.

Statistical analysis

A final dataset including all the variables was constructed in SAS (SAS v9.3 Institute Inc., Cary, NC). Statistical analyses were performed using the MIXED procedure of SAS. A linear mixed model was used to explore the associations between THI regimens and variables of interest. Treatment variable (THI) was forced into the model. Parity was not significant ($P>0.6$) in the models and was removed. The covariate days in milk was left in the models. The covariance structure that yielded the lowest corrected Akaike information criterion was the compound symmetry and was used in the models. Cow was treated as a random effect. Residual distribution was evaluated for normality and homoscedasticity and variables were transformed if necessary. A log transformation was used for the variables NEFA, milk somatic cell count (SCC), and GGT for better homogeneity of the distribution of residuals. Means shown in tables for these variables are back transformed. Least square means were separated using the Tukey adjustment statement in SAS.

Table 1 Least square means and associated standard errors for clinical indicators in Holstein cows submitted to natural heat stress in southern Brazil

Indicator	THI=59 (control)	THI 75–81 (low)	THI 81–82 (moderate)	THI 83–90 (severe)
Rectal temperature (°C)	38.5±0.1a	39.3±0.1b	39.4±0.1b	39.4±0.1b
Respiratory rate (breaths/min)	37.9±1.4a	76.4±1.0b	79.9±0.8c	80.8±0.8c

Different letters indicate significant difference (P<0.001) among groups of heat stress

THI temperature humidity index

Results

Results corresponding to clinical indicators are shown in Table 1. Clinically, the effect of heat stress on cows with THI above 80, expressed by the increased rectal temperature (above 39.2 °C) and more evident by the incremented respiratory rate (above 76 breaths/min) was evident. All animals under heat stress showed body temperature and respiratory rate significantly higher than the cows under thermal comfort.

Performance indicators are shown in Table 2. The main recognized alteration that occurred in cows under heat stress, which was a reduction in milk yield, was evident in the animals under severe heat stress, but not in the cows under low or moderate heat stress. The cows under moderate and severe heat stress had the lowest values of body condition score. There was a higher SCC in the milk of the cows under moderate and severe stress than CON.

Blood metabolic indicators are shown in Table 3. Increased total protein was observed in all the cows under heat stress, along with increased albumin, but not globulins. Glucose and cholesterol concentrations were higher in heat-stressed cows than CON. The value of AST was higher in the cows under severe heat stress than CON. Blood gas indicators are shown in Table 4. The blood pH was lower while urine pH was higher in cows under heat stress than CON. Cows under thermal comfort had the higher values for bicarbonate and total CO_2, while the cows under severe heat stress had the highest values of pO_2. The concentration of sodium was higher only in cows under moderate heat stress, and potassium values were lower in cows under severe heat stress.

Discussion

The temperature-humidity index (THI) has been considered a good indicator of heat stress in cows in relation to its physiological effects (Dikmen and Hansen 2009). In the present study, the three categories of THI studied for determining heat stress (low, moderate, and severe) had THI differences among them (P<0.0001).

Alterations in rectal temperature and in respiratory rate are considered the most recognized physiological parameters for assessing the animal comfort and the adaptability to adverse environments (Ferreira et al. 2009). Respiratory rate higher than 60 breaths/min indicates heat stress, once cows utilize evapotranspiration as the main mechanism for losing body heat (Kadzere et al. 2002). In our experiment, the cows under thermal comfort showed a mean respiratory rate of 38 breaths/min, while the cows under heat stress showed values about 80 breaths/min.

The cows under low and moderate heat stress did not have decreased milk yield, maybe due to mellow heat during night that allowed the animals to dissipate the cumulated heat and therefore stabilized their body temperature. The cows under severe stress had lower milk yield than cows in control. Increased maintenance energy and reduced feed intake during severe heat stress are considered to be the cause for accentuating the negative energy balance and, consequently, reduced milk yield (Bertoni 1998).

