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Short-term physiological responses to moderate heat stress in grazing dairy cows in temperate climate

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ABSTRACT

Even in temperate climate regions, an increase in ambient temperature and exposure to solar radiation can cause heat stress in lactating dairy cows. We hypothesised that grazing dairy cows exhibit short-term physiological changes due to increasing heat load under moderate climate conditions. Over two consecutive summers, 38 lactating Holstein dairy cows were studied in a full-time grazing system. Data were collected in 10 experimental periods of up to three consecutive days with a moderate comprehensive climate index (**CCI**). The individual animals' vaginal temperature (**VT**), heart rate, and locomotor activity data were automatically monitored with sensors. Blood samples and proportional whole milk samples were collected at afternoon milking. The concentrations of beta-hydroxybutyrate, glucose, non-esterified fatty acids, urea nitrogen, plasma thyroxine and triiodothyronine were analysed in blood plasma, and fat, protein, lactose, urea nitrogen, cortisol, Na^+, K^+, and Cl^- concentrations were analysed in milk. The daily distribution of VT recordings greater than 39 °C showed a circadian rhythm with a proportion of recordings of 2% and lower during the night and a percentage of 10% or higher in the afternoon. The cows' maximal daily vaginal temperature (**VT_{MAX}**) between 0830 and 1430 h was positively related to the mean daily CCI in the same time period (**CCI_{MEAN}**; mean and SD 23.6 ± 5.4 °C). Cows with greater VT_{MAX} had an increased mean heart rate, plasma glucose and milk cortisol concentrations and decreased concentrations of plasma thyroxine and triiodothyronine. The concentration of Na^+ in milk was lower, and the concentration of K^+ in milk tended to be higher in cows with increased VT_{MAX}. For beta-hydroxybutyrate, non-esterified fatty acids and urea nitrogen concentrations in plasma and fat and lactose concentrations in milk no relationships were found in terms of increasing VT. For milk urea nitrogen and protein concentrations, the proportion of total variance explained by inter-individual or -period variance was high. In conclusion, changes observed in milk and blood likely reflected short-term physiological responses to moderate heat stress. In particular, milk cortisol and Na^+ may be useful traits for timely monitoring of heat stress in individual cows because their inter-individual variances were relatively small and samples can be collected non-invasively.

Implications

A moderate heat load can affect the health and welfare of grazing dairy cows, even in temperate regions. Because the susceptibility of individual cows to heat stress can vary greatly, it is necessary to monitor animal-related traits sensitive to heat load. In particular, cortisol and electrolytes in milk are potential physiological indicators to assess heat stress in the short term. Electrolytes could even be measured automatically in the future.

Introduction

Consumer demand for sustainable animal production that takes into account the natural behaviour of animals is constantly increasing (Arnott et al., 2017). Pasture-based milk production systems appear to be ideal to fulfil this demand (Arnott et al., 2017). In addition, grazing can be a very cost-efficient way to produce milk

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(Taweel et al., 2006) and concomitantly helps reduce the competition between feed for livestock and food for people.

There is evidence that even moderate increases in ambient temperature under temperate climate conditions cause heat stress in lactating dairy cows (Van Laer et al., 2015). Grazing dairy cows are particularly vulnerable to heat stress, as they are exposed not only to rising ambient temperature but also to direct solar radiation (West, 2003). Therefore, Mader et al. (2010 and 2011) have proposed using the comprehensive climate index (**CCI**), which considers not only ambient temperature and relative humidity but also wind speed and solar radiation, to assess climatic effects on grazing cows.

Although CCI is a comprehensive environmental indicator to assess heat load, CCI cannot reflect individual factors that further determine the actual heat stress experienced by an individual animal. In addition to climatic conditions, the susceptibility and responses of individual animals to heat stress depend on various other factors such as milk production, stage of lactation, breed, and coat colour (Polsky and von Keyserlingk, 2017; Hoffmann et al., 2019). To allow for the timely initiation of heat stress mitigation measures, continuous monitoring of traits indicating the heat load on, and the response of individual animals is required. An increased core body temperature as an indicator of heat stress has been demonstrated in grazing dairy cows (Veissier et al., 2018). Core body temperatures greater than 39 °C are an indication of heat stress (Kadokawa et al., 2012; Veissier et al., 2018). Vaginal temperature (**VT**) is often considered as a proxy for assessing core body temperature (Idris et al., 2021). Heart rate recording also is used as an indicator of heat stress. Increased heart rate during exposure to short-term heat stress was observed in loose-housed cattle (Jo et al., 2021). Furthermore, heat-stressed dairy cows have been shown to decrease feed intake in order to reduce metabolic heat production (West, 2003). Whereas a direct detection of reduced feed intake on pasture is not feasible, it might be indirectly assessed by changes in the concentrations of blood metabolites and metabolic hormones. Reduced feed intake in heat-stressed dairy cows leads to higher beta-hydroxybutyrate concentrations (Tian et al., 2016) due to the mobilisation of body fat. Furthermore, Shehab-El-Deen et al. (2010) observed lower plasma glucose concentrations and higher plasma non-esterified fatty acid concentrations in dairy cows during the hottest part of the day and suggested that this was related to a lower feed intake. Elevated plasma urea nitrogen concentration have been observed due to lacking energy in the rumen for microbial protein synthesis (Shehab-El-Deen et al., 2010). Both plasma thyroxine (**T4**) and triiodothyronine (**T3**) have been shown to be decreased in cows during heat stress (Magdub et al., 1981; Aleena et al., 2016). These previous studies were conducted in the barn under controlled conditions, and dairy cows were exposed to tropical heat stress conditions.

By contrast to invasive blood sampling, collecting milk samples is easy and analyses can be automated. Information on metabolic changes can be derived from milk samples collected during routine milking. Depression in milk yield and alterations in concentrations of fat, lactose, protein, and urea nitrogen (**MUN**) have been observed after a few days of heat stress exposure (Van Laer et al., 2015). Increased sweating and respiratory alkalosis during heat stress can affect the homeostasis of electrolytes in blood and milk (Calamari et al., 2007). Therefore, electrolyte concentrations in milk may reveal short-term physiological responses related to heat stress. Cortisol plays a key role in maintaining or restoring energy homeostasis in response to various stressors. Because plasma cortisol is transferred into milk (Mormède et al., 2007), milk cortisol reflects the average amount of cortisol released into circulation during the period between two milkings.

The utility of the mentioned traits in blood and milk as short-term indicators of heat stress in dairy cows on pasture in temperate climates is still unclear. The objective of the present study was therefore to identify short-term physiological changes associated with increased core body temperature in grazing dairy cows in temperate climates. We hypothesised that a rising CCI under moderate climate conditions would induce varying degrees of increase in the individuals' VT. Accordingly, we expected changes in heart rate, in the concentration of plasma metabolites and hormones, and in the concentrations of milk components, cortisol and electrolytes to accompany increases in VT.

