Rumination time during the summer season and its relationships with metabolic conditions and milk production

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ABSTRACT

The main objective of this experiment was to monitor the rumination pattern during the summer season in lactating dairy cows and to investigate its relationships with metabolic conditions and physiological markers of heat stress. The study was carried out in an experimental freestall barn located near Piacenza, Italy (45°01'N, 9°40'E; 68 m above sea level), and involved 21 Italian Friesian cows (11 primiparous and 10 multiparous) during the summer season. Rumination time (RT) was recorded by using an automatic system and data were calculated and summarized in 2-h intervals. Microclimatic conditions (temperature and relative humidity) inside the barn were recorded during the trial, and the temperature-humidity index (THI) was calculated. Breathing rates and rectal temperatures were recorded following stable meteorological periods characterized by lower and higher temperatures. At the same times, blood samples were collected to assess biochemical variables related to energy, protein, and mineral metabolism, as well as markers of inflammatory conditions and enzyme activity. Daily milk yield, body weight, nutritional condition, and health status were also recorded. The average RT was 501 min/d, with no significant differences between primiparous and multiparous cows. According to the microclimatic conditions and physiological markers of heat stress, the cows suffered mild to moderate heat stress during the summer. A negative relationship between daily maximum THI and RT was observed ($r = -0.32$), with a reduction of 2.2 min of RT for every daily maximum THI unit over the threshold of daily maximum THI of 76. Most of the rumination occurred during the night (on average the nighttime RT was 63.2% of daytime and nighttime RT); moreover, the proportion of nighttime RT slightly but significantly increased as THI increased. Rumination time throughout the trial was negatively related to breathing rate and positively related to milk yield.

Daily maximum THI was negatively correlated with plasma glucose ($r = -0.52$) and positively correlated with plasma β -hydroxybutyric acid ($r = 0.26$). Values of plasma β -hydroxybutyric acid were positively related to RT through the trial. Our results indicate that hot conditions negatively affect RT and modify its daily pattern. The relationship between RT and the physiological markers used in our trial support the use of RT as a marker of heat stress.

Key words: heat stress, dairy cow, rumination time, metabolic profile

INTRODUCTION

The adverse effects of hot environmental conditions on the performance and welfare of dairy cows are well known and, sometime, quantitatively predictable (St-Pierre et al., 2003). Animals suffer heat stress when the core body temperature exceeds the range specified for normal activity because of a total heat load greater than the capacity for heat dissipation (Bernabucci et al., 2010). In an attempt to maintain body temperature, increased heat loss and reduced heat production are promoted by means of physiological and behavioral responses. The initial responses are homeostatic mechanisms and include increased water intake, increased loss of body fluids due to sweating and panting, and reduction in fecal and urinary water losses, reduced feed intake, and increased heart rate during short-term exposure to heat, which would slow down if heat stress persists (Horowitz, 2002; Kazdere et al., 2002). When the exposure to thermal load is prolonged, heat acclimation is achieved and includes reduction of growth hormone, catecholamine, glucocorticoid, and thyroid hormones, with consequent reduction of the basal metabolic rate and thus heat production (Johnson, 1980).

During heat stress, rumination is reduced in cows (Tapk and Sahin, 2006; Acatincai et al., 2009; Moallem et al., 2010), and a lower blood flow to rumen epithelium is observed (Hales et al., 1984). Acatincai et al. (2009) observed that with an increase in air temperature above 27 to 28°C, the rumination process was severely affected in Romanian Black and White mul-

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tiparous cows. In heat-stressed animals, the fractional rate of digesta passage in the gastrointestinal tract is slower, which reflects reduced intake, ruminal activity, and motility (Silanikove, 1992). Nevertheless, changes in microclimatic conditions can induce alterations in the activity and function of the digestive system, which are independent of changes in feed intake (Kazdere et al., 2002). Moallem et al. (2010) indicated that the primary negative effect of high temperature-humidity index (**THI**) is a depression of rumination time (**RT**), which subsequently led to a reduction of DMI, followed by a decline in milk yield. Those authors suggested that rumination time plays a pivotal role in the negative effect of heat stress on DMI and, consequently, on milk production. However, Bernabucci et al. (2010) recently showed that reduced nutrient intake (an indirect effect of heat) accounts for only about 35% of the heat stress-induced decrease in milk synthesis.

Heat production increases during and after feeding, and shifting a great part of feed intake to night hours, when nonevaporative heat loss from the animal to the environment is more efficient, results in lower energy expenditure during the daytime (Aharoni et al., 2005); thus, less metabolic heat is produced during the hotter hours of the day and heat loads are reduced (Brosh et al., 1998). However, shifting from morning to evening feeding in cows exposed to short-term moderate heat stress did not alleviate production losses associated with heat stress (Ominski et al., 2002). A different daily pattern of feed intake was observed in heat-stressed cows (Ronchi, 1998) and, considering the relationship between DMI and rumination time (Miron et al., 2003; Adin et al., 2009), a different daily behavior of rumination time in the hot season could be expected.

The recent introduction of an indirect method to measure rumination time, based on the analysis of vocal signs (HR-Tag rumination monitoring system, SCR Engineers Ltd., Netanya, Israel), allows automatic measurement of RT and analysis of rumination behavior during the daytime and nighttime. Our hypothesis was that the severity of heat stress would affect the daily behavior of rumination activity and that the effect is related to the decrease in milk yield. The aim of our trial was to study rumination activity in dairy cows during the hot season and to study the relationships with physiological markers of heat stress.