Rhoads et al. (2009) reported that heat stress increased milk fat concentration, which was not found in our experiment. Increased milk fat might be related to rising free fatty acids

Table 2 Least square means and associated standard errors for performance indicators in Holstein cows submitted to natural heat stress in southern Brazil

Indicator	THI=59 (control)	THI 75–81 (low)	THI 81–82 (moderate)	THI 83–90 (severe)
Body condition score (scale 1–5)	3.75±0.1a	3.65±0.1a	3.51±0.1b	3.45±0.1b
Milk yield (L/cow/day)	25.2±1.0a	24.95±0.8a	25.0±0.7a	20.8±0.7b
Milk fat (%)	3.5±0.2	3.4±0.1	3.3±0.1	3.5±0.1
Milk protein (%)	3.4±0.1a	3.1±0.1c	3.2±0.1bc	3.3±0.1b
Milk lactose (%)	4.4±0.1a	4.4±0.1a	4.4±0.1a	4.3±0.5b
Milk total solids (%)	12.6±0.2a	11.4±0.1c	11.8±0.1bc	11.9±0.1b
Somatic cells (10^3/mL)	552±159b	571±114b	594±98ab	644±100a

Different letters indicate significant difference (P<0.05) among groups of heat stress

THI temperature humidity index

Table 3 Least squares means and associated standard errors for blood metabolic indicators in Holstein cows submitted to natural heat stress in southern Brazil

Indicator	THI=59 (control)	THI 75–81 (low)	THI 81–82 (moderate)	THI 83–90 (severe)
Total protein (g/L)	71.8±1.3c	80.8±0.9a	77.9±0.7b	77.0±0.7b
Albumin (g/L)	25.5±0.9c	30.5±0.6a	27.9±0.5b	30.1±0.5a
Globulins (g/L)	46.2±1.5	50.2±1.0	49.9±0.8	46.9±0.8
Urea (mg/dL)	37.1±1.7	33.2±1.1	33.3±0.1	32.7±0.9
Creatinine (mg/dL)	1.06±0.1a	1.07±0.1a	0.91±0.1b	1.12±0.1a
Glucose (mg/dL)	53.6±1.9b	60.2±1.3a	58.5±1.25ab	59.9±1.2a
Cholesterol (mg/dL)	89.5±6.7b	151.5±4.5a	163.6±3.8a	147.2±3.8b
Triglycerides (mg/dL)	12.3±0.9	13.6±0.6	13.6±0.5	13.8±0.5
β-OH-butyrate (mmol/L)	0.70±0.1	0.82±0.1	0.82±0.1	0.76±0.1
Lactate (mmol/L)	n.a.	1.64±0.1a	1.39±0.1b	1.01±0.1c
AST (U/L)	60.6±7.3b	130.7±4.9a	123.0±4.3a	115.7±4.8a
GGT (U/L)	37.6±2.9	33.4±1.7	34.2±1.6	32.9±2.6
ALP (U/L)	75.2±13.3	95.1±8.7	101.6±8.1	98.4±8.5

Different letters indicate significant difference ($P<0.05$) among groups of heat stress

n.a. not available, *THI* temperature humidity index, *AST* aspartate transaminase, *GGT* gamma-glutamyl transferase, *ALP* alkaline phosphatase

in negative energy balance, as in the case of severe heat stress. On the contrary, Kadzere et al. (2002) observed decreased milk fat in heat-stressed cows. Rhoads et al. (2009) also reported decreased milk protein in heat-stressed cows due to a lower synthesis of casein formation enzymes. In our study, milk protein decreased in moderate and severe heat stress. The values of total solids in milk were reduced in all groups of heat-stressed cows as a consequence of the reduction in protein and lactose, in agreement with the findings of Kadzere et al. (2002).

