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Behavioural responses related to increasing core body temperature of grazing dairy cows experiencing moderate heat stress

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ABSTRACT

Exposure to direct solar radiation, high ambient temperature, lack of wind movement, coupled with own metabolic heat production, makes grazing dairy cows vulnerable to heat stress. In pastures, it would be beneficial to monitor heat stress by observable changes in behaviour. We hypothesised that grazing dairy cows exhibit behavioural changes due to increasing heat load in temperate climate. Over two consecutive summers, 38 full-time grazing Holstein dairy cows were investigated in 12 experimental periods of up to 3 consecutive days where the cows were repeatedly exposed to various levels of moderate heat load determined by the comprehensive climate index (**CCI**). The CCI defines the ambient climate conditions, combining air temperature, relative humidity, solar radiation and wind speed. Vaginal temperature (**VT**) was automatically measured as an indicator of heat stress. In addition, as a less invasive method, we investigated if reticular temperature (**RET**) can be indicative of heat stress on pastures. Walking activity, lying-, feeding, and ruminating durations were recorded continuously with sensors. Respiration rate (**RR**), proximity to and competition at the water trough, social licking, self-licking, inter-individual distance, and fly intensity were directly observed. Data were analysed in the morning (0900–1100 h) and during the hottest time of day when cows were on pasture (1230–1430 h). The VT and RET showed similar patterns in relation to the CCI, suggesting that RET can be suitable for continuous monitoring of heat stress on pastures. In the morning, the cow's VT and RET did not relevantly react to the CCI. During the period 1230–1430 h, the cow's mean VT (mean vaginal temperature (**VT_{MEAN}**); range: 37.7–40.3 °C) and mean RET (mean reticular temperature; range: 37.0–41.1 °C) were positively related to the mean CCI (mean comprehensive climate index) in this period (mean ± SD: 25.9 ± 5.71 °C). For cows with greater VT_{MEAN}, an increased mean RR and decreased durations of walking, lying, feeding, and ruminating were found. These cows were also more likely to be in proximity to the water trough and to have small inter-individual distances. Changes in these traits seem to reflect behavioural adaptations to heat stress in a temperate climate and could be used to detect the heat stress in individual dairy cows on pastures.

Implications

Exposure to a moderate heat load can affect the production, health, and welfare of grazing dairy cows in regions with temperate climates. Because individual cows' heat stress susceptibility can be highly variable, it would be useful to detect even moderate heat stress through behavioural changes. We demonstrated that reticular temperature can be suitable for monitoring heat stress on pastures. Proximity to the water trough and small inter-individual distances seem to be suitable indicators of heat stress.

Introduction

Heat stress occurs when the heat load caused by environmental conditions and the organism's metabolic processes exceed its thermoregulation capacity, causing an increase in core body temperature (Bernabucci et al., 2010). Even moderate increases in ambient

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temperature under temperate conditions can cause heat stress in lactating dairy cows (Van Laer et al., 2015a; Pontiggia et al., 2023). Dairy cows on pasture are particularly vulnerable to heat stress because of their continuous direct exposure to ambient climatic conditions, including solar radiation, coupled with their own metabolic heat production related to ruminal fermentation and milk synthesis (West, 2003; West et al., 2003). This is especially true for high-producing dairy cows, in which a high milk yield via maximised herbage intake on pasture is targeted, as metabolic heat production increases with greater dietary fibre content and with increasing milk production (Kadzere et al., 2002). Additionally, in intensive grazing systems, such as strip grazing, it is often difficult to provide shade for animals to protect them from solar radiation.

Heat stress leads to losses in milk yield (Ammer et al., 2018) and alterations in milk composition (Van Laer et al., 2015a) and is a threat to animal health and welfare (Silanikove, 2000). In order to apply efficient cooling strategies, farmers should monitor the heat stress of grazing dairy cows reliably and in a timely manner. The comprehensive climate index (**CCI**), which considers ambient temperature, relative humidity, wind speed and solar radiation, can thoroughly assess climatic effects on grazing cows (Mader et al.,2010 and, 2011). However, individual reactions to heat load can vary considerably (Pontiggia et al., 2023), and climatic indices cannot reflect the actual heat stress that an individual cow is experiencing (Hoffmann et al., 2020). Animal-related indicators are therefore necessary to monitor heat stress in individual cows (Hoffmann et al., 2020).

Animals react to heat load with homeostatic responses, which aim to keep their core body temperature at the set value (Silanikove, 2000). Responses that are clearly observable would be of great use in taking timely action to mitigate heat stress. For example, to dissipate endogenous heat, cows increase their respiration rate (**RR**) (Kadzere et al., 2002), which is currently used in an index assessing moderate to high heat stress (Van Laer et al., 2015b). Further, based on the results of on-farm studies on pasture and in the barn, several behavioural responses could be expected in heat-stressed grazing dairy cows. One reaction through which cows reduce their metabolic heat production is to lower their activity in the barn (Tapkı and Şahin, 2006; Herbut and Angrecka, 2018). Moreover, they decrease the time spent lying to improve heat dissipation while standing (Allen et al., 2015). Further, they lower metabolic heat production, by reducing feed intake (Kadzere et al., 2002), which is shown by the reduced time spent feeding (Tapkı and Şahin, 2006; Ammer et al., 2018) and ruminating (Tapkı and Şahin, 2006; Moretti et al., 2017). Especially during hot periods, when animals need to drink more water, dairy cows can be observed longer near the trough on pasture (Schütz et al., 2010). Heat-stressed dairy cows in free-stall systems visit the watering places more frequently to compensate for water loss due to sweating and panting and to obtain a direct cooling effect (Ammer et al., 2018). They also display more competitive events at the water trough (McDonald et al., 2020). Although less studied to date, further behaviours seem worthy of exploration in this context. If shade is not available, crowding behaviour is anecdotally reported by farmers in relation to heat load (Gaughan et al., 2002; Polsky and von Keyserlingk, 2017). An explanation for this behaviour could be that grazing cows seek shade by keeping their heads underneath the bodies of other individuals. However, crowding might lead to increased social stress when the required social distances cannot be maintained (Proudfoot and Habing, 2015). Consequently, social licking might occur more often, a behaviour that can serve to reduce social tension (Sato et al., 1991). In addition, heat-stressed cattle might increase the frequency of self-licking as a mean of enhancing cooling (Beatty et al., 2006).