MATERIALS AND METHODS

Animal and Management Conditions

The Italian Friesian dairy cows involved in this study were raised in a freestall barn at the experimental farm "Vittorio Tadini," located near Piacenza, Italy (45°01'N,

9°40'E; altitude 68 m above sea level). Cows were raised in pens holding a variable number of cows each (12 to 32), consisting of a resting area with cubicles. Fresh potable water was available ad libitum in each pen. All cows were inspected daily and all health-related problems and incidents that occurred and any treatments administered throughout the trial were recorded. Cows were milked twice daily (0330 and 1500 h) and milk yield of each milking was recorded daily (Alpro, DeLaval, Tumba, Sweden). Cows were fed a TMR once daily ad libitum at 0730 h. To ensure cows had ad libitum access to the TMR, the amount offered to the cows was assessed daily with the aim of producing 3 to 8% refusals. The TMR offered and the refusals were recorded daily.

Furthermore, each pen was equipped with 2 axial flow fans (0.75 kW; 90 cm in diameter; 22,500 m³/h maximum airflow rate) installed along the feed drive-way. Fans were mounted at a height of approximately 2.5 m and angled downward at about 10° from vertical. The variable speed fans were thermostatically controlled and were switched on at 23°C and reached maximum flow rate at 27°C. Sprinklers (delivery rate of 4 L/min and a pressure of 150–200 kPa) spaced at 150-cm intervals were placed perpendicular to the airflow of the fans along the feed alley. The sprinklers were thermostatically controlled to 27°C in an uneven way: 50 s of showering and ventilation followed by 5 min of ventilation alone.

The trial was carried out in the summer season (from June 15 to August 24) and involved 21 Holstein Friesian dairy cows (11 primiparous, **PR**, and 10 multiparous, **PL**) of good genetic merit (Production, Functionality and Type index of 481, on average) and kept in a pen. At the start of the trial the DIM of these cows was 116 ± 33 d in PR and 99 ± 42 d in PL, BW averaged 565.0 ± 35.5 kg for PR cows and 645.0 ± 22.4 kg for in PL cows, and milk yield averaged 31.9 ± 4.7 kg/d in PR and 35.1 ± 5.6 kg/d in PL.

Measurements and Analyses

Microclimatic Conditions. Temperature and relative humidity of the inside barn were recorded daily during the study period using 2 electronic probes (Gemini Data Logger, Chichester, UK) connected to a data logger programmed to record every 30 min. Mean daily temperature and humidity and daily minimum and maximum temperature and humidity were calculated from temperature and relative humidity data recorded throughout the trial. Data were used to compute a composite climatic welfare index, the THI, according to the formula of Kelly and Bond (1971), as reported by Ingraham et al. (1979). Mean daily THI (**Avg THI**),

daily minimum THI (**Min THI**), and daily maximum THI (**Max THI**) were calculated throughout the trial, and heat stress was estimated according to Armstrong (1994). In addition, the occurrence of heat waves was detected according to Hahn et al. (1999).

Feed and Diets. Representative samples of forages and concentrate mixes were collected twice monthly. All samples were pooled monthly for analyses. Chemical (moisture, fat, CP, NDF, ADF, ADL, starch, and ash) composition were determined using standard procedures, and nutritive values were calculated according to NRC (2001). The chemical and nutritive characteristics of the diet of lactating cows were calculated monthly. Samples of TMR were collected weekly and analyzed for moisture. Furthermore, particle size distribution on these samples was determined by the new Penn State Particle Separator system (**PSPS**; Kononoff et al., 2003). Average daily feed intake was calculated daily by subtracting the refusals measured before TMR distribution from the total fresh TMR offered the day before. The DM content of the TMR for that week was then applied to both offered and refused feeds to estimate the daily DMI. In these calculations, the DM contents of the offered and refused TMR were assumed the same.

Rumination Time. The RT was measured using the HR-Tag rumination monitoring system (SCR Engineers Ltd.). The system consisted of rumination loggers, stationary readers, and software for processing the electronic data (Data Flow Software, SCR Engineers Ltd.). A neck collar positioned the logger on the left side of the neck. The logger contained a microphone that was able to record the distinctive sounds of regurgitation and rumination. Data were calculated and summarized in 2-h intervals and stored in the memory of the logger.

Blood. Blood samples were collected 5 times during the experimental period in accordance with the trends of THI as shown in Figure 1. In particular, to obtain blood samples under different microclimatic conditions, each blood collection was done after heat waves as well as after stable meteorological periods characterized by lower temperatures. Blood samples were collected in the morning, before feed distribution, by venipuncture from the jugular vein, using 10-mL lithium heparin-treated tubes (containing 18 IU/mL of Li-heparin, Vacuette, Kremsmünster, Austria). Samples were immediately cooled in ice water after collection.