Material and methods

Animals and experimental outline

The experiment was carried out during two subsequent summers (June 6th to September 7th, 2018, and June 15th to September 1st, 2019) on the Agroscope experimental farm in Posieux, Switzerland (46°46.01′N 7°6.03′E) with a total of 38 dairy cows (51% Holstein and 49% Red-Holstein). There were 24 cows in each experimental year, with 10 cows in the experiment in both 2018 and 2019. Due to circumstances not associated with the present study, one cow died during the summer of 2019 and was replaced. At the onset of the experiment in 2018, the cows were (mean ± SD) 103 ± 26 days in milk and in the 2.8 ± 1.4 lactation, had a BW of 647 ± 54 kg, and produced 35.2 ± 5.4 kg of milk per day. In 2019, the cows were 125 ± 20 days in milk and in the 2.5 ± 1.4 lactation, had a BW of 654 ± 77 kg, and produced 32.1 ± 6.1 kg of milk per day.

To select only clinically healthy cows for the experiment, a medical check-up including vital parameters, as well as udder and claw health, was performed. During the grazing periods, all cows were treated monthly with an insecticide (Butox Protect 7.5 pour-on, MSD Animal Health GmbH, Luzern, Switzerland) that was applied along the dorsal line from the neck to the root of the tail.

Data were collected during 10 experimental periods of 2–3 consecutive days (four experimental periods in 2018 and six in 2019), depending on weather conditions. In order to evaluate the dairy cows' short-term physiological reaction to various levels of moderate heat load, an experimental period started when the weather forecast announced a dry period with a daily increasing mean in ambient temperature for the next few days and with a minimal ambient temperature of $\geq$ 6 °C and a maximal ambient temperature of $\geq$ 15 °C within 24 h on the starting day. A new experimental period started after an interruption of at least 4 days.

Cows stayed on pasture from 0800 to 1430 h and from 1800 to 0400 h in experimental groups of four cows each. Milking times were from 0440 to 0550 h and from 1550 to 1640 h. After milking, the cows received an energy-rich concentrate (min. 1.8 kg/day under 25 kg/milk per day, max. 4.2 kg/day from 45 kg/milk per day onwards) in a transponder feeding station (Insentec, Marknesse, Netherlands) according to their current milk yield (Agroscope, 2018). The concentrate contained (g/kg): maize grain, 440; wheat grain, 220; barley grain, 110; maize gluten, 90; mixed fat, 30; molasses, 20; $CaCO_3$, 33; NaCl, 30; and trace elements-vitamin mix, 27. Non-iodised cattle salt was provided *ad libitum* on pasture. Shading systems were not provided on pasture, and neither fans nor sprinklers were used inside the barn or milking parlour.

Grazing management

Experimental groups were grazed in adjacent paddocks using a set stocking system. Paddock size varied between 1.0 and 1.3 ha and was adapted over the grazing period based on herbage growth.

The sward was composed of 87% grasses, 2% legumes, and 10% herbs. Pasture height was measured before and once during every experimental period, using an electronic rising plate meter (FARMWORKS Plate Meter F200, Jenquip, Feilding, New Zealand) and was (mean ± SD) 9.1 ± 0.8 cm in 2018 and 10.1 ± 0.6 cm in 2019. The estimated average herbage mass over an assumed postgrazing sward height of 4 cm was 1133 ± 108 kg DM per ha in 2018 and 1284 ± 196 kg DM per ha in 2019. Each paddock was equipped with a water trough (volume 5 L, floater-controlled valve; LA BUVETTE Lac 5, Tournes, France), and the water temperature which was continuously recorded between 0830 and 1430 using a thermometer (HOBO 64K Pendant UA-001-64, Onset, Bourne, Massachusetts, USA) was 21.3 ± 2.90 °C.

Sampling, laboratory analysis, feed data recording

Samples of herbage were collected every other day at multiple sites across the paddocks during each experimental period by using electrical shears (Husqvarna GARDENA AB S-561, Husqvarna, Sweden), imitating the grazing behaviour of the cows. Herbage samples were then chopped and lyophilised (Delta 1–24 LSC, Christ, Osterode, Germany). For each experimental period, concentrate samples were collected and subsequently dried at 105 °C for 3 h for the estimation of DM content. Analysis of the chemical composition of the herbage and concentrate was performed by following the methods described by Heublein et al. (2017). The net energy for lactation content and the content of absorbable protein at the duodenum of herbage were calculated according to Agroscope (2018) using a multi-step approach based on the estimated digestibility of organic matter. The net energy for lactation and absorbable protein at the duodenum contents of the concentrate were determined from the tabled values of the individual components (Agroscope, 2018). The results of the analyses and calculations are reported in Table 1.

Climate data

A mobile weather station (Onset, Bourne, Massachusetts, USA) was used on pasture to record the ambient temperature in °C, relative humidity in %, WS in m/s, and solar radiation in W/m^2 every minute. The weather station was located on the second of six paddocks, and the furthest paddock was 200 m away. The sensors were placed two meters above the ground. Due to technical problems in experimental periods 9 and 10, the climate data of the meteorological station in Grangeneuve (Meteo-Schweiz, Station Fribourg/Posieux, Switzerland), located approximately 1 km from the experimental pasture, were collected. The climate data were used to calculate the CCI, which reflects the perceived ambient temperature in °C. The CCI provides an adjustment to ambient temperature for relative humidity (Eq. (1)), wind speed (Eq. (2)), and solar radiation (Eq. (3)) and was calculated using the following equation (Mader et al., 2010 and 2011):

CCI = ambient temperature + Eq. (1) + Eq. (2) + Eq. (3)

$$e^{(0.00182*\text{relative humidity}+1.8*10^{-5}*\text{ambient temperature}*\text{relative humidity})} * \left(0.000054 * \text{ambient temperature}^2 + 0.00192 * \text{ambient temperature} - 0.0246\right) * (\text{relative humidity} - 30) \tag{1}$$

$$\frac{-6.56}{e^{\frac{1}{(2.26*\text{wind speed}+0.23)^{0.45}}*\left(2.9+1.14*10^{-6}*\text{wind speed}^{2.5}-\log_{0.3}(2.26*\text{wind speed}+0.33)^{-2}\right)}} - 0.00566\text{wind speed}^2 + 3.33 \tag{2}$$

$$(0.0076 * \text{solar radiation}) - (0.00002 * \text{solar radiation} * \text{ambient temperature}) + \left(0.00005 \times \text{ambient temperature}^2 * \text{solar radiation}^{1/2}\right) + 0.1 * \text{ambient temperature} - 2 \tag{3}$$

The temperature humidity index (**THI**) was calculated according to the formula proposed by NRC (1971).