In a parallel study, we showed that a rising daily mean CCI (mean comprehensive climate index (**CCI_{MEAN}**)) in temperate climates induced varying degrees of increased vaginal temperature (**VT**) and further changes in physiological traits in grazing dairy cows. Moreover, we demonstrated that physiological changes can already occur at relatively low ambient temperatures (16–20 °C). These changes varied considerably among cows and were physiological responses indicative of moderate heat stress (Pontiggia et al., 2023). Increased reticular temperature (**RET**) can be indicative of heat stress for dairy cows in barns (Ammer et al., 2016a). However, it is unclear whether RET measurements can be suitable for monitoring heat stress in cows on pastures, because the feeding behaviour on pasture differs from that inside the barn, and also the heat production of the animals due to the physical activity (Dohme-Meier et al., 2014).

The first objective of the present study was to investigate whether RET reacts to heat stress in a similar way to VT in cows on pastures. The second objective was to investigate whether behavioural changes occur as a response to heat stress experienced by individual animals at relatively low ambient temperatures. In the present study, we explored in two time windows (in the morning and during the hottest time of day when cows were on pasture) if the individual cows showed behavioural changes associated with increased body temperature in temperate climate. For this purpose, we investigated already familiar behavioural indicators (RR, proximity to and competition at the water trough, walking activity, feeding-, ruminating- and lying durations) and explored behaviours that had been less studied (inter-individual distances, social licking, and self-licking).

Material and methods

Experimental design

Animals and grazing management

The experiment was conducted during summer 2018 (6 June to 7 September) and summer 2019 (15 June to 1 September) on the experimental farm of Agroscope in Posieux, Switzerland (46°46.01′N, 7°6.03′E; 676 m above sea level). It was conducted with a total of 38 black (51%) and red (49%) Holstein dairy cows. In 2018, the dairy cows were (mean ± SD) in the 2.8 ± 1.4 lactation, 103 ± 26 days in milk and produced 35.2 ± 5.42 kg of milk per day at the onset of the experiment and 27.0 ± 4.80 kg of milk per day at the end of the experiment. In 2019, the dairy cows were in 2.5 ± 1.4 lactation, 125 ± 20 days in milk and produced 32.1 ± 6.11 kg of milk per day at the beginning of the experiment and 24 ± 3.73 kg of milk per day at the end of the experiment. As described in Pontiggia et al. (2023), 24 dairy cows were studied each year, and 10 individuals were enrolled in both 2018 and 2019. Data were collected during 12 experimental periods of 2 to 3 consecutive days. This resulted in a total of 32 days where each animal was exposed to several days with differing levels of moderate heat load. As explained in Pontiggia et al. (2023), a medical check-up was carried out and only clinically healthy animals were included in the study. During the grazing periods, an insecticide (Butox Protect 7.5 pour-on, MSD Animal Health GmbH, Luzern, Switzerland) was applied monthly along the dorsal line from the neck to the root of the tail.

In each year, two groups of 12 animals were formed that were comparable in traits known to influence heat stress susceptibility (milk production, BW, coat colour and lactation stage). Within these two groups, pairs of cows were formed consisting of animals that should differ as much as possible in these traits. The two individuals in a pair always grazed together throughout the duration of a summer. The composition of the pairs in 2019 was different from the one in 2018. Two pairs each were combined to form six experimental groups of four animals. The composition of the two pairs within the experimental groups changed randomly every second experimental period. Animals were observed in experimental groups to facilitate direct behavioural observation, and to limit interdependencies by herd effects. The cows grazed in their experimental group at least 2 days before starting an experimental period and during the experimental period. For the rest of time, all cows were managed as one herd.

The animals were on pasture from 0800 h to 1430h and from 1800 h to 0400 h and were milked between 0440 and 0550 h and between 1540 and 1640 h in a milking parlour with individual milk yield recording (Pulsameter 2, SAC, Kolding, Denmark). Shading systems were not provided on pasture, and fans or sprinklers were not used inside the barn or milking parlour. Cows in heat were removed from the herd during the experimental periods (5 cows, 4 experimental days in 4 experimental periods). Experimental groups grazed using a set stocking system in adjacent paddocks which were provided with a water trough (volume 5 L, floater-controlled valve; LA BUVETTE Lac 5, Tournes, France) located at one end of the pasture, near the fence. Paddock size varied between 1.0 and 1.3 ha and was adapted over the season based on the current herbage offer. The sward was composed of 87% grasses, 2% legumes and 10% herbs. After milking, the cows were offered a concentrate feed according to their current milk yield (Agroscope, 2018), and non-iodised cattle salt was provided *ad libitum* on pasture (for details, see Pontiggia et al., 2023).

Climate data

Ambient temperature (°C), relative humidity (%), wind speed (m/s) and solar radiation (W/m^2) were recorded every minute by using a mobile weather station with sensors (HOBO U30-NRC, Onset, Bourne, MA) set up at the pasture site. The sensor of ambient temperature had a measurement range from −40 to 75 °C and an accuracy of 0.2 °C (Temperature/RH Smart Sensor, S-THB-M00x). The relative humidity could be recorded from 0 to 100% with an accuracy of ± 2.5% (Temperature/RH Smart Sensor, S-THB-M00x). The sensor of wind speed had a measurement range from 0 to 45 m/sec and an accuracy of ± 1.1 m/sec (Wind Speed Smart Sensor, S-WSA-M003). Solar radiation could be measured from 0 to 1280 W/m^2 with an accuracy of ± 10 W/m^2 (Silicon Pyranometer Smart Sensor, Part # S-LIB-M003). The climate data were used to calculate the CCI following Mader et al., (2010 and and 2011); equation see Supplementary Material S1).