A small amount of blood was used for determination of hematocrit (packed cell volume) after high-speed

Figure 1. Daily minimum and maximum temperature-humidity index (THI) and the potential for heat stress during the trial. Index values >72, 78, and 88 reflect the potential for slight, high, and severe heat stress, respectively. The arrows indicate the days when blood samples were collected.

centrifugation ($15,000 \times g$ for 10 min at room temperature) in a capillary tube. The remainder of the blood was centrifuged ($3,500 \times g$ for 16 min at 4°C) and the obtained plasma was separated into several aliquots and stored at -20°C for further analysis. Plasma metabolites were analyzed at 37°C by automated clinical analyzer (ILAB 600, Instrumentation Laboratory, Lexington, MA). Commercial kits were used to measure glucose, total cholesterol, urea, calcium, inorganic phosphorus, magnesium, total protein, albumin, total bilirubin, and creatinine (Instrumentation Laboratory), NEFA and zinc (Wako Chemicals GmbH, Neuss, Germany), and BHBA (BHBA kit Randox, Crumlin, UK). A potentiometric system with specific electrodes was used to measure Na, K, and Cl. Kinetic analysis was adopted to determine activity of alkaline phosphatase (**AP**, EC 3.1.3.1), aspartate aminotransferase (**AST**, EC 2.6.1.1), and γ -glutamyltransferase (**GGT**, EC 2.3.2.2) using commercial kits (Instrumentation Laboratory). Ceruloplasmin and haptoglobin were determined with reagents prepared according to the method reported by Bertoni et al. (1998). Total plasma reactive oxygen metabolites (**ROM**) were measured using a commercial kit (d-ROMs test, Diacron International, Grosseto, Italy), according to Bernabucci et al. (2005), and results were expressed as milligrams of hydrogen peroxide per 100 mL of plasma. This reagent kit measures not only ROM existing in the matrix but also ROM species developing during the Fenton reaction (Oriani et al., 2001).

Physiological Measurements. Rectal temperatures and breathing rates were measured weekly on cows in the feeding area before milking (1500 h) on the day before blood sampling. For measurement of rectal temperature, a digital thermometer was used; the measurement was terminated when an acoustic signal was emitted by the digital thermometer and the probe was retracted from the rectum. Breathing rate was determined by counting uninterrupted flank movements in 30 s and multiplying it by 2 to obtain breaths per minute.

Data Processing. The RT data obtained with the automatic system were summarized in 2-h intervals and used to calculate the following variables: total day-nighttime RT (**RT_{dn}**), by adding the 2-h interval values recorded from 0800 to 0800 h of the following day; daytime RT (**RT_d**), by adding the 2-h intervals values recorded from 0800 to 2000 h; and nighttime (**RT_n**), from 2000 to 0800 h of the following day. These values were also expressed as percentage of **RT_{dn}**. The data collected during the experimental period were grouped in 3 classes according to maximum daily THI: **LTHI** (<80), **MTHI** (from 80 to 85), and **HTHI** (>85). The thresholds were based on those proposed by Hahn et al. (1999) to define the severity of heat waves.

Statistical Analysis

All analyses were performed using the statistical software package SAS 9.2 (SAS Inst. Inc., Cary, NC). Data were tested for non-normality by the Shapiro test (SAS Inst. Inc.). In case of non-normality, parameters were normalized by log or exponential transformation. Transformations were performed for total bilirubin, haptoglobin, AST, GGT, NEFA, and BHBA.

The RT variables, milk yield, and plasma indices were submitted to repeated-measures variance analysis using a mixed model (MIXED procedure of SAS). The model included the fixed effect of parity (primiparous and multiparous), THI level, sampling date within THI level, and individual variability (within cow $\times$ RT level). The analysis was carried out using 3 covariance structures: autoregressive order, compound symmetry, and spatial power. These were ranked according to their Akaike and Schwarz Bayesian information criterion, with the one having the least information criterion being eventually chosen (Littell et al., 1998). For each treatment, least squares means were computed and preplanned pairwise comparisons (PDIF option, SAS Inst. Inc.) were conducted when the F-test of one of the main factors was significant at $P < 0.10$. Statistical significance was designated as $P < 0.05$ and tendencies were declared at $P < 0.10$.

The effect of THI above threshold on RT was calculated according to Bohmanova et al. (2007). The slope of a decline represents the reduction of rumination time per degree of THI above threshold. Different thresholds, ranging from THI 58 to 90 of Min, Avg, and Max THI, were tested in the model, and the one that provided the highest R^2 was selected.

The linear relationships between RT variables (**RT_{dn}**, **RT_d**, **RT_n**, and **RT_d:RT_{dn}** ratio) and blood metabolites were calculated by using linear regressions. In each model, RT variables were used as dependent variables and blood metabolites as independent variables. The data of each variable were expressed as difference between each actual value and the average value of each cow. Pearson correlations between RT variables (using average values of the previous 3 d) and milk yield as well as plasma indices were calculated.

RESULTS

Microclimatic Conditions

The course of daily Min and Max THI during the experimental period is shown in Figure 1. Every day during the trial, the cows experienced daily Max THI >72 . Cows were exposed to daily Min THI >72 on only 2 consecutive days in August (August 21 and 22), and

to daily Min THI around 70 for 4 consecutive days in July (July 10, 11, 12, and 13), and for more than a week in August (August 14 to 24). During the hotter period of July, a THI <72 was recorded for 13 h on 3 consecutive days (July 10, 11, and 12). During those days, a THI >79 was observed for 25 h. During the hotter period of August, a THI <72 was recorded for 6 h on 4 consecutive days (August 21, 22, 23, and 24). During those days, a THI >79 was observed for 34 h. Furthermore, during this latter period, the daily Max THI was continuously around 90 and greater than the values observed previously.