Vaginal temperature

The VT of each cow was recorded continuously every 10 min with a microprocessor temperature logger (DST micro-T logger, Star-Oddi, Garðabær, Iceland) as described by Suthar et al. (2012). The logger had dimensions of 25.4 mm × 8.3 mm (length × diameter), a weight of 3.3 g, and an accuracy of ±0.06 °C. The logger was attached to a progesterone-free modified vaginal controlled internal drug-release device (Eazi-Breed CIDR, Zoetis, Parsippany, USA; length, 13.5 cm; wingspan, 15.0 cm) and inserted about 30 cm deep into the vaginal cavity at the onset of every experimental period. At the completion of every experimental period, the CIDR devices were extracted from the vaginal cavity, the loggers were connected to a computer (through a USB commu-

Table 1
Chemical composition of the herbage and the concentrate for dairy cows.

	Herbage[a]				Concentrate[b]	
	2018		2019			
Item	Mean	SD	Mean	SD	Mean	SD
DM (g/kg of wet weight)	199	25.9	236	36.2	895	3.70
Analysed nutrient and chemical composition (g/kg DM)						
Organic matter	901	10.9	910	7.90	895	15.7
CP	223	34.6	172	18.7	142	8.20
Crude fat	–	–	–	–	66	3.64
NDF	427	24.8	455	23.3	108	10.6
ADF	237	16.6	247	13.9	38.4	3.54
Starch	–	–	–	–	563	23.9
Estimated energy and protein supply per kg of DM[c]						
Net energy for lactation (MJ)	6.58	0.242	6.19	0.197	7.10	-
Absorbable protein at the duodenum (g)	114	6.70	105	4.76	110	-

[a] Means of 58 hand-collected herbage samples.

[b] Means of 11 samples.

[c] Using a multi-step approach based on the estimated digestible organic matter according to Agroscope (2018) for herbage and calculated from tabled values of the individual components for the concentrate.

nication box), and collected data were retrieved and stored in CSV files.

Heart rate and locomotor activity

Heart rate measurements in beats/min were recorded using a PolarTeam Pro system (Polar Electro Oy, Kempele, Finland). A data logger and a chest belt with two embedded electrodes are included in the system. During each experimental period, every cow was equipped with a chest belt and logger after the morning milking (between 0600 and 0700 h), and data recording started at 0830 h (30 min after cows had arrived on pasture). To increase electrical conductivity, the belts were soaked in water before being placed on the cows. In addition, the cows themselves were wetted with water where the electrodes were positioned. A foam pad (20 cm × 20 cm) was placed between the cow and the belt to protect the withers from lesions due to friction. Moreover, an equine elastic blanket belt (Felix Bühler, Lenzburg, Switzerland) was fitted to the chest belt to prevent the belt from slipping. The chest belts were removed starting from 1445 h, and collected raw data were stored in a cloud database and successively transferred into CSV files.

Locomotor activity of the cows was recorded continuously as a potential confounder influencing heart rate by using an accelerometer (MSR145 data logger, MSR Electronics GmbH, Seuzach, Switzerland) that recorded vertical and horizontal accelerations continuously at a rate of 1 Hz and a maximum acceleration of ±16 *g*. The day before every experimental period, each cow was equipped with an accelerometer that was attached at the metatarsus of the left hind leg as described by Weigele et al. (2018). For technical reasons, data were collected from the second experimental period onward. The accelerometer was removed at the completion of each experimental period, and raw data were transmitted via MSR software (version 5.28.14, MSR Electronics GmbH, Seuzach, Switzerland) to a computer as CSV files.

Blood constituents

During each experimental period, blood was collected from every cow between 1500 and 1550 h by puncturing the jugular vein and using EDTA-tubes (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). After sampling, tubes were kept in an ice bath until centrifugation at 1000*g* for 15 min. The retrieved plasma was stored at −20 °C for later analyses of beta-hydroxybutyrate, glucose, non-esterified fatty acid, urea nitrogen, T4, and T3. Beta-hydroxybutyrate, glucose, non-esterified fatty acid, and urea nitrogen were analysed enzymatically using kits as described by Graber et al. (2010) and T4 and T3 using radioimmunoassay as described earlier (Vicari et al., 2008).

Milk constituents

Milk yield was recorded at each milking (Pulsameter 2, SAC, Kolding, Denmark). Proportional whole milk samples were taken from every cow at each afternoon milking. Samples were preserved with Broad-Spectrum Microtabs II (Gerber Instruments AG, Effretikon, Switzerland) and stored at 5 °C for later analysis of fat, protein, lactose, MUN, and somatic cell count. The contents of fat, protein, lactose, and MUN were determined using infrared spectrometry (MilkoScan™ 7 RM, FOSS, Hillerod, Denmark), and somatic cell count was assessed using fluorescence flow cytometry (Fossomatic™ FC, FOSS, Hillerod, Denmark). A second milk sample per cow was taken and stored at −18 °C for analysis of cortisol, Na^+, K^+, and Cl^-. After thawing, whole milk was skimmed by centrifugation at 3 000*g* for 15 min, and the concentration of cortisol was analysed using radioimmunoassay as described by Schwinn et al. (2016). A subsample of the skim milk was taken and centrifuged again (14 000*g* for 30 min) to obtain milk serum. This was used to measure Na^+, K^+, and Cl^- by ion-selective electrodes of the ISE Module of the Roche Cobas Mira (Roche Diagnostics, Rotkreuz, Switzerland).

Data analysis

Data processing

Data were collected for a total of 26 days. Cows in estrus were removed from the herd during the experimental period (four cows, three experimental days in three experimental periods). Some VT (8.9%, animals lost the logger) and heart rate data (6.7%, technical problems) were lost. In addition, values of the VT below 37.3 and above 40.4 °C (< 0.1%) and heart rate values below 57 and above 170 beats/min (3.1%) were considered measurement errors according to Ammer et al. (2016) and Wierig et al. (2018) and were excluded from the dataset. Based on acceleration data, the mean activity per hour (*g*-force per hour) between 0830 and 1430 h was calculated with R (version 4.0.2; R Core Team, 2021) according to Weigele et al. (2018).

To overcome autocorrelation and pseudoreplication among the values within a day, CCI and heart rate were reduced to single observations per day per animal by using their mean from 0830 to 1430 h (**CCI_{MEAN}**, **heart $rate_{MEAN}$**). For VT, the maximal value (**VT_{MAX}**) per animal per day in the same time period was considered. To avoid analysing data containing non-plausible extremes, the difference between VT_{MAX} and the previous (10 min before) and following (10 min after) VT value was calculated (mean difference across cows: 0.054 ± 0.016 °C).