Body temperature

Body temperature, as a physiological indicator of heat stress, was measured continuously and recorded every 10 min during the experimental periods by using VT and RET. The VT of each cow was measured using a microprocessor temperature logger (DST micro-T logger, Star-Oddi, Garðabær, Iceland; see Pontiggia et al., 2023) that 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 was inserted about 30 cm deep into the vaginal cavity at the beginning of every experimental period. The RET was recorded using a temperature sensor bolus (BASIC BOLUS, smaXtec, Graz, Austria). The bolus had a dimension of 105 × 35 mm (length × diameter) and recorded RET with an accuracy of ± 0.05 °C. At the onset of the experiment, the boli were orally inserted into the oesophagus of each cow with the help of an applicator and were swallowed into the reticulum. Each bolus was provided with an internal microprocessor, a memory space, a battery and an antenna communicating with an external receiver station (smaXtec, Graz, Austria) installed at the milking parlour.

The collected data were stored in a cloud database and successively transferred into an Excel file. Some data of the VT were missing (8.9%) because the animals lost the loggers. Furthermore, values of the RET below 32.0 and above 43.0 °C (< 0.1%) and values of the VT below 37.3 and above 40.4 °C (< 0.1%) were considered measurement errors according to Ammer et al., (2016a and 2016b) and were excluded from the dataset.

Direct observations

The cows were directly observed on pasture by two trained observers who collected behavioural data, full breaths and fly intensity (defined in Table 1). The cows were accustomed to peo-

Table 1
Ethogram used for direct observations of dairy cows.

Items	Description	Unit	Time of collection
Inter-individual distance	The shortest space between two standing cows	Categorical Small (distance ≤ 1 body length) Large (distance > 1 body length)	At the beginning of the 10-min interval
Full breaths	The duration of 10 uninterrupted full breaths	S	After inter-individual distance
Fly intensity	Number of flies around each eye (eye + 5 cm)	Categorical Weak (number of flies ≤ 10) Strong (number of flies > 10)	In parallel to full breaths
Water proximity[1]	At least two feet are within the rectangular area (3 × 5 m) around the water trough, at least once during an interval	Yes, No	During whole 10-min interval
Water competition	A cow displaces another cow drinking from the water trough; the displacing cow then begins to drink	Frequency	During whole 10-min interval
Social licking	A cow touches the body of another cow with her tongue at least once during an interval	Yes, No	During whole 10-min interval
Self-licking	A cow touches her body with her tongue: a new licking event started if the cow licked a new body part or continued with the same after a pause of at least 10 s	Frequency	During whole 10-min interval

[1] Determined from the second experimental period onwards.

ple's handling and close contact to humans from previous experiments. To minimise the risk that the observers' proximity to the cows would affect the cows' behaviour, the observers kept a distance of at least 3 m from each cow. For easy identification, each animal had an individual number (50 × 60 cm) sprayed on both flanks.

Observations took place during two time windows of 2 h each, in the morning between 0900 and 1100 h, and in the afternoon during the hottest time of day when cows were on pasture between 1230 and 1430 h (Pontiggia et al., 2023). During the experimental day, each of the two observers observed three experimental groups. Within each time window, the observer switched between the experimental groups in intervals of 10 min. This led to four observation intervals per experimental group, summing up to a total of 40 min per cow and per experimental group in each time window. The order in which the experimental groups were observed and the observer of the experimental group switched randomly every experimental day.

In each 10-min interval, the inter-individual distance of each animal to the three other individuals in the experimental group was assessed first before entering the paddock to avoid any recording bias in the position of the cows due to the observer's presence (Table 1). Second, full breaths were recorded by using a stopwatch (Tresoldi et al., 2016). Only the full breaths of resting or standing cows were recorded because full breaths were not sufficiently detectable in grazing or walking cows. In parallel, as a potential confounder influencing behaviour, fly intensity was evaluated. Assessing full breaths and fly intensity took about 2–3 min. Afterwards during the rest of each 10-min interval, the occurrence of water proximity and social licking as well as all events of water competition and self-licking were monitored simultaneously. To decide on water proximity, a rectangular area (3 × 5 m) around the water trough was marked by two plastic stakes in front of the trough and by the fence on the backside of the water trough.

Recorded full breaths were converted to breaths per minute reflecting RR (Tresoldi et al., 2016). Because only data of resting or standing cows could be collected, the number of measurements per cow varied between 0 and 4 in a time window, resulting in 37.4% missing values in the data set (n = 481 from 768 potential data points). Because inter-individual distance was assessed only for standing cows, the number of measurements per cow varied between zero and four in a time window (8.6% missing values). Fly intensity was analysed considering the eye with the higher categorical value during each 10-min interval. Water competition and social licking occurred less than once per time window in all cows; therefore, these behaviours were not further analysed.

Behavioural traits automatically measured

The activity of the cows was measured continuously using an accelerometer (MSR145 data logger, MSR Electronics GmbH, Seuzach, Switzerland). The device was attached to the metatarsus of the left hind leg as described by Weigele et al. (2018), the day before an experimental period started. Owing to technical reasons, data were collected from the second experimental period onwards. One animal did not tolerate the accelerometer, the device was removed, and data were not generated. Raw data were transmitted via MSR software (version 5.28.14, MSR Electronics GmbH, Seuzach, Switzerland) to a computer as CSV files.

Feeding behaviour was continuously recorded by the RumiWatch recording device (Itin + Hoch GmbH, Liestal, Switzerland) as described by Rombach et al. (2019). The halters were put on the day before every experimental period. Raw data were transmitted via RumiWatch Manager software (Itin + Hoch GmbH, Liestal, Switzerland), to a computer as CSV files. Owing to malfunctions of the logger, 22% of the collected data were not included in the analysis.