Diet Characteristics, DMI, and Milk Yield

The diet used for lactating cows was based on corn silage, alfalfa, and grass hay, and was characterized by a balanced forage:concentrate ratio and a high content of NDF from forage (Table 1). The analysis of TMR obtained with the PSPS was characterized by optimal separation of particles with each sieve. On average, the daily DMI during the trial was 22.7 ± 0.9 kg.

The pattern of milk yield through the trial (Figure 2) highlights a different rate of milk yield reduction during

Table 1. Composition, chemical analysis, and nutritional value of diets fed to lactating cows (% of DM)

Item	%
Ingredient	
Forage	56.24
Corn silage	33.29
Triticale silage	6.62
Alfalfa and grass hay	16.33
Concentrate	43.76
Corn meal	23.10
Soybean meal	5.11
Commercial concentrate mix ¹	14.62
Sodium bicarbonate	0.93
Nutrient	
NE _L , ² MJ/kg of DM	6.65
CP	15.83
Ether extract	3.27
Starch	29.64
NDF	32.38
Forage NDF	25.33
PSPS, ³ % of TMR retained on sieves	
Upper sieve, particles >19 mm	8
Middle sieve, particles >8 mm	36.1
Bottom sieve, particles >1.18 mm	35.3
Bottom pan, particles <1.18 mm	20.6

¹Based on soybean meal, sunflower meal, and barley; 1 kg contained 27 g of NaHCO₃, 26.8 g of CaCO₃, 6.7 g of NaCl, 6.7 g of Ca(H₂PO₄), 10 g of MgO, 1 g of ZnSO₄·7H₂O, 13.9 mg of Cu, 37.0 mg of Mn, 139 mg of Zn, 0.19 mg of Co, 3.2 mg of I, 0.37 mg of Se, 28,000 IU of vitamin A, 2,800 IU of vitamin D, and 139 mg of vitamin E.
²According to NRC (2001) and calculated using Razio-Best software of the Istituto di Zootecnica (Piacenza, Italy).
³Particle size distributions, measured using the new Penn State Particle Separator (PSPS; Kononoff et al., 2003).

Figure 2. Milk yield measured during the trial. Values are least squares means ± SEM.

the trial. The average rate of milk yield reduction was −0.13 kg/d. During July (from July 5 to 10), the rate of milk yield reduction was −0.53 kg/d. From August 16 to 22, a high rate of milk yield reduction was observed (−0.50 kg/d). A lower rate of milk yield reduction was observed during other days, with an increase of milk yield from July 18 to 28, days that were characterized by lower THI values. A positive correlation between milk yield and RT_{dn} was observed (r = 0.43; P < 0.001).

Rectal Temperature and Breathing Rate

Both parameters showed significant differences between THI classes, and both increased as THI increased (Figure 3). In particular, only the values observed in LTHI (including the observations collected when the daily Max THI was <80) were always significantly different from those observed in MTHI and HTHI (the latter including the observations collected when the daily Max THI was >85). Moreover, only in the HTHI class was the mean rectal temperature greater than 39°C. A slight difference in breathing rate was observed between MTHI and HTHI classes, with an average value around 70 breaths/min.

Rumination Time

The mean day-nighttime RT observed during the trial was 501 min/d, without significant differences between primiparous and multiparous cows. The RT and its daily behavior were affected by THI. The behavior of RT_{dn} during the trial is shown in Figure 4. A negative correlation between RT_{dn} and daily Max THI was observed (r = −0.32; P < 0.05), with a reduction, calculated using linear regression, of 2.2 min for every

Figure 3. Rectal temperature (black bars) and breathing rate (white bars) categorized by 3 levels of daily maximum temperature-humidity index (THI). Values are least squares means $\pm$ SEM.

daily Max THI unit over the threshold of daily Max THI of 76 (Table 2). A significant correlation was found between RT_d and daily Max THI ($r = -0.41$; $P < 0.01$), with a reduction of 2.1 min for every daily Max THI unit over the threshold of daily Max THI of 77 (Table 2). Conversely, nighttime RT was not correlated with THI. The greater effect of THI on RT_d affected the

proportion of RT during the day and night, with a significant reduction of the $RT_d:RT_{dn}$ ratio as daily Max THI increased (Figure 5).

The pattern of RT during the trial was related to that of DMI and both were related to THI. A weak negative correlation was observed between daily DMI and daily Max THI ($r = -0.24$; $P < 0.05$). Positive correlations between RT variables and DMI of the subsequent 1 to 3 d were observed ($P < 0.05$). Conversely, RT variables were not significantly related to DMI of the actual day.

Most rumination occurred during the night; on average, the proportion of RT_n was 63.2% of RT_{dn} (Figure 6a). A slight but significant reduction of the proportion of daytime RT occurred during the day with greater THI (37.4, 36.9, and 36.1% in LTHI, MTHI, and HTHI, respectively). The RT in each 4-h interval was also affected by THI, when expressed both in minutes of RT in each 4-h interval and as values expressed on daytime and nighttime RT (Figure 6b and 6c).

The daily behavior of RT according to THI classes is shown in Figure 7. The RT in each 2-h interval was negatively affected by THI in 6 intervals (4 daytime and 2 nighttime). Conversely, the RT in each interval expressed as a percentage of RT_{dn} was negatively affected by THI only in the 2-h interval after TMR delivery (between 0800 and 1000 h).