High-serum total protein and albumin observed in the heat-stressed cows in the present study may have been related to a mild dehydration. Albumin can diminish in situations of low protein intake, as usually occurs in heat stress conditions where the animals have lower feed intake, thus the dehydration takes on more transcendence to explain the rise in albumin. Globulins concentration did not change what suggests metabolic adaptation to maintain plasma oncotic pressure. Urea is also a good indicator of dehydration, but its concentration may be affected by a series of additional factors mainly nutritional. In the present study, no differences were observed in urea concentrations among the groups of heat-stressed cows albeit cows on thermal comfort had higher values of urea.

Increased blood glucose concentrations in cows under heat stress may have been related to a higher cortisol secretion in those conditions, which is a stimulus for gluconeogenesis (Wise et al. 1988). Higher cholesterol concentration in heat-stressed cows could have reflected a lower secretion of thyroxine that is a common finding in animals suffering heat stress as a mechanism for reduction of metabolic heat production (West 2003). Our results drastically differed from the

Table 4 Least squares means and associated standard errors for blood gas indicators in Holstein cows submitted to natural heat stress in southern Brazil

Indicator	THI=59 (control)	THI 75–81 (low)	THI 81–82 (moderate)	THI 83–90 (severe)
Blood pH	7.46±0.01a	7.42±0.01b	7.43±0.01b	7.41±0.01b
Urine pH	7.80±0.04b	8.01±0.02a	7.95±0.02a	7.98±0.02a
Bicarbonate (mmol/L)	29±0.6a	26.6±0.4b	25.4±0.4b	26±0.3b
pCO$_2$ (mmHg)	40.1±1.4	42.3±0.9	39.6±0.8	41.1±0.8
Total CO$_2$ (mmol/L)	30±0.6a	27.9±0.4b	26.5±0.3b**	27.1±0.4b
pO$_2$ (mmHg)	44.6±7.9	58.9±5.2	61.3±4.4	67.9±4.5*
Sodium (mmol/L)	n.a.	140.4±0.3b	142.2±0.3a	139.6±0.3b
Potassium (mmol/L)	n.a.	4.18±0.1a	4.1±0.1a	3.77±0.1b

Different letters indicate significant difference ($P<0.05$) among groups of heat stress

n.a. not available, *THI* temperature humidity index

*$P<0.1$, tendency; **$P=0.07$, for difference with low group

findings of Ronchi et al. (1999) who suggested that heat stress would bring a reduction in liver activity, leading to lower values of glucose and cholesterol. Our results for triglycerides and BHB concentrations also differed from those of Ronchi et al. (1999), who found increased BHB concentrations in cows under heat stress.

In the present experiment, cows under severe heat stress had decreased lactate. Deshpande et al. (2000), working with rats having septic processes, observed decreased blood lactate concentrations when animals were submitted to heat stress. The authors attributed the decrease in lactate concentrations to a greater availability of tissue oxygen in heat-stressed animals as a consequence of the augmented respiratory rate. In our experiment, this concept was consistent with the higher pO_2 found in the cows under severe heat stress.

Rhoads et al. (2009) stated that heat stress in dairy cows does not bring about the typical metabolic profile of negative energy balance, but the values of BHB in the present study revealed that energy balance was not highly negative. A lower blood pH along with a higher urine pH in cows under heat stress was apparently paradoxical. However, in heat-stressed animals, the increment in the respiratory rate here manifested by higher values of pO_2 and lower values of total CO_2 may have been responsible for a respiratory alkalosis. The blood buffer system ($CO_2 \leftrightarrow HCO_3$) may have been displaced to the left to compensate the loss of CO_2. This event may explain the lower values of bicarbonate in the heat-stressed cows, which would have been excreted in the urine. Thus, seems that the development of a compensatory metabolic acidosis with more alkaline urine, as it was described by West (2003), occurred. The lower blood potassium concentrations in animals under severe heat stress may have been related to the loss of this electrolyte through sweat (West 2003).