Validation and quality assurance

As physiological indicators of heat stress, the VT and the RET were used because both have been validated to reliably reflect core body temperature in dairy cows (Suthar et al., 2012; Ammer et al., 2016a). The temperature sensor boli applied to record the RET were used in several previous studies to monitoring the temperature under heat stress (Ammer et al., 2016a and 2016b). The vaginal sensors DST micro-T logger were validated to reliably measure VT (Suthar et al., 2013).

Inter-observer reliability was assessed for the directly observed data (behaviour, fly intensity and full breaths) by calculating Pearson correlations. They were between 0.8 and 1.0 (all $P < 0.01$). The lying duration (min) and the activity of standing or walking (mean walking activity [*g*-force/hour]) were measured with accelerometers and calculated with R (version 4.0.2; R Core Team, 2021) according to Weigele et al. (2018). The accelerometer records the vertical and horizontal accelerations continuously with a rate of 1 Hz and a maximum acceleration of ± 16 *g*. Based on these data, the vertical position of the hind leg is determined and, hence, if the cow is lying or not. Furthermore, acceleration data can assess the activity the cows produced by moving the hind leg while standing or walking (=not lying).

The feeding and ruminating durations (min) were calculated using the RumiWatch Converter (version 0.7.3.36) according to Rombach et al. (2019). Raw data were checked for completeness and plausibility (see for each variable its corresponding paragraph in sub-chapter Experimental design).

Statistical analysis of results

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 (reflected in the random effects of the respective model). To minimise autocorrelation among the values within a day, all variables were reduced to one single observation per animal, day and time window, so that the effects could be analysed on the level of the individual (n = 768 data points: 24 cows/time window*32 experimental days; assigned to n = 38 cows with an unbalanced number of days/time windows per cow). The residuals of the data were visually inspected for normal distribution and homoscedasticity, and all the variables were acceptable for the analysis without transformations (see R-code in Supplementary Material S1).

Relation of comprehensive climate index to vaginal temperature, reticular temperature, and fly intensity

For CCI, VT and RET, their mean values recorded between 0900 and 1100 h and between 1230 and 1430 h (morning and afternoon, respectively) were used (CCI_{MEAN}, mean vaginal temperature (**VT_{MEAN}**), mean reticular temperature (**RET_{MEAN}**)). To explore the relation between core body temperature (VT_{MEAN} and RET_{MEAN}) and CCI_{MEAN}, one model was specified for every trait. In the models, the CCI_{MEAN} and time window (coded as a two-level factorial variable, i.e. morning and afternoon) and their interaction were inserted as explanatory variables. The effect of CCI_{MEAN} was estimated using natural splines (splines package; R Core Team, 2021) to allow for a non-linear regression. Animal identity within pair identity within experimental period identity was added as random intercept. Additionally, the experimental group identity was coded as a crossed random intercept.

In the morning, VT_{MEAN} did not relevantly increase in relation to CCI_{MEAN}. Therefore, further analyses are presented only for the time window 1230–1430 h. Fly intensity was analysed as the proportion of strong fly intensity in relation to the total number of observed values within the time window. To check whether fly intensity was related to the CCI_{MEAN}, a logistic regression model was fitted (glmer function; R Core Team, 2021). Animal identity within pair identity within the experimental period identity was added as a random intercept. Additionally, the experimental group identity was coded as a crossed random intercept. Fly intensity was strongly correlated to CCI_{MEAN}. To avoid problems of multicollinearity, fly intensity was not included as a confounding variable in the models analysing the effect of VT_{MEAN} on behavioural indicators.

Relations of vaginal temperature to respiration rate and to behaviours observed during 1230–1430 h

For RR, self-licking, and walking activity, the mean value was used. For lying, feeding, and ruminating, the duration a cow had spent with these behaviours within the time window was taken. For inter-individual distance and water proximity, the proportion of one category (small inter-individual distance = yes; water proximity = yes) in relation to the total observed values within the time window was calculated.

One regression model using VT_{MEAN} as explanatory variable was specified for every trait. Mean RR (breaths per minute), mean self-licking (number of events), mean walking activity (*g*-force/hour), and lying, feeding, and ruminating durations (min/2h) were analysed by fitting mixed regression models (lmer function; Bates et al., 2015). Inter-individual distance and water proximity were analysed by fitting logistic regression models (glmer function; R Core Team, 2021). The models included animal identity within pair identity within experimental period identity as a random intercept. The experimental group identity was also coded consecutively as a random intercept.

Results

Relation of comprehensive climate index to vaginal temperature, reticular temperature, and fly intensity

Values for the CCI and its components ambient temperature, relative humidity, wind speed and solar radiation, are summarised in Table 2. The VT_{MEAN} and RET_{MEAN} showed similar patterns in relation to CCI_{MEAN}, but RET_{MEAN} fluctuated more strongly than VT_{MEAN}. The VT_{MEAN} was estimated to increase differently with rising CCI_{MEAN} in the two time windows (interaction $P < 0.01$) (Fig. 1A). In the morning, in the range of a recorded CCI_{MEAN} of 13.7–31.2 °C, VT_{MEAN} was estimated to increase from 38.3 °C [38.2–38.4 °C] to 38.4 °C [38.3–38.5 °C] (estimate, 95% confidence interval). In the afternoon, according to the non-linear model, VT_{MEAN} was estimated to remain at a similar level up to CCI = 25 °C and then rise to 38.9 °C [38.9–39.0 °C]. Overall, the CCI_{MEAN} had an influence on VT_{MEAN} ($P < 0.01$) and during the afternoon, VT_{MEAN} was higher than during the morning ($P < 0.01$).