Figure 4. Values of daily maximum temperature-humidity index (THI; solid line with black circles), day-nighttime rumination time (solid line), daytime rumination time (dotted line), and nighttime rumination time (dashed line) during the trial. Values are least squares means $\pm$ SEM.

Figure 5. Relationship between daytime rumination time (RT; % of day-nighttime rumination time) and maximum temperature-humidity index (THI) ($r = 0.84$; $P < 0.01$). Each symbol represents the average value of the percentage in classes of THI according to 2-unit intervals of THI.

Blood Variables

Among blood variables related to energy metabolism, only plasma glucose and BHBA were affected by THI (Table 3). Plasma glucose differed statistically between THI classes, with greater values in LTHI compared with MTHI ($P < 0.05$). Furthermore, plasma glucose showed a high correlation with daily Max THI ($r = -0.52$; $P < 0.001$). Even though BHBA was only statistically greater in MTHI compared with LTHI and HTHI, a positive correlation with daily Max THI was observed ($r = 0.26$; $P < 0.01$). Variables related to protein metabolism (plasma urea and creatinine) had lower values in LTHI compared with MTHI and HTHI ($P < 0.05$). Nevertheless, only creatinine was correlated

with daily Max THI ($r = 0.28$; $P < 0.01$). Among blood variables related to mineral metabolism, only Na had lower values in HTHI compared with LTHI and MTHI ($P < 0.05$).

Lower activity of AP was observed in HTHI (not significant) and MTHI ($P < 0.05$) compared with LTHI. Furthermore, the activity of this enzyme was negatively correlated with daily Max THI ($r = -0.35$; $P < 0.001$). The activity of the other enzymes measured in this trial was not affected by THI.

The positive and negative acute phase proteins were not statistically different between THI classes. However, plasma haptoglobin was positively correlated with daily Max THI ($r = 0.23$; $P < 0.05$), whereas plasma ceruloplasmin was negatively correlated ($r = -0.27$; $P < 0.01$).

Relationships Between RT, Milk Yield, and Physiological Responses

Among the blood variables, only Mg showed a high correlation with RT variables (with RT_{dn} , r was 0.38; $P < 0.001$). Mild positive correlations were observed between RT_{dn} and albumin ($r = 0.22$; $P < 0.05$) and between RT_{dn} and AP ($r = 0.23$; $P < 0.05$).

The results obtained from the analysis of the linear relationships between the blood and RT variables, calculated by using for each variable the difference between each actual value and the average value of each cow, are shown in Table 4. All blood variables related to energy metabolism were related to the RT variables. In particular, BHBA levels throughout the trial were positively related to RT_{dn} ($P = 0.02$) and RT_n ($P = 0.02$). Plasma total cholesterol showed the same relationships, with a tendency to be significant ($P = 0.07$). Among

Table 2. Rate of decline (slope) per unit of increase of temperature-humidity index (Max THI: daily maximum THI; Min THI: daily minimum THI; Avg THI: daily average THI) above the threshold¹

Dependent variable ²	Independent variable	Threshold	Slope	SE _{slope}	<i>t</i>	<i>P</i> -value
RT_{dn} , min/d	Max THI	76	-2.228	0.463	4.80	<0.0001
	Min THI	62	-2.686	0.447	6.01	<0.0001
	Avg THI	73	-4.973	0.853	5.83	<0.0001
RT_d , min/daytime	Max THI	77	-2.099	0.282	7.45	<0.0001
	Min THI	63	-1.351	0.293	4.61	<0.0001
	Avg THI	75	-5.191	1.024	5.07	<0.0001
RT_n , min/nighttime	Max THI	NS	NS	NS	NS	NS
	Min THI	61	-1.317	0.293	4.49	<0.0001
	Avg THI	68	-1.128	0.353	3.20	0.0014
RT_d , % of RT_{dn}	Max THI	78	-0.217	0.040	5.40	<0.0001
	Min THI	NS	NS	NS	NS	NS
	Avg THI	74	-0.532	0.095	5.59	<0.0001

¹During the trial, the Min THI ranged from 59 to 73, the Avg THI ranged from 68 to 78, and the Max THI ranged from 75 to 90.

² RT_{dn} = values of day-nighttime rumination time; RT_d = values of daytime rumination time, and RT_n = values of nighttime rumination time.

Figure 6. (a) Daytime and nighttime rumination time (RT), and rumination time in minutes per 4 h during (b) daytime and (c) nighttime categorized into 3 levels of daily maximum temperature-humidity index (THI). Values are least squares means $\pm$ SEM. Values in each bar indicate the percentage of day-nighttime rumination time (a), daytime rumination time (b), and nighttime rumination time (c). ^{a,b}Means (percentage of RT in each daytime and nighttime interval) within the same interval without common superscripts differ ($P < 0.05$).

the blood variables related to mineral metabolism, a positive relationship was observed between inorganic P and Mg and RT variables, and a mild negative relationship was observed between Cl and RT_n ($P = 0.09$).

The pattern of milk yield through the trial was positively related to RT variables (Table 4). A negative relationship between breathing rate and RT variables was observed. The pattern of rectal temperature through the trial was negatively related only to RT_n (Table 4).