Conclusion

Our study indicates that heat stress had profound effects on clinical indicators and caused a reduction in milk yield in Holstein cows. Also, heat stress was associated with lower concentrations of milk protein and lactose and increased somatic cells count but did not affect milk fat content. The more evident effect of heat stress on metabolic indicators was an increase of total protein and albumin, probably due to dehydration. Increased glucose and cholesterol concentrations suggest that endocrine effects are related to heat stress. The respiratory alkalosis produced by heat stress was compensated through a metabolic acidosis.

References

Abeni, F., Calamari, L., Stefanini, L., 2007. Metabolic condition of lactating Friesian cows during the hot season in the Po Valley. 1. Blood indicators of heat stress. International Journal of Biometeorology. 52, 87–96.

Armstrong, D.V., 1994. Heat stress interaction with shade and cooling. Journal of Dairy Science. 77, 2044–2050.

Bertoni, G., 1998. Effects of heat stress on endocrine-metabolic and reproductive status of the dairy cows. Zootecnica e Nutrizione Animale. 24, 273–282.

Deshpande, G.G., Heideman, S.M., Sarnaik, A.P., 2000. Heat stress is associated with decreased lactic acidemia in rat sepsis. Critical Care. 4, 45–49.

Dikmen, S., Hansen, P.J., 2009. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? Journal of Dairy Science. 92, 109–116.

Ferguson, J.D., Galligan, D.T., Thomsen, N., 1994. Principal description of body condition score in Holstein cows. Journal of Dairy Science. 77, 695–2703.

Ferreira, F., Campos, W.E., Carvalho, A.U., Pires, M.F.A., Martinez, M.L., Silva, M.V., Verneque, R.S., Silva, P.F., 2009. Parâmetros clínicos, hematológicos, bioquímicos e hormonais de bovinos submetidos ao estresse calórico. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 61, 769–776.

Honig, H., Miron, J., Lehrer, H., Jackoby, S., Zachut, M., Zinou, A., Portnick, Y., Moallem, U., 2012. Performance and welfare of high-yielding dairy cows subjected to 5 or 8 cooling sessions daily under hot and humid climate. Journal of Dairy Science. 95, 3736–3742.

Kadzere, C.T., Murphy, M.R., Silanikove, N., Maltz, E., 2002. Heat stress in lactating dairy cows: a review. Livestock Production Science. 77, 59–91.

McDowell, D., Hooven, N., Cameron, K., 1979. Effects of climate on performance of Holsteins in first lactation. Journal of Dairy Science. 68, 2418–2435.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. (Washington, D.C.)

Rhoads, M.L., Rhoads, R.P., Van Baale, M.J., Collier, R.J., Sanders, S.R., Weber, W.J., Crooker, B.A., Baumgard, L.H., 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: 1. Production, metabolism, and aspects of circulating somatotropin. Journal of Dairy Science. 92, 1986–1997.

Rhoads, R.P., Baumgard, L.H, Suagee, J.K., Sanders, S.R., 2013. Nutritional interventions to alleviate the negative consequences of heat stress. Advances in Nutrition. 4, 267–276.

Ronchi, B., Bernabucci, U., Lacetera, N., Verini Supplizi, A., Nardone, A., 1999. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. Zootecnica e Nutrizione Animale. 25, 11–20.

Samal, L., 2013. Heat Stress in Dairy Cows: Reproductive Problems and Control Measures. International Journal of Livestock Research. 3, 14–23.

Smith, D.L., Smith, T., Rude, B.J., Ward, S.H., 2013. Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. Journal of Dairy Science. 96, 3028–3033.

West, J.W., 2003. Effects of heat stress on production in dairy cattle. Journal of Dairy Science. 86, 2131–2144.

Wise, M.E., Armstrong, D.V., Huber, J.T., Hunter, R., Wiersma, F., 1988. Hormonal alterations in the lactating dairy cow in response to thermal stress. Journal of Dairy Science. 71, 2480–2485.