Statistical analyses

The statistical analysis was conducted with R (version 4.0.2; R Core Team, 2021) by using the lme4 package for calculating linear mixed-effects models (lmer; Bates et al., 2015). Mixed-effects models were chosen to account for dependencies in the data due to the experimental design. Because of the non-normal distribution of residuals of non-esterified fatty acid, cortisol, and Na^+ concentrations, these models were run with log10 transformed variables. The relation between VT and CCI was analysed with VT_{MAX} as the outcome variable and included CCI_{MEAN} and CCI^2_{MEAN} as explanatory variables, as the relation did not show a linear regression. To explore whether an individual's heart $rate_{MEAN}$, concentrations of plasma hormones (T4 and T3), plasma metabolites (beta-hydroxybutyrate, glucose, non-esterified fatty acid, and urea nitrogen), milk components (fat, lactose, protein, and MUN), milk cortisol, and milk electrolytes (Cl^-, K^+, and Na^+) were related to each cow's daily level of heat stress, one linear regression model using VT_{MAX} as an explanatory variable was specified for every trait. As nutritional status and lactational stage of the cow are expected to impact blood metabolites, grass CP content per experimental period (g/kg DM), individual daily consumption of concentrate feed (kg), and days in milk were considered as covariates in all models. All models also included individual daily milk yield (kg) after checking that there was no direct relation between milk yield and VT_{MAX}. Because somatic cell counts and electrolytes depend on blood barrier integrity (Wellnitz and Bruckmaier, 2021), and as they were correlated in the present data, the models for Cl^-, K^+, and Na^+ included the log-transformed individual somatic cell count (cells/mL) as an additional covariate. The model for heart $rate_{MEAN}$ took the individual mean activity (*g*-force per hour) into account as an additional covariate.

As animals were repeatedly measured over days within an experimental period and a different composition of 24 animals contributed to the 10 experimental periods, animal and experimental period identities were inserted as random effects in all models. For every model, the proportion of the total variance explained by each random effect was estimated by calculating the Intraclass Correlation Coefficient (**ICC**, lme4 package). Information on the complete models with results for all covariates are shown in supplementary Table S1.

Results

Daily values for the CCI and its components' ambient temperature, relative humidity, solar radiation, and wind speed are presented in Table 2. The CCI and likewise the THI on pasture followed a circadian rhythm during the course of the day, with low values during the night and constantly increasing values from the morning until the cows entered the barn for milking in the afternoon (Fig. 1A). The daily distribution of VT recordings greater than 39 °C showed a proportion of 2% and lower during the night, a percentage of 10% or higher in the afternoon and a peak of 20% after milking (Fig. 1B). The higher the daily CCI_{MEAN} was, the more cows showed increased values of VT_{MAX} (regression coefficients ± SE: $\beta_{linear} = -0.107 \pm 0.019$, $\beta_{quadratic} = 0.003 \pm 0.0004$; $P < 0.001$; Fig. 2).

Overall means, estimates of the regression slopes, and *P*-values for mean heart $rate_{MEAN}$, blood plasma metabolites and hormones, and milk traits in relation to the VT_{MAX} are shown in Table 3. Heart $rate_{MEAN}$ ($P < 0.001$), the concentration of plasma glucose ($P < 0.001$), plasma T4 ($P < 0.001$), plasma T3 ($P < 0.01$), MUN ($P = 0.021$), milk protein ($P < 0.01$), milk cortisol ($P < 0.01$), and milk Na^+ ($P < 0.01$) were related to cow's VT_{MAX}. There tended to be a relation between milk K^+ concentration and VT_{MAX} ($P = 0.085$). No relations of the cows' daily VT_{MAX} with the plasma concentrations of beta-hydroxybutyrate ($P = 0.668$), non-esterified fatty acid ($P = 0.100$), urea nitrogen ($P = 0.680$), and the concentrations of fat ($P = 0.115$), lactose ($P = 0.538$), or Cl^- ($P = 0.619$) in milk were detected.

The range of explained total variance of the random factor animal identity (ICC_{animal}) was between 0.096 (plasma urea nitrogen) and 0.773 (Cl^-). The ICC for experimental period (ICC_{period}) ranged from 0.035 (T4) to 0.799 (plasma urea nitrogen). In scale, for non-esterified fatty acid and milk protein, ICC_{animal} and ICC_{period} were on similar levels. For plasma urea nitrogen and MUN, more variance was explained by ICC_{period} than ICC_{animal}. For all other models, more variance was explained by ICC_{animal} than ICC_{period}.

Heart $rate_{MEAN}$ and the concentration of plasma glucose (Fig. 3A, B), MUN (Fig. 4A), milk cortisol and milk K^+ (Fig. 5A, B) were shown to rise in cows with greater VT_{MAX}. In the range of a VT_{MAX} of 38.2–40.4 °C (recorded range of VT_{MAX} between 0830 and 1430 h), the estimated increase was 12% for heart $rate_{MEAN}$, 7% for plasma glucose, 10% for MUN, 45% for milk cortisol, and 3% for milk K^+ (Table 3). Concentrations of plasma T4 and T3 (Fig. 3C, D), milk protein (Fig. 4B) and milk Na^+ (Fig. 5C) were estimated to decrease in cows with increased VT_{MAX}. In the range between 38.2 °C and 40.4 °C, the estimated decrease was 16% for plasma T4, 20% for plasma T3, 3% for milk protein, and 9% for milk Na^+.

Discussion

In the present exploratory study, dairy cows were exposed repeatedly to days with varying levels of moderate heat load which resulted in the increasing number of cows with elevated levels of VT_{MAX} with rising daily CCI_{MEAN}. We also observed that several physiological changes were associated with increased daily levels of VT_{MAX} and appear to reflect short-term responses to moderate heat stress. Physiological responses to heat stress vary considerably among cows. This underlines the need to assess heat stress on an individual basis, and to focus on responses with low inter-animal variability.

Heat stress under moderate climate conditions

As even moderate increases in ambient temperature under temperate climate conditions can cause heat stress in lactating dairy cows (Van Laer et al., 2015), our data collection started at comparatively low ambient temperatures (minimal daily ambient temperature ≥ 6 °C and maximal ambient temperature ≥ 15 °C). Each animal was exposed to several days with differing but moderate heat load corresponding to days with a CCI_{MEAN} (from 0830 to 1430 h) between 13 and 33 °C. Core body temperature is considered a sensitive physiological indicator of heat stress (Hoffmann et al., 2019). Recordings greater than 39.0 °C would indicate a state of mild hyperthermia and thus be associated with impairment of milk production and fertility in dairy cows (Kadokawa et al., 2012). Moreover, above 39.5 °C is a strong indication of heat stress (Veissier et al., 2018). The percentage of values greater than 39.0 °C in the present study indicates that in particular, in the afternoon, cows suffered from heat stress whereas the peak VT values greater than 39 °C around 1830 h may have been additionally influenced by the heat production due to the locomotor activity when the cows walked a distance of 350–1 500 m to the pasture. At night, VT data greater than 39 °C showed a quite constant distribution and low abundance (around 2–2.5%), which suggested that the cows were able to recover from the heat load experienced during the day. We can therefore argue that the cows in our study were repeatedly exposed to a short-term moderate heat load.