DISCUSSION

Rumination is a cyclical process characterized by re-gurgitation, remastication, and reswallowing, which is measurable by visual observation or indirect methods. In our study, a new system was used to measure RT in dairy cows (HR-Tag rumination monitoring system, SCR Engineers Ltd.) to study the influence of the weather on RT. This system is based on a microphone that detects chewing actions by analyzing vocal signals. This device has been described and validated by Schirmann et al. (2009) and used on heifers (Burfeind et al., 2011), as well as early lactating cows fed diets differing in roughage-NDF (Adin et al., 2009).

Most rumination activity occurs during night hours and occurs with other activities such as walking, nursing, scratching, defecating, and urinating, in both lying and standing position (Beauchemin, 1991). Rumination activity is influenced by many factors but mainly diet characteristics and nutritional factors such as digestibility of the feeds, NDF intake, and forage quality (Welch and Smith, 1970). In our study, the diet was characterized by a forage:concentrate ratio of 56:44 (DM basis), and an NDF content of 32.4% on DM basis (25.3% on DM basis as NDF from forage). The RT of cows in this study, producing on average 32 kg/d of milk, averaged 515 ± 60 min/d, without a significant effect of parity. The range of RT found for high-producing cows during lactation reported in literature was between 340 and 540 min/d (Kononoff et al., 2003; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006). The RT observed in the current study agree with those of DeVries et al. (2009), who found an average RT of 555 min/d in mid-lactating cows fed a 60:40 forage:concentrate ratio and producing 34 kg/d of milk. In addition, our data agree with those of other researchers who used a diet characterized by an NDF content ranging between 31 and 35%, similar to ours (32.4% DM) and who found RT to range between 428 and 518 min/d (Adin et al., 2009; Storm and Kristensen, 2011). The effect of NDF on RT was highlighted by Dado and Allen (1994) in early lactating Holstein cows. Those authors observed an increase in RT from 380 to 510 min/d by increasing the NDF content of the diet (from 25 to 35% of DM).

Figure 7. Daily pattern of rumination time expressed in minutes per 2 h (least squares means $\pm$ SEM) in 3 levels of daily maximum temperature-humidity index (THI). White bars = THI <80; bars with vertical lines = THI from 80 to 85; black bars = THI >85.

Rumination activity is also affected by stress factors. Rumination activity is negatively influenced by acute stress (Herskin et al., 2004), disease (Hansen et al., 2003), and high stocking density (Grant and Albright, 2001). Kazdere et al. (2002) observed a reduction of RT in heat-stressed cows. This reduction plays a pivotal role in the deleterious effect of heat stress in dairy cows (Moallem et al., 2010).

In our study, the microclimatic conditions and the variables used as markers of heat stress indicated that cows suffered heat stress during some periods during the trial. According to Armstrong (1994), heat stress is considered negligible in dairy cows when THI values are <72, whereas THI of >72, >78, and >88 are indicative of mild, high, and severe levels of heat stress. The values of daily Max THI recorded during our trial were always above the critical value of 72, indicating conditions capable of inducing moderate heat stress every day during the trial (Armstrong, 1994). According to the definition of Hahn et al. (1999), 2 heat waves (a period of at least 3 consecutive days with fewer than 10 recovery hours with a THI <72) occurred in our trial. The first wave was recorded between the July 10 and 12 and was classified as slight to mild heat stress. The

second wave was recorded between August 21 and 24 and was classified as mild to moderate heat stress.

Although cows in the current study were exposed to a THI >72 and even >79 for long periods, the potential heat stress was not severe. The average values of rectal temperature over the normal range of dairy cows (between 38.3 and 38.7°C; Abeni et al., 2007) observed when the daily Max THI was >80 indicated the presence of heat stress and insufficient thermoregulation. Also, breathing rate increased to average values of around 70 breaths/min when the daily Max THI was >80. The prevalence of breathing rates of 70 to 80 may indicate the need for heat stress relief measures (Berman, 2005).

Heat stress involves hormonal and metabolic changes, as anabolic hormones decline and catabolic hormones increase, which alter energy balance. High temperature stress affects production and reproduction performance by decreasing antioxidant enzyme activity, which increases oxidative damage in the tissues, and by changing carbohydrate, lipid, and protein metabolism. In response to reduced feed intake and consequent negative energy balance, insulin levels decrease, allowing for adipose lipolysis and increased circulating NEFA. On

Table 3. Least squares means of plasma variables according to daily maximum temperature-humidity index (THI)

Item ¹	THI class ²			SEM
	<80	80–85	>85	
Packed cell volume, L/L	0.309 ^a	0.300 ^a	0.316 ^b	0.0062
Glucose, mmol/L	3.96 ^b	3.77 ^a	3.90 ^b	0.0616
NEFA, mmol/L	0.102	0.088	0.096	0.0146
BHBA, mmol/L	0.448 ^a	0.576 ^b	0.424 ^a	0.0359
Total cholesterol, mmol/L	5.33 ^b	5.13 ^a	5.44 ^b	0.2319
Urea, mmol/L	6.18 ^a	6.64 ^b	6.76 ^b	0.2712
Creatinine, μ mol/L	91.6	92.1	93.4	1.5316
Ca, mmol/L	2.59 ^a	2.59 ^a	2.66 ^b	0.0425
Inorganic P, mmol/L	1.96 ^{ab}	2.09 ^b	1.84 ^a	0.0967
Mg, mmol/L	1.01	1.02	1.01	0.0237
Na, mmol/L	144.8 ^b	145.5 ^b	140.9 ^a	0.5595
K, mmol/L	3.98	4.04	3.99	0.0990
Cl, mmol/L	102.4	103.1	102.4	0.6340
Zn, μ mol/L	12.4	11.6	12.0	0.5122
Ceruloplasmin, μ mol/L	3.19	3.12	3.14	0.1706
Total protein, g/L	84.0 ^a	82.9 ^a	86.3 ^b	1.1231
Albumin, g/L	36.8	36.7	36.5	0.5904
Globulin, g/L	47.1 ^b	46.3 ^b	49.8 ^a	1.3856
Haptoglobin, g/L	0.18 ^b	0.18 ^b	0.06 ^a	0.0659
Total bilirubin, μ mol/L	1.12	1.16	1.19	0.1019
ROM, mg of H ₂ O ₂ /dL	14.00	13.81	13.31	0.5208
AST, U/L	110.1	108.5	108.3	8.1175
GGT, U/L	31.1	31.1	31.3	2.2241
AP, U/L	47.9 ^b	42.8 ^a	44.4 ^{ab}	3.4670