The RET_{MEAN} was estimated to change differently with rising CCI_{MEAN} in the two time windows (interaction $P < 0.01$) (Fig. 1B). In the morning, RET_{MEAN} was estimated to decrease from 38.7 °C [38.6–38.8 °C] to 38.5 °C [38.3–38.6 °C]. In the afternoon, RET_{MEAN} was estimated to remain on a similar level up to CCI = 25 °C and then rise to 39.5 °C [39.3–39.6 °C] °C. During the afternoon, RET_{MEAN} was higher than during the morning ($P < 0.01$). Owing to the interaction between CCI and the time windows, there was no consistent effect of CCI_{MEAN} on RET_{MEAN} ($P = 0.086$). The proportion of intervals in which the animals were exposed to a strong fly intensity was estimated to rise with increasing CCI_{MEAN} (regression coefficient ± SE: 16.6 ± 0.35; $P < 0.001$).

Relation of vaginal temperature to respiration rate and to behaviours observed during 1230–1430 h

Overall means, estimates of the regression slopes and *P*-values for mean RR and behaviours in relation to the VT_{MEAN} recorded between 1230 and 1430 h are shown in Table 3. Mean RR ($P < 0.001$), mean walking activity ($P < 0.001$), lying- ($P = 0.036$), feeding- ($P < 0.001$) and ruminating durations ($P < 0.01$), small inter-individual distances ($P < 0.001$) and water proximity ($P < 0.001$) were related to a cow's VT_{MEAN}. No relation was detected between the cows' VT_{MEAN} and the mean number of self-licking events ($P = 0.573$).

The mean RR, the proportion of intervals an animal showed small inter-individual distances, and water proximity were estimated to be higher in cows with increased VT_{MEAN} (Fig. 2A, B, and C). In the VT_{MEAN} range of 37.7–40.3 °C (recorded range of VT_{MEAN} between 1230 and 1430 h), the estimated increase was more than threefold for the mean RR and for the proportion of intervals an animal showed small inter-individual distances, and more than tenfold for the proportion of intervals an animal showed water proximity (Table 3). Mean walking activity and lying, feeding, and ruminating durations were estimated to decrease in cows with increased VT_{MEAN} (Fig. 3A, B, C, and D). In the VT_{MEAN} range of 37.7–40.3 °C, the estimated decrease was 44% for walking activity, 63% for lying duration, 40% for feeding duration and 68% for ruminating duration.

Discussion

In this explorative study, dairy cows on pastures were repeatedly subjected to days with varying levels of heat load under temperate climate conditions. This resulted in cows with elevated levels of VT_{MEAN} and RET_{MEAN} in relation to the CCI_{MEAN} in the afternoon, suggesting a cumulative effect of the heat load over the day. We also showed that during the afternoon, RR and several behavioural changes were associated with increased levels of VT_{MEAN}, which could be used to monitor heat stress on pasture on an individual basis.

Table 2
Values (mean and SD, minimum, maximum) of 32 days 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 12 experimental periods with dairy cows during the time windows (0900–1100 h and 1230–1430 h).

	0900–1100 h				1230–1430 h			
Items	Mean	SD	Min	Max	Mean	SD	Min	Max
CCI (°C)	22.1	4.78	13.7	31.2	25.9	5.71	12.0	35.3
Ambient temperature (°C)	17.5	2.76	12.2	22.4	20.5	3.12	12.8	26.2
Relative humidity (%)	77.1	11.80	53.8	95.0	66.0	13.71	39.7	85.8
Wind speed (m/s)	1.3	1.27	0.0	4.87	1.9	1.27	0.13	5.6
Solar radiation (W/m^2)	404	214.0	91	809	632	250.0	91	959

Abbreviation: CCI = comprehensive climate index.