We observed that the higher the CCI_{MEAN} between 0830 and 1430 h, the more cows showed increased levels of VT_{MAX}. In particular, from 1000 to 1430 h, records of a VT > 39 °C increased from 0.6 to 13%. Apparently, at least some of the individuals had difficulty maintaining their core body temperature in the normal range between 38.5 and 39 °C (Veissier et al., 2018), although they were similar in milk yield which is known to be related to heat stress susceptibility (West, 2003). On days with a CCI_{MEAN} greater than

Table 2
Daily values (mean and SD, minimum, maximum) for the comprehensive climate index (°C) and its components: ambient temperature (°C), relative humidity (%), wind speed (m/s) and solar radiation (W/m^2) assessed in 10 experimental periods with dairy cows during the observation period (0830–1430 h) and over 24 h.

	0830–1430 h				24 h			
Item	Mean	SD	Min	Max	Mean	SD	Min	Max
CCI (°C)	23.6	5.4	13.0	33.1	20.7	4.4	12.3	30.4
Ambient temperature (°C)	18.7	3.0	13.1	24.1	17.3	2.8	12.2	23.5
Relative humidity (%)	73.2	12.6	47.2	90.8	77.6	8.6	54.3	89.4
Wind speed (m/s)	1.45	1.30	0.16	4.77	1.1	1.2	0.15	4.9
Solar radiation (W/m^2)	479	211	126	837	237	88	83	451

Abbreviation: CCI = comprehensive climate index.

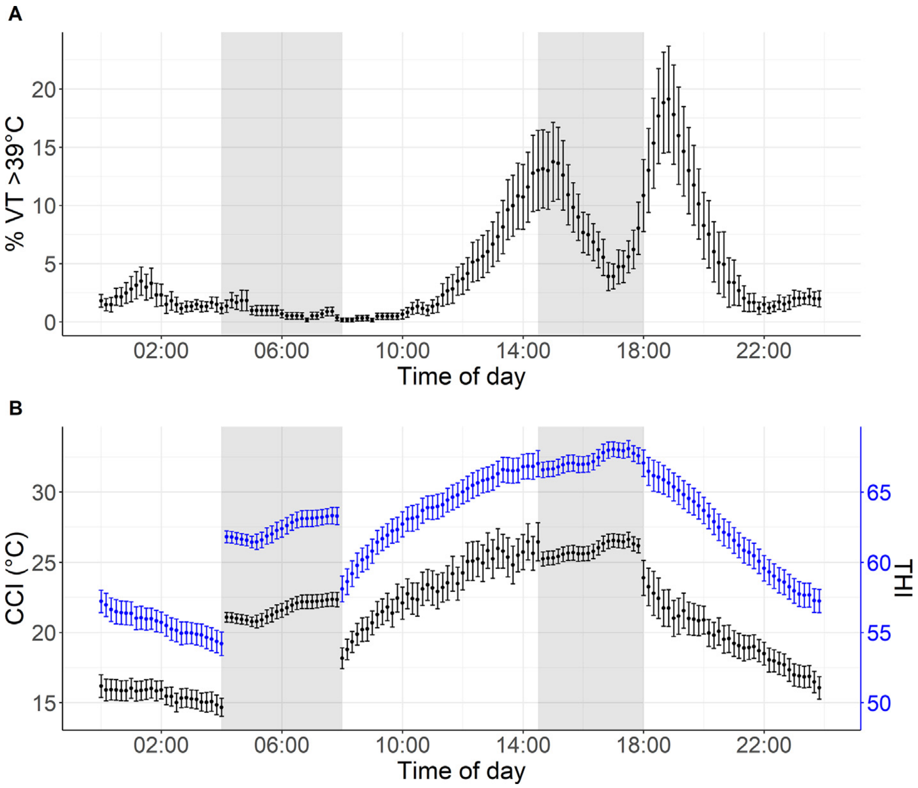

Fig. 1. Distribution (mean and SE of 26 experimental days) of the vaginal temperature recordings greater than 39 °C during the course of the day (A), distribution of the comprehensive climate index (°C) and distribution of the temperature humidity index on pasture (0800–1430 h and 1800–0400 h) or inside the barn (1430–1800 h and 0400–0800 h) (B). The white areas represent time periods where cows were on pasture, the grey areas represent two time periods where cows were inside the barn for milking. Abbreviations: VT = vaginal temperature; CCI = comprehensive climate index; THI = temperature humidity index.

25 °C, variation in VT_{MAX} increased. Some individuals reached values greater than 40.0 °C, whereas others were still able to maintain values below 39 °C. Instead of relying on climatic conditions, animal-based indicators are therefore necessary to adequately assess the individuals' heat stress. However, the risk of logger loss and potential inflammation of the vaginal wall make VT sensors inappropriate for continuous monitoring (Hoffmann et al., 2019).

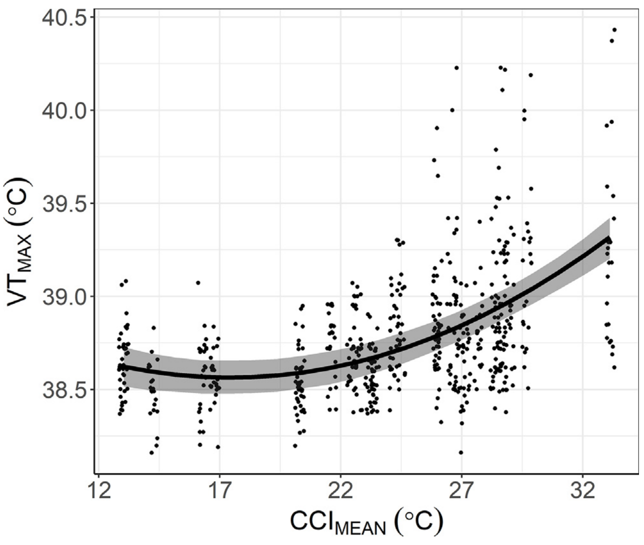

Fig. 2. Maximal vaginal temperature (°C) of individual dairy cows in relation to the mean comprehensive climate index (°C) between 0830 and 1430 h at each experimental day (n = 26). The line represents the model estimate (with the confidence intervals in grey). Abbreviations: VT_{MAX} = maximal vaginal temperature; CCI_{MEAN} = mean comprehensive climate index.