^{a-c}Least squares means within a row without common superscripts differ ($P < 0.05$).

¹ROM = reactive oxygen metabolites; AST = aspartate aminotransferase; GGT = γ -glutamyltransferase; AP = alkaline phosphatase.

²The numbers of observations were 42, 42, and 21 in <80, 80–85, and >85 THI classes, respectively.

Table 4. Relationships between rumination time (RT) variables and rectal temperature, breathing rate, milk yield, and blood metabolites¹

Item ²	RT ³			
	RT _{dn}	RT _d	RT _n	RT _d /RT _{dn}
Rectal temperature	NS	NS	0.02 (–)	0.01 (+)
Breathing rate	<0.001 (–)	0.01 (–)	0.01 (–)	NS
Milk yield	<0.001 (+)	<0.001 (+)	0.11 (+)	<0.001 (+)
Blood				
Glucose	NS	0.13 (+)	NS	NS
NEFA	0.10 (–)	NS	0.14 (+)	NS
BHBA	0.02 (–)	0.13 (–)	0.02 (–)	NS
Total cholesterol	0.07 (+)	NS	0.08 (+)	NS
Urea	NS	NS	NS	0.12 (–)
Inorganic P	0.05 (+)	NS	0.06 (+)	NS
Mg	0.06 (+)	0.06 (+)	NS	NS
Na	NS	NS	NS	0.13 (+)
Cl	NS	NS	0.09 (–)	0.06 (+)
Total protein	NS	0.03 (–)	NS	0.09 (–)
Globulin	NS	0.02 (–)	NS	0.03 (–)
Haptoglobin	NS	NS	0.12 (–)	0.05 (+)
AST	NS	NS	NS	0.11 (–)
GGT	0.10 (+)	NS	0.08 (+)	NS

¹The linear relationships were calculated by using, for each variable, the difference between each actual value and the average value of each cow. Values in the table are P -values: (+) indicates a positive relationship between row and column variables; (–) indicates a negative relationship between the row and column variables.

²AST = aspartate aminotransferase; GGT = γ -glutamyltransferase.

³RT_{dn} = day-nighttime RT; RT_d = daytime RT; RT_n = nighttime RT.

the other hand, heat-stressed cows, despite inadequate nutrient intake, exhibit increased basal insulin levels, lack of increase of NEFA levels, and increased plasma urea nitrogen (Bernabucci et al., 2010).

In the current study, the values of all blood metabolites and enzymatic activities were within the normal reference intervals (Bertoni et al., 2000). Nevertheless, the blood variables used as markers of heat stress confirmed that heat stress occurred during the hotter periods. Significant variations of glucose, BHBA, and urea were observed in the current study. The effect of THI on energy metabolism observed in the current study agrees with results obtained in heat-stressed cows (Abeni et al., 2007). These effects can be explained by different factors: (1) decreased energy intake because of the reduction in DMI; (2) increased cost for thermoregulation; (3) nutrient partitioning; (4) negative effect of the heat on gluconeogenesis, as an endocrine acclimation to hot conditions. Despite the reduced feed intake, an increase of plasma NEFA was not observed, agreeing with results of other authors obtained in heat-stressed animals (Rhoads et al., 2009; Shwartz et al., 2009). This could indicate that heat stress directly (not mediated by feed intake) affects energetics (Bernabucci et al., 2010). The positive relationship between THI and plasma urea observed in the current study agrees with the observations of Bernabucci et al. (2010), confirming that heat stress occurred during the hotter periods.

Among the measured enzymes, only plasma AP showed lower values during the hotter periods. Similar behavior of AP in hot conditions was observed by Vazhapilly et al. (1992) and Abeni et al. (2007). Reduction of AP was also observed by Ronchi et al. (1999) in heifers exposed to stressful temperatures and in cows exposed to high temperatures by Broucek et al. (1986). The reduction of AP during heat stress could be related to the endocrine acclimation response of cattle to the hot environment and to related variations in energy metabolism, from both liver and gut activity reduction, as suggested by Ronchi et al. (1999). Abeni et al. (2007) proposed AP activity as a heat-stress plasma marker in dairy cattle.

