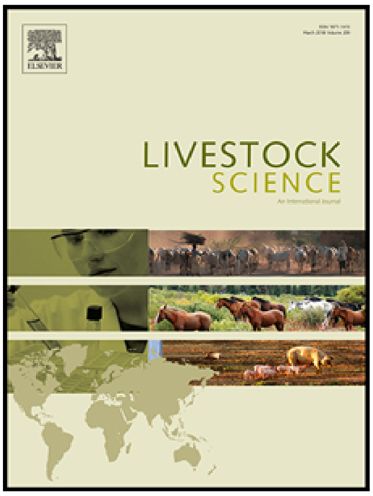

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Livestock Science

Behavioural changes to moderate heat load in grazing dairy cows under on-farm conditions

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HIGHLIGHTS

- Heat stress increasingly threatens dairy cows’ welfare, health and productivity
- On hot days, dairy cows on pasture show changes in their daily behavioural patterns
- They show less lying, more activity and a decrease in inter-individual distances
- These changes can be used as indicators to monitor heat stress of grazing cows

A B S T R A C T

Heat stress poses an increasing risk to welfare, health and productivity of dairy cows, especially for cows on pasture. To apply timely mitigation strategies for grazing cows, simple indicators are needed that signal heat stress. We conducted an exploratory study on the behaviour of grazing dairy cows in relation to the environmental heat load on four commercial dairy farms in Switzerland with herd sizes ranging from 20 to 57 cows. In a scan-sampling procedure standing/lying, feeding/ruminating, low inter-individual distances, proximity to drinker, use of natural shade and insect infestation were observed during 30 days (5-9 days/per farm). Additionally, 10 focal cows per farm were equipped with accelerometers to analyse lying duration and locomotor activity during on average 46 days per farm. On one farm all cows (N=57) were equipped with GPS devices which were used to calculate inter-individual distances among cows continuously during 69 days. Air temperature and relative humidity were recorded to calculate the temperature-humidity index (THI). For behaviours recorded in direct observations, a principal component analysis was performed for variable reduction. The first three principal components (PC) as well as the variables from automatic measurements were used as outcome variables in mixed effects models with daily maximum THI (THI_{max}), time of day (continuous, in 10-min intervals) and their interaction as explanatory variables. The three PCs could be described as: “feeding and standing”, “proximity to drinker” and “standing in close proximity and seeking shade”. The daily pattern of these PC’s differed by THI_{max} (interaction time of day * THI_{max}; all $p<0.01$). On days with high THI_{max} compared to days with lower THI_{max} cows were seen more often close to the drinker in the morning, but not in the afternoon when they were observed standing close to each other and in the shade. On days with high THI_{max}, cows also were lying less and increased their locomotor activity towards noon (interaction time of day * THI_{max}; $p<0.001$). Data from GPS devices confirmed the findings: On days with high THI_{max}, cows reduced their inter-individual distances over the course of the day, while this was not observed on days with lower $\mathrm{THI}_{\max}$ (interaction time of day * $\mathrm{THI}_{\max}$; $p<0.001$). Insect infestation increased with higher $\mathrm{THI}_{\max}$. We conclude that a distinct change in daily behavioural patterns, especially a reduction of lying behaviour, an increase in locomotor activity and a decrease in inter-individual distances could be used to monitor heat stress of dairy cows on pasture.

Keywords: THI, behaviour, inter-individual distance, climate, welfare

1. Introduction

Global warming is leading to higher average temperatures and an increase in the duration and frequency of hot spells. In Switzerland, located in the moderate climate zone of Central Europe, the average temperature in summer is expected to further increase by 0.7–7.2 °C until the end of the century, depending on climate scenario and region (CH2018, 2018). Simultaneously it is expected that the average precipitation during summer months decreases and solar radiation increases (CH2018, 2018). Climate change affects all aspects of livestock production, including fodder production, reproductive and growth performance as well as animal health of various species. Apart from these economically relevant consequences, increasing temperatures also pose a great risk for animal welfare. Heat stress occurs when the heat load (internal production and ambient environmental conditions) exceeds the capacity of an animal for heat dissipation, resulting in physiological and behavioural adaptations and finally an increase in the core body temperature (Bernabucci et al., 2010). Dairy cows are especially susceptible to heat stress due to their high internal metabolic heat production associated with fermentation and milk synthesis. The susceptibility increases with an increased milk production (Ammer et al., 2016) and varies depending on breed or stage of pregnancy (reviewed by Kadzere et al., 2002; Renaudeau et al., 2012). The environmental heat load is usually quantified as Temperature-Humidity-Index (THI), which is calculated from ambient air temperature and relative humidity. Different formulas are available, taking into account regional climatic differences (Bohmanova et al., 2007; Brügemann et al., 2012).

Heat stressed cows show several behavioural reactions (reviewed by Hoffmann et al., 2020). Similar to all mammalian species, cows reduce their feed intake in order to minimize heat production from digestion by adapting meal duration, meal size and feeding duration (Ammer et al., 2018; Eslamizad et al., 2015). Cows also adapt their behaviour by seeking shade or cooler areas (Heinicke et al., 2018; Schütz et al., 2010). They reduce their time spent lying, probably to increase heat dissipation by air circulation (Allen et al., 2015). Standing time as well as activity (number of steps) increase with increasing THI (Heinicke et al., 2018). Cows have also been observed to move closer to the water trough and drink more (Ammer et al., 2018; McDonald et al., 2020; Pontiggia et al., 2023; Schütz et al., 2010). A further behavioural reaction to heat stress that has been mentioned in anecdotal reports is a behaviour called grouping, bunching, clustering or crowding behaviour (Gaughan et al., 2002; Polsky and von Keyserlingk, 2017). Multiple causes and influential factors have been discussed to induce such behaviour in outdoor systems: e.g. increasing heat load and time of day (Erbez et al., 2012; Javorová et al., 2014) or biting insects (El Ashmawy et al., 2019). Recently, Pontiggia et al. (2023) provided evidence of this behaviour in an experimental setting on pasture. They found that cows were more likely to show small inter-individual distances when their vaginal temperature was elevated due to the existing heat load.