Relation between maximal vaginal temperature and other physiological traits

In line with our expectations, heart $rate_{MEAN}$ was increased in cows with increased VT_{MAX}. A similar increase in heart rate was observed in a previous experiment with dairy cows subjected to 4 days with continuous ambient temperature of 31 °C (Jo et al., 2021). Our results suggest that heart rate may be assessed as a stress response of dairy cows not only during exposure to severe heat stress conditions but also under moderate short-term heat load exposure. For the majority of the cows, the estimated individual slopes were in the order of magnitude or even higher than the model estimate (12% estimated increase between 38.2 °C and 40.4 °C). As inter-individual variation in heart $rate_{MEAN}$ was rather small, a day-to-day comparison of heart rate might be suitable for monitoring the heat stress of individuals.

Besides heart rate, other physiological traits such as thyroid hormones are of interest in relation to heat stress (Weitzel et al., 2017). Thyroxine and T3 determine the basal metabolic rate of the organism and are involved in thermoregulatory mechanisms (Aleena et al., 2016). In a study with dairy cows subjected to 10 days with ambient temperature = 31.2 °C and relative humidity 65%, decreases in T4 and T3 plasma concentration of about 25 and 42%, respectively, were found compared with cows subjected to 10 days with ambient temperature = 17.6 °C and relative humidity 60% (Magdub et al., 1981). As their study was conducted under much hotter climate conditions, it is not surprising that the estimated decrease was two times higher than those recorded in the present study (16% for T4 and 10% for T3). We may assume that the dairy cows in our study exhibited physiological responses that would tend to reduce heat production derived from metabolic processes. However, the majority of the variation in the data was

Table 3

Relation of the dairy cows' daily maximal vaginal temperature (°C) to mean heart rate (beats/min) in the time period 0830–1430 h and to traits in blood plasma and milk traits (harvested during afternoon milking).

Item	Overall mean ± SD	Estimate of the regression slope ± SE[1]	*P*-value	$\mathrm{VT_{MAX}}$ 38.2 – 40.4 °C[2]	ICC[3] for animal identity	ICC[3] for experimental period
Heart $\mathrm{rate_{MEAN}}$ (beats/min)[4]	75.3 ± 8.0	4.5 ± 0.95[4]	<0.001[4]	72.6 [70.1, 74.9] – 81.6 [78.6, 85.7]	0.342	0.097
Blood hormones and metabolites[5]						
Beta-hydroxybutyrate (mmol/L)	0.640 ± 0.103	−0.007 ± 0.02	0.668	0.637 [0.581, 0.701] – 0.620 [0.526, 0.688]	0.373	0.144
Glucose (mmol/L)	3.08 ± 0.155	0.088 ± 0.025	<0.001	3.03 [2.96, 3.09] – 3.23 [3.12, 3.38]	0.422	0.086
Non-esterified fatty acid (mmol/L)	0.125 ± 0.066	0.054 ± 0.035[6]	0.120[6]	0.078 [0.054, 0.12] – 0.104[6] [0.072, 0.153]	0.311	0.363
Plasma urea nitrogen (mmol/L)	7.83 ± 1.32	−0.047 ± 0.11	0.680	7.74 [6.65, 9.03] – 7.65 [6.44, 8.94]	0.096	0.799
T4 (nmol/L)	38.6 ± 8.60	−2.97 ± 0.699	<0.001	40.3 [37.0, 42.8] – 33.7 [30.7, 37.8]	0.745	0.035
T3 (nmol/L)	2.08 ± 0.267	−0.095 ± 0.029	<0.01	2.14 [2.04, 2.23] – 1.93 [1.76, 2.08]	0.610	0.053
Milk components, cortisol and electrolytes[7]						
Fat (%)	3.97 ± 0.43	−0.09 ± 0.06	0.115	4.05 [3.86, 4.25] – 3.82 [3.54, 4.11]	0.530	0.116
Lactose (%)	4.63 ± 0.15	0.009 ± 0.01	0.538	4.62 [4.56, 4.70] – 4.65 [4.57, 4.73]	0.621	0.156
MUN (mg/kg)	19.6 ± 2.98	0.78 ± 0.34	0.021	18.8 [15.9, 21.2] – 20.6 [17.2, 23.0]	0.146	0.700
Protein (%)	3.26 ± 0.17	−0.05 ± 0.02	<0.01	3.29 [3.18, 3.43] – 3.18 [3.04, 3.35]	0.385	0.450
Cortisol (ng/ml)	0.675 ± 0.174	0.11 ± 0.025[6]	<0.001[6]	0.520 [0.460, 0.596] – 0.942[6] [0.728, 1.21]	0.200	0.090
$\mathrm{Cl^-}$ (mmol/L)[8]	28.0 ± 4.0	0.24 ± 0.27	0.352	28.2 [26.3, 29.7] – 28.6 [27.0, 30.3]	0.773	0.074
$\mathrm{K^+}$ (mmol/L)[8]	38.1 ± 2.69	0.43 ± 0.25	0.085	37.8 [36.2, 39.3] – 39.0 [37.0, 40.2]	0.553	0.262
$\mathrm{Na^+}$ (mmol/L)[8]	16.4 ± 2.10	−0.033 ± 0.011[6]	<0.01[6]	16.6 [15.5, 17.4] – 15.5[6] [13.8, 17.0]	0.538	0.197

Abbreviations: $\mathrm{VT_{MAX}}$ = maximal vaginal temperature; heart $\mathrm{rate_{MEAN}}$ = mean heart rate; T4 = thyroxine; T3 = triiodothyronine; MUN = milk urea nitrogen.

[1] Slopes derived from multivariate model including also the individual daily milk yield (kg), days in milk, individual daily consumption of concentrate feed (kg), and mean content of CP in the grass (g/kg DM) as covariates and animal and experimental period identities as random intercept.

[2] Values of outcome variables when $\mathrm{VT_{MAX}}$ = 38.2 °C (the lowest recorded temperature between 0830 and 1430 h) and when $\mathrm{VT_{MAX}}$ = 40.4 °C (the highest recorded temperature between 0830 and 1430 h) according to model estimates.

[3] Intraclass Correlation Coefficient (ICC) estimates the proportion of the total variance explained by the random effect.

[4] The model included the individual mean locomotor activity (*g*-force per hour) as additional covariate.

[5] Blood was sampled between 1500 and 1550 h.

[6] Model based on log-transformed data.

[7] Milk was collected between 1550 and 1640 h.

[8] The model included the log-transformed individual somatic cell count (cells/ml) as additional covariate.