In the current study, lower values of RT during the hotter periods were observed, and a negative relationship between the daily Max THI and rumination time was observed. These results agree with those of other authors (Bertoni et al., 1998; Kazdere et al., 2002; Moallem et al., 2010). Moallem et al. (2010) observed, through principal component analysis, that the primary negative effect of high THI was depression of RT, which subsequently led to a decline in DMI followed by reduced milk production. In the current study, the reduction of DMI as THI increased was observed in

the days following the reduction of RT, confirming the hypothesis of Moallem et al. (2010). Furthermore, a close and positive relationship between RT and milk yield was observed throughout the trial.

The system used in our trial to measure RT allowed us to record the pattern of RT during daytime and nighttime. During the hotter periods, RT decreased during both daytime and nighttime, with greater reductions in RT during the daytime. The reduction of RT_d during hotter periods was mainly related to the daily Max THI; conversely, the reduction of RT_n was not related to the daily Max THI but was related to the daily Min THI. The different effect of microclimatic conditions on daytime and nighttime RT could be related to the pattern of microclimatic conditions during day and night, with higher temperature and more unfavorable conditions occurring during the daytime. Cow behavior studies demonstrate that cows suffering heat stress eat more frequent, smaller meals and, to increase the surface area available for dissipating body heat, stand for a longer periods (Frazzi et al., 2000), and pant as ambient temperature increases (Kazdere et al., 2002). We can hypothesize that chewing and rumination are impaired by panting in heat-stressed cows, mainly during the daytime. A negative relationship between breathing rate and RT throughout the trial was observed in the current study. Depressing RT might delay passage of the rumen digesta through the digestive tract, which would reduce the capacity of rumen for additional feed and thereby decrease feed consumption (Church, 1988). Shiao et al. (2011) compared the effect of different cooling systems and observed a lower daytime intake activity in cows that had suffered a greater heat stress. The reduction of DMI because of the reduction of RT in turn negatively influences the RT. Adin et al. (2009), analyzing the patterns of daily rumination and feed consumption, observed a delay of approximately 1 to 2 h between eating and rumination peaks. The results obtained in our study, besides confirming that heat stress reduces daytime and nighttime RT, highlight that heat stress modifies the proportion between daytime and nighttime RT, with a slight but significant reduction of the $RT_d:RT_{dn}$ ratio as the THI increases. In the current study, we also observed an effect of THI on the daily pattern of RT.

The behavior of RT throughout the trial was negatively related to the behavior of BHBA. This relationship may be related to the effect of hot conditions on RT and DMI, with a negative energy balance. The effect of heat stress on the proportion between daytime and nighttime RT, and probably on the proportion between daytime and nighttime DMI, could also help to explain the relationship between RT and BHBA observed in

our study. There is a meal effect on plasma NEFA and BHBA, with a reduction in the former and an increase in the latter after a meal (ASPA Commission, 1999).

In our trial, a significant and positive correlation between plasma Mg and RT was observed in the current study. Furthermore, levels of plasma Mg through the trial were positively related to RT. Plasma Mg is influenced by Mg intake (ASPA Commission, 1999) and thus its relationship with RT could be related to the relationship between RT and DMI. Magnesium is mainly, perhaps only, absorbed in the rumen and reticulum (NRC, 2001). Magnesium absorption from the rumen depends on the concentration of magnesium in solution in the rumen fluid (important to both the active and passive transport of magnesium across the ruminal wall) and the integrity of the magnesium transport mechanism (NRC, 2001). During heat stress, the lower RT and the lower DMI often reported in literature (Ronchi, 1998), associated with an increase in water content of digesta in the rumen (Silanikove, 1992), could lead to inadequate magnesium in the ruminal fluid, reducing magnesium absorption. In addition, increased utilization of orally administered magnesium when supplied with oral glucose was reported, suggesting that glucose supplied the ruminal epithelium with a source of energy to power active transport of Mg (NRC, 2001). The relationship between plasma variables of the energy metabolism and the pattern of RT through the trial could imply an effect of energy balance on the reduction of plasma Mg in cows with lower RT.

A negative relationship between RT and Cl was observed throughout the trial. The cow subjected to hot climatic conditions can have acid-base disturbances resulting from respiratory alkalosis caused by the increased breathing rate, subsequent renal compensation by increasing urinary excretion of bicarbonate and Na, and renal conservation of K (Collier et al., 1982). The higher values of plasma Cl during some hotter periods are related to the higher excretions of HCO_3^- in the urine (Calamari et al., 2007). The negative relationship between Cl and RT observed in our trial appeared to be mediated by breathing rate: greater reductions of RT occurred as breathing rate increased.

CONCLUSIONS

The lactating dairy cows used in this study suffered mild to moderate heat stress in some periods of the trial. The physiological markers of heat stress used in this trial showed, besides an increase in breathing rate and rectal temperature, an effect of hot conditions on plasma variables related to energy, protein, mineral metabolism, and enzyme activity (i.e., alkaline phosphatase). Rumination time was negatively affected by hot

conditions, and RT began to decrease over a threshold of 76 to 77 daily Max THI. The negative effect of daily Max THI on daytime RT was particularly evident; this resulted in a reduction in the daytime:nighttime RT ratio as the THI increased. These results could be related to the effect of heat stress on energy balance and DMI and the daily pattern of eating. Further research is necessary to verify whether the primary negative effect of high THI is the depression of RT, which subsequently leads to a reduction of DMI, which in turn negatively influences RT. Our results support the use of RT as a heat stress marker; its measurement during the summer season provides useful information on the potential levels of heat stress that often affect cows.

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