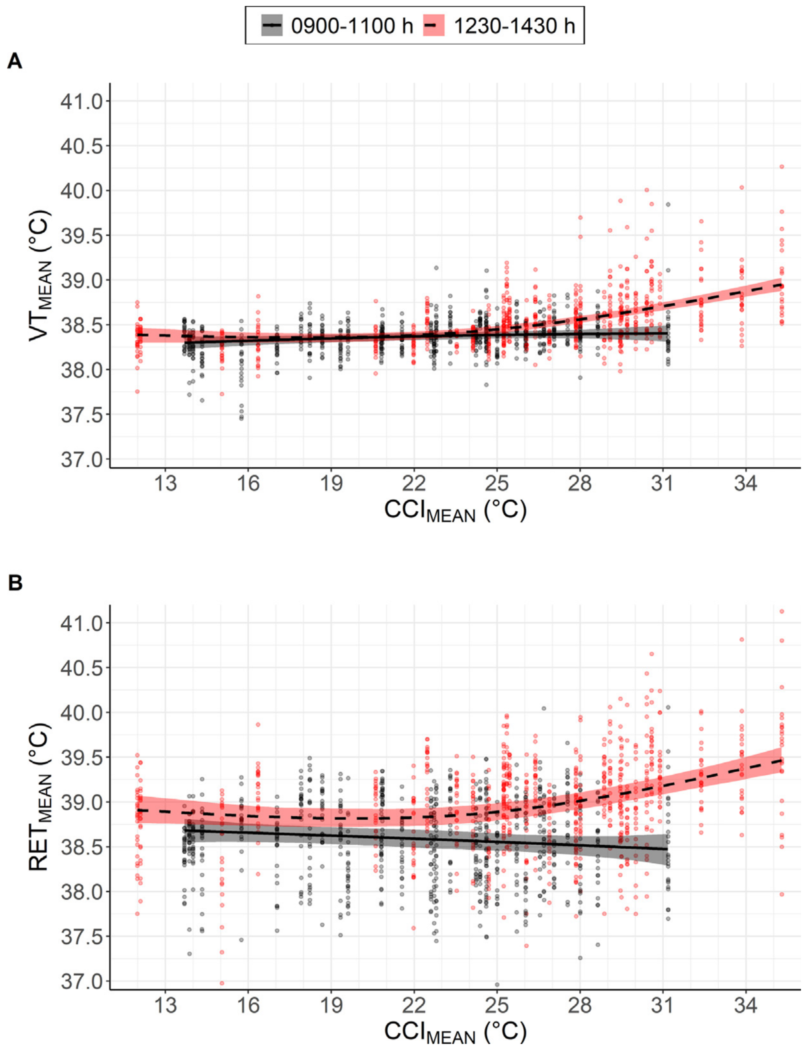

Fig. 1. Mean vaginal temperature (°C) (A) and reticular temperature (°C) (B) of individual dairy cows in relation to the mean comprehensive climate index (°C) in the time windows 0900–1100 h (n = 702 for VT, n = 763 for RET) and 1230–1430 h (n = 703 for VT, n = 764 for RET) of 32 experimental days. The lines represent the model estimates (with confidence intervals in grey and red). Abbreviations: VT_{MEAN} = mean vaginal temperature; RET_{MEAN} = mean reticular temperature; CCI_{MEAN} = mean comprehensive climate index.

Table 3
Relation of the dairy cows' mean vaginal temperature (°C) to mean respiration rate (breaths per minute) and behavioural traits in the period 1230–1430 h.

Items	Overall mean ± SD	Estimate of the regression slope ± SE[1]	P-value	Estimated values for VTMEAN 37.7–40.3 °C[2]
RR (breaths per minute)	47.2 ± 17.65	29.8 ± 2.15	<0.001	24.7–100
Small inter-individual distance (% of intervals each animal showed distance ≤ 1 body length)	13.1 ± 22.39	83.7 ± 18.15	<0.001	7.27–33.4
Water proximity (% of intervals with water proximity per animal)	10.7 ± 19.24	127 ± 22.6	<0.001	3.86–47.2
Walking activity (g-force/hour)	602 ± 184.1	−117 ± 22.0	<0.001	678–383
Lying duration (min/2 h)	33.2 ± 30.02	−8.32 ± 3.876	0.036	41.6–15.6
Feeding duration (min/2 h)	76.9 ± 28.76	−14.4 ± 3.59	<0.001	83.5–49.9
Ruminating duration (min/2 h)	20.8 ± 19.77	−7.52 ± 2.526	<0.01	25.9–8.26
Self-licking (number of events)	0.54 ± 0.770	−0.05 ± 0.092	0.573	0.54–0.41

Abbreviations: RR = respiration rate; VT_{MEAN} = mean vaginal temperature.
[1] Slopes derived from the model including animal, pair, group, and experimental period identities as random intercept.
[2] Estimated values of outcome variables when VT_{MEAN} = 37.7 °C (the lowest recorded temperature between 1230 and 1430 h) and when VT_{MEAN} = 40.3 °C (the highest recorded temperature between 1230 and 1430 h), according to the model estimates.

Effect of heat load on cows' body temperature in the morning and afternoon

For the timely implementation of countermeasures, it would be advantageous if behavioural changes were identified before severe heat stress occurs. In both time windows explored in this study, RET_{MEAN} and VT_{MEAN} showed similar patterns in relation to the CCI_{MEAN} in these periods. Compared with VT_{MEAN}, the influence of feed and water intake likely explains the presence of larger variations in RET_{MEAN} (Ammer et al., 2016a), and might also explain why