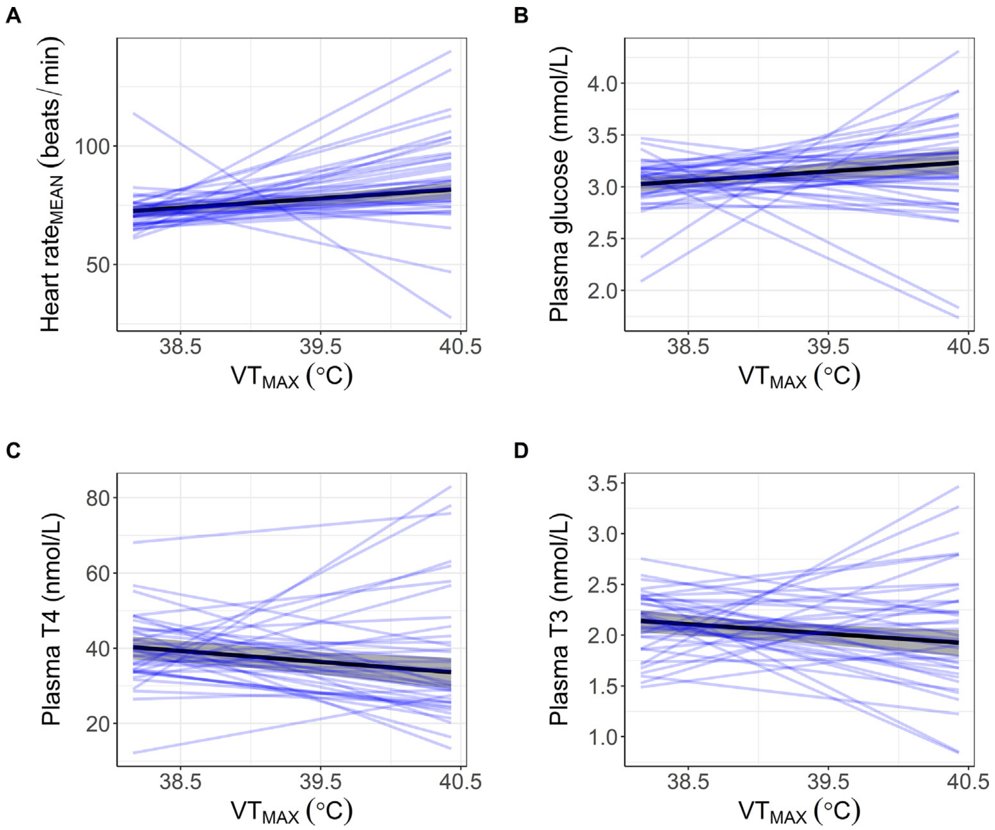

Fig. 3. Mean heart rate (beats/min) (A), concentration of the plasma glucose (mmol/L) (B), plasma thyroxine (nmol/L) (C), and plasma triiodothyronine (nmol/L) (D) of individual dairy cows in relation to the maximal vaginal temperature (°C) recorded between 0830 and 1430 h of each experimental day (n = 26). The black lines represent the model estimate with the confidence intervals in grey and the blue lines the estimated slope for each individual. Abbreviations: heart $\mathrm{rate_{MEAN}}$ = mean heart rate; T4 = thyroxine; T3 = triiodothyronine; $\mathrm{VT_{MAX}}$ = maximal vaginal temperature.

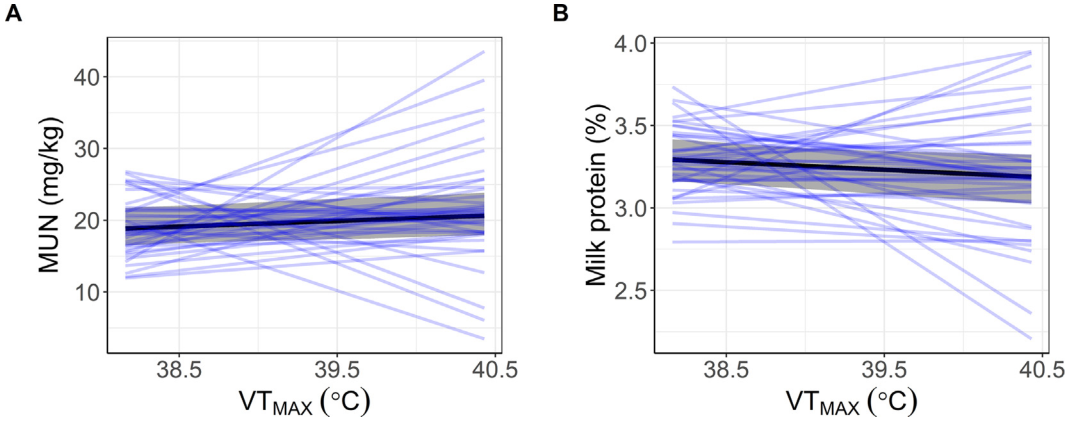

Fig. 4. Concentration of milk urea nitrogen (mg/kg) (A), and milk protein (%) (B) of individual dairy cows in relation to the individuals' maximal vaginal temperature (°C) recorded between 0830 and 1430 h of each experimental day (n = 26). The black lines represent the model estimate with the confidence intervals in grey and the blue lines the estimated slope for each individual. Abbreviations: MUN = milk urea nitrogen; VT_{MAX} = maximal vaginal temperature.