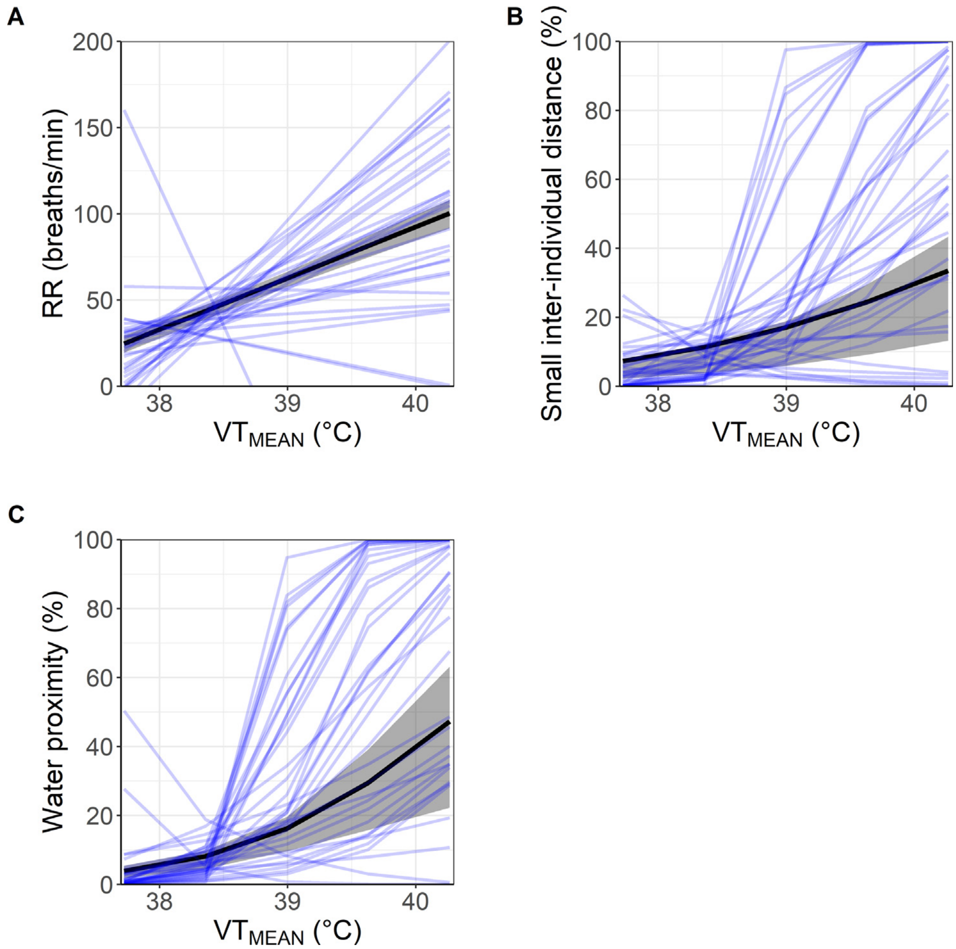

Fig. 2. Mean RR (breaths per minute) (n = 481) (A) and proportion of intervals an individual dairy cow showed small inter-individual distance (distance ≤ 1 body length) (n = 702) (B) and water proximity (n = 702) (C) in relation to the mean vaginal temperature (°C) recorded in the time window 1230–1430 h of 32 experimental days. The black lines represent the model estimates with the confidence intervals in grey, and the blue lines represent the estimated slopes for each individual. Abbreviations: RR = respiration rate; VT$_{MEAN}$ = mean vaginal temperature.

RET$_{MEAN}$ slightly decreased with increasing CCI$_{MEAN}$ in the morning. With increasing CCI$_{MEAN,}$ cows might have had more drinking events; however, specific analyses that would link drops in RET$_{MEAN}$ with drinking or eating events were not performed in this study. Although VT is more accurate than RET and is preferred in research (Kendall et al., 2006), it is not appropriate for long-term monitoring on commercial dairy farms because of the risk of logger loss and potential inflammation of the vaginal wall (Hoffmann et al., 2020). In addition, retrospective data download limits their use in practice. By contrast, the bolus for measuring RET can be applied to cows permanently with a battery life of up to 4 years. Despite the presence of interferences in the reticulorumen environment, RET could be a suitable indicator of heat stress on commercial dairy farms and for studies requiring long-term monitoring of dairy cows when considering mean values based on longer observation periods over the course of the day.

The individuals' heat stress level differed in the two time windows investigated. In the morning, the body temperature of the cows did not remarkably increase biologically with the heat load. In the afternoon at a CCI$_{MEAN}$ above 25 °C, an increase in both VT$_{MEAN}$ and RET$_{MEAN}$ was observed, which was particularly pronounced in some individuals. A CCI of 25 °C corresponds to an ambient temperature of 16–20 °C, depending on wind speed, solar radiation, and relative humidity. Previous studies have suggested an accumulation of heat load over the day (Langbein and Nichelmann, 1993), which could also explain why we found an effect of heat load on VT$_{MEAN}$ and RET$_{MEAN}$ in the afternoon only. Cooler conditions prevailed in the morning compared with the afternoon on pasture, and during the night, the cows recovered from the heat load experienced during the day (Pontiggia et al., 2023). Furthermore, the cows were predominantly grazing in the morning and predominantly ruminating in the afternoon, which is a typical pattern for cows on pasture (Gibb et al., 1998; Gregorini et al., 2012). During rumination, metabolic heat production rises through feed digestion (Kadzere et al., 2002), which could additionally explain why our animals were affected in the afternoon. The individual variation and the differing reactions of VT$_{MEAN}$ and RET$_{MEAN}$ to the CCI$_{MEAN}$ in the morning and afternoon could therefore be explained by the cows' feeding behaviour and the individuals' susceptibility to heat stress that accentuated throughout the day.

Relation of mean vaginal temperature to respiration rate and to behavioural traits during 1230–1430 h

We could show that the mean RR and several behavioural traits were related to the cows' VT$_{MEAN}$ during 1230–1430 h indicating that they were adaptations to heat stress. In line with our expectations, mean walking activity and the lying duration were reduced in cows with increased VT$_{MEAN}$. Similar results were previously found in dairy cows in free-stall systems (Allen et al., 2015; Herbut and Angrecka, 2018). In addition, ruminating and feeding

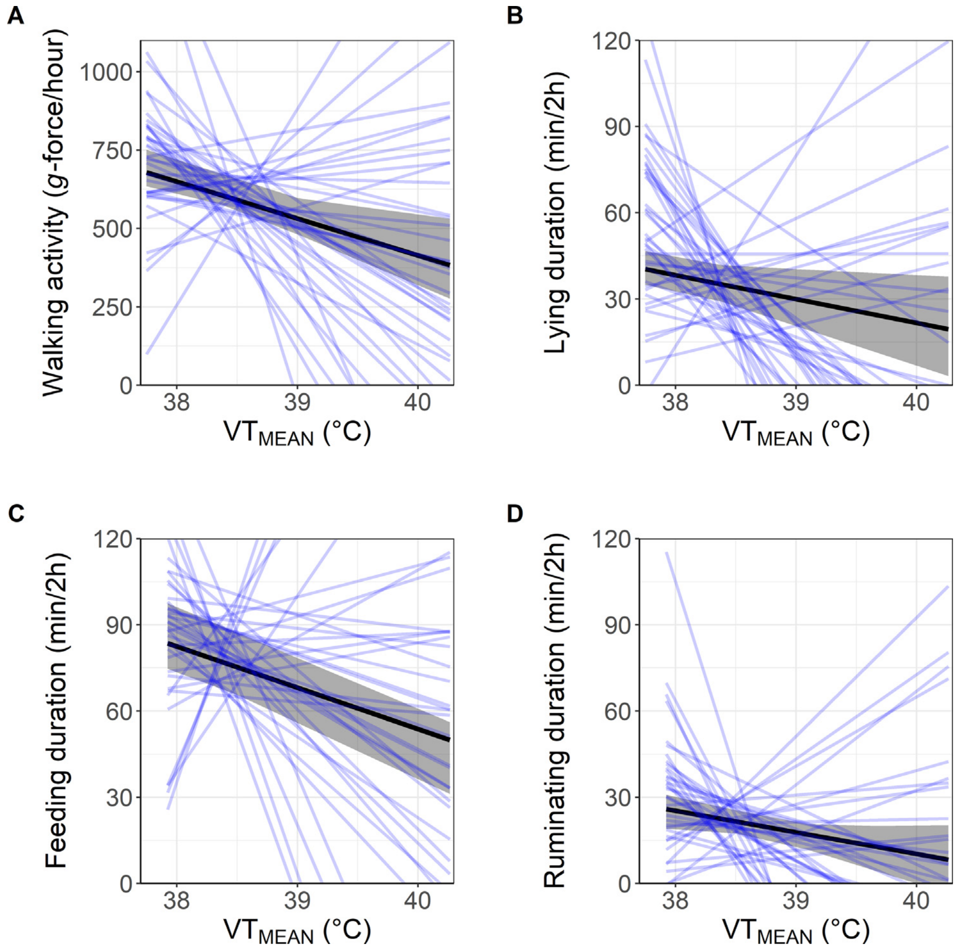

Fig. 3. Mean walking activity (g-force/hour) (n = 602) (A), lying duration (min/2h) (n = 602) (B), feeding duration (min/2h) (n = 591) (C) and ruminating duration (min/2h) (n = 591) (D) of individual dairy cows in relation to the mean vaginal temperature (°C) recorded in the time window 1230–1430 h of 32 experimental days. The black lines represent the model estimates with the confidence intervals in grey, and the blue lines represent the estimated slopes for each individual. Abbreviation: VT_{MEAN} = mean vaginal temperature.

durations decreased in cows with increased VT_{MEAN} and similar results were previously found in heat-stressed dairy cows in free-stall systems (Tapkı and Şahin, 2006; Moretti et al., 2017; Ammer et al., 2018). Animals' core behaviours are multifactorial and strongly depend on each other. Considering the variability and effect size, these indicators seem to be rather limited for the detection of the individual heat stress.

Although the cows reduced their walking activity, the mean RR increased in cows with increased VT_{MEAN}. Therefore, increased RRs indicated heat stress, as previously observed under similar climate conditions (Van Laer et al., 2015b). Li et al. (2020) previously classified RRs < 48 breaths per minute as normal. Indeed, we observed values of RRs > 48 breaths per minute in all individual dairy cows with a VT_{MEAN} > 39.0 °C. Because recordings of body temperature > 39.0 °C indicate heat stress in dairy cows (Kadokawa et al., 2012), these threshold values for respiration seem suitable to monitor heat stress. Full breaths can be recorded rapidly (approx. 60 s per cow, Dißmann et al., 2022). However, such observations require that the cow is not walking and that the observer is in proximity to the cow, which is challenging on pastures.

Proximity to the water trough and small inter-individual distances occurred more often when a cow had an increased VT_{MEAN}. Previous studies found dairy cows to be more frequently near water troughs when the heat load increased (Schütz et al., 2010; McDonald et al., 2020), but to date, none have linked this outcome to the individual cows' actual heat stress. Several explanations are possible for this behaviour. Heat-stressed dairy cows need to consume more water as they increase their heat loss through evaporating processes such as sweating and panting (Kadzere et al., 2002). Moreover, consumption of chilled water (water temperature <22 °C) has a direct cooling effect on cattle (Stermer et al., 1986). Furthermore, Schütz et al. (2010) speculated that water evaporation could have created a cooler environment around the trough in their study. Given that we used water troughs with much smaller surface areas, we reasoned whether this might explain why our cows stood closer to the water trough when their heat stress increased. Further studies are needed to clarify what biological advantage cows derive from proximity to water trough. The same also applies to the smaller inter-individual distances that we observed when the cows had elevated levels of VT_{MEAN}. During our behavioural observations, it was not possible to collect corresponding data that could verify whether the reduced inter-individual distances served to take advantage of the shadow cast by herd members or if there were other reasons, e.g. warding off insects.

Irritation from insects may be an additional stressor that is not directly related to heat (Woolley et al., 2018). However, the number of insects is known to be positively associated with ambient temperature. Therefore, we considered it a potential confounder and could show that fly intensity was very closely related to the CCI_{MEAN}. Owing to this relation, it was not possible to separate the effect exerted by flies from the effect of the heat load. Although the animals were periodically treated with fly repellent in our study, flies were always present on the pasture. Cows are known to exhibit a variety of behaviours to avoid flies, such as self-licking, crowding, or going to an area with reduced fly pressure (Woolley et al., 2018). Further, time devoted to fly-avoidance behaviours is not used to perform other activities, such as grazing, and this time allocation may influence feeding and ruminating durations (Woolley et al., 2018). Therefore, inter-individual distance, activity of standing and walking cows, and feeding and self-licking behaviours were likely to be influenced by the presence of flies. This stresses the need to consider fly intensity as a confounder in further studies on heat stress in cows on pastures.

Even if the above-discussed aspects are not yet completely clarified, inter-individual distance and proximity to the water trough could be useful in farming practice to detect heat stress that can arise in individual cows already at ambient temperatures of about 18 °C. The model estimated distinct changes for most cows; thus, these behaviours should be straightforward to observe, do not require continuous observation, and can be assessed even from large distances. Regarding the application of these findings, further research is required to determine the threshold of the behavioural changes in a herd to implement preventive measures (e.g. bringing the animals into the barn). Furthermore, because our results were obtained from only one herd at one site, on-farm experiments with dairy cows of other breeds and performance levels are needed for a broader generalisation of the results found in the present study (see Holinger et al., 2024).

Conclusions

Reticular temperature can be suitable for continuous monitoring of heat stress in grazing dairy cows on pastures. Grazing dairy cows with increased VT_{MEAN} responded with changes in several behavioural traits in this study. Changes in these traits seem to reflect behavioural adaptations to heat stress in temperate climate conditions on pastures. Especially the inter-individual distances and the proximity to the water trough are promising indicators for monitoring heat stress in cows on pastures. Further research is required to validate these indicators for feasibility in on-farm studies.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101097.

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, but they are available upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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