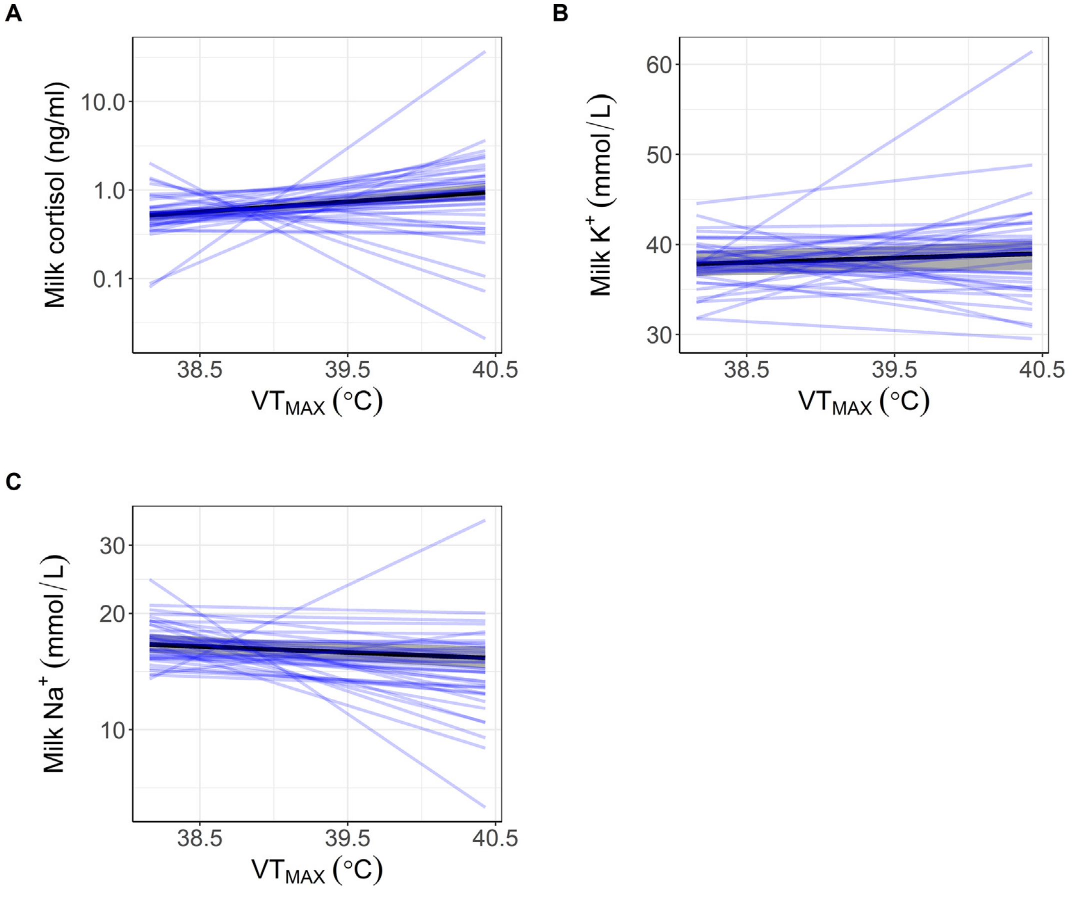

Fig. 5. Concentration of milk cortisol (ng/ml) (A), K^+ (mmol/L) (B), and Na^+ (mmol/L) (C) of individual dairy cows in relation to the individuals' maximal vaginal temperature (°C) recorded between 0830 and 1430 h of each experimental day (n = 26). The black lines represent the model estimate with the confidence intervals in grey and the blue lines the estimated slope for each individual. The y-axes of cortisol and Na^+ are presented on a logarithmic scale with scale values re-transformed. Abbreviations: VT_{MAX} = maximal vaginal temperature.

explained by differences between individuals (75% for T3 and 61% for T4), which rather limits the suitability of these traits for the individual assessment of heat stress.

Furthermore, decreased T4 and T3 concentrations can also be explained by reduced feed intakes (Aleena et al., 2016). Although data on individual feed intake are unavailable in the present study, we expected that decreasing feed consumption during heat stress would result in changes in the concentration of some plasma metabolites (Shehab-El-Deen et al., 2010; Van Laer et al., 2015; Tian et al., 2016). However, we did not detect a relation between beta-hydroxybutyrate, non-esterified fatty acid, or plasma urea nitrogen and elevated levels of VT_{MAX}. These results suggest that the dairy cows in our study did not yet relevantly reduce their feed intake and/or mobilise fat reserves, because plasma metabolites react within hours when the energy supply is reduced (Müller et al., 2018). Moreover, Baumgard and Rhoads (2011) postulated that heat-stressed dairy cows are not able to mobilise adipose tissue which also could explain the lack of changes in non-esterified fatty acid in our cows. The increased MUN concentration in relation to elevated levels of VT_{MAX} is difficult to interpret because usually, an increase in MUN follows an increase in plasma urea nitrogen (Spek et al., 2013). Furthermore, the majority of the variation in the data was explained by differences between the experimental periods (70% for MUN, 80% for plasma urea nitrogen). The unexpected rise in plasma glucose concentration with the increase in VT_{MAX} might be explained by a gluconeogenetic effect of cortisol during stressful situations (Mormède et al., 2007).

Cortisol is released in blood within seconds to minutes after the impact of a stressor; therefore, this hormone has been extensively used in research as a measure of farm animal welfare (Palme, 2012). In contrast to blood sampling which can be stressful for the animal (Palme, 2012), milk cortisol data can be obtained non-invasively and reflects the stress situation up to 2 hours before sampling (Verkerk et al., 1998). In line with our expectations, milk cortisol concentration was increased in cows with increased VT_{MAX}, similar to results previously found by Veissier et al. (2018). Furthermore, in the present study, only 20% of the variation in the data was explained by differences between individual cows, suggesting that the estimated increase in milk cortisol concentration would reliably indicate moderate short-term heat stress in individual cows.

Regarding milk components, only milk protein concentration was related to VT_{MAX}. The observed decrease was three times less steep than observed in a study with grazing cows subjected to daily average ambient temperature between 16 and 32 °C for 11 days (Van Laer et al., 2015). Furthermore, the vast majority of the total variance in the data was explained by the random effects. Changes in milk composition may therefore occur only after prolonged exposure to heat stress (Van Laer et al., 2015) and would need to be observed over a longer period of time due to possible delays in the response.

Electrolytes are involved in acid–base regulation, and alterations may occur in heat-stressed dairy cows (Calamari et al., 2007). The few studies on this subject have investigated electrolyte changes occurring in plasma only (Calamari et al., 2007; Van Laer et al., 2015). They hypothesised that hot conditions cause alterations in the acid–base balance due to increased respiratory alkalosis and that dairy cows compensate for these disturbances by increasing urinary excretion of Na^+ and renal conservation of K^+ (Calamari et al., 2007). Dairy cows subjected to constant ambient temperature = 27 or 32 °C in climate chambers for 2 weeks had reduced K^+ concentration in milk, whereas no changes in Na^+ were found compared to cows compared with cows subjected to ambient temperature = 18 °C (Kamal et al., 1961). Those findings were not in line with our results, which indicated that as VT_{MAX} increased, the milk concentration of Na^+ decreased and K^+ concentration tended to increase. However, in our study, the focus was on short-term reactions to moderate heat load and cows were probably able to recover during the night. To our knowledge, our study is the first to investigate electrolytes in milk in relation to VT. Because milk can be collected non-invasively and electrolytes possibly analysed automatically by ion-selective electrodes, further investigation of this relationship is worthwhile. Such investigations would have to include more animals with elevated body temperatures, as our study is methodologically based on a limited number of these animals.

Conclusion

The daily maximal vaginal temperature (VT_{MAX}) of dairy cows was positively associated with the heat load (CCI_{MEAN}) in the same time span. Cows with increased VT_{MAX} also responded with changes in heart $rate_{MEAN}$, plasma hormones (T4 and T3), milk cortisol, and milk electrolytes (K^+ and Na^+). Changes in these traits likely reflected short-term physiological responses to moderate heat stress. In particular, milk cortisol and Na^+ may be useful for timely monitoring of heat stress in individual cows because their inter-individual variances were relatively small and samples can be collected non-invasively. Milk Na^+ could be even analysed automatically in future. Further research is needed to corroborate these results.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100718.

Ethics approval

All experimental procedures were approved (2018_04_FR) by the Committee of Animal Experiments of the Canton of Fribourg (Switzerland) and were in accordance with the Swiss guidelines for animal welfare.

Data and model availability statement

None of the data were deposited in an official repository. The data/models that support the study findings are available from the authors upon request.

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Declaration of interest

None.

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