Measures against heat stress for cows housed in barns include shading, passive or active ventilation or the use of sprinklers. For cows that are kept on pasture for most of the day – as it is common in Switzerland – such protective measures are more difficult to implement. Shade can be provided naturally (trees or bushes) or artificially (shelters, tarps). Natural shading is often not available in intensive grazing systems or is not sufficient for the whole herd and artificial shade structures may also be difficult to set up for large herd sizes and rotational grazing systems. As an additional measure, grazing times can be shifted to the night or the early morning in order to avoid the times with highest temperature and solar radiation (Silanikove, 2000). For this latter measure in particular, simple behavioural indicators are needed so that farmers can determine the optimal time to bring the cows into the barn while optimizing feed intake on pasture and minimizing heat stress.

However, most of the reports on behavioural changes in cows due to heat stress are either anecdotal or were obtained in indoor studies or studies in different climatic zones, as in tropical or subtropical regions. There is not much knowledge about the behaviour of dairy cows under heat stress on pasture in moderate climate zones. Additionally, most of the studies were performed at research facilities while the present study aims to provide results based on commercial on-farm conditions. As stated in the literature review of Aubé et al. (2022) reliable indicators and time-budgets of cows on pasture depending on the ambient climate conditions still have to be developed. For practical reasons and for the application of heat stress assessment on-farm, a combination of indicator variables for heat stress detection might be useful in order to account for diverse conditions on farms. We therefore conducted an explorative, multi-centre on-farm study with the aim to identify behavioural indicators for early heat stress in grazing dairy cows that can be used in farming practice. Our hypothesis was that potential behavioural indicators of heat stress would show distinctly different daily patterns during hot days compared to cooler days, implying a behavioural response to the heat load experienced.

2. Animals, materials and methods

2.1. Study design

An observational study was carried out on four farms with dairy cows in North-western Switzerland between June and September 2021. This region belongs to the temperate climate zone at the transition between oceanic and continental climate, without extreme weather conditions and temperatures. Farms were selected by the type of feeding, which had to be predominantly pasture-based in summer. Additionally, the grazing management had to be rotational grazing or continuous grazing. Characteristics of the dairy farms (A-D) are presented in Table 1. Herd size ranged from 19 to 57 and changed slightly on the individual farms during the course of the study due to slaughtering of cows or because some of them were sent to alpine pasture regions for the summer months. Size of the used pastures during the observations ranged between 0.14 ha and 2.6 ha. The cows were grazed on each farm during the day, partly also at night. The cows were taken out to pasture in the morning between 06:30 and 11:00. On average, the animals spent 5.5 h (min–max: 2.0 – 8.8 h) on pasture during the day. Thus, the cows were brought back to the barn between 10:00 and 17:45. It was up to the farmers to decide at what time the animals were brought into the barn, in order not to disturb the usual management routines. All farms had a milking parlour inside the barn and milked the cows twice a day.

Farms were visited on 5 to 9 observation days per farm (Table 1). The observation days were determined according to the following criteria, based on the forecast of the Federal Office of Meteorology and Climatology (MeteoSwiss): an expected daily minimum temperature of 18 °C, sunny or overcast skies, probability of precipitation max. 50 %, expected precipitation amount max. 5 l/m^2. Water was provided through a mobile drinking trough on Farm A and D. Farm C used fixed installed watering troughs and Farm B changed between mobile and fixed troughs depending on pasture. There was always one drinker per pasture. On two observation days (on one farm) there was no drinker on pasture. On 17 observation days (across all farms) the cows had access to natural shade (trees or bushes).

The experiment was approved by the Cantonal Veterinary Office (Granges-Paccot, Fribourg, Switzerland; approval no. 2018_04_FR).

Table 1: Dairy farm characteristic description

Farm	Herd size	Breed[a]	Altitude [m.a.s.l]	Feeding and grazing system	Horn status	Average milk yield per standard lactation	Observation days
A	20–21	SI	789	Grazing during the day, supplemented with hay at night	Horned	6890	5
B	37–57	SF / KC	521	Full grazing	Dehorned	5760	8
C	19–24	SI	441	Grazing during the day, supplemented with hay at night	Horned	5000	9
D	37–44	HO	377	Full grazing	Dehorned	4700	8

[a] SI = Simmental, SF = Swiss Fleckvieh, KC = New Zealand Kiwi Cross, HO = New Zealand Holstein

2.2. Measurement of climatic conditions

Air temperature (in °C, T) and relative humidity (in %, RH) were measured every 10 min on pasture during the observations (TinyTag Ultra 2, Gemini Data Loggers, Chichester UK). The logger was installed at a height of approximately 1 meter, protected by a white plastic cover from direct sunlight. Additionally, two identical loggers were permanently installed on every farm at covered places in close proximity to the barn and set to measure T and RH in 20 min intervals throughout the study period. The temperature-humidity-index was calculated according to the formula by the NRC (1971):

$$THI = (1.8 * T + 32) – (0.55 – 0.0055 * RH) * (1.8 * T – 26)$$

2.3. Direct behavioural observations

In total, 30 observation days took place within the study. Direct behavioural observations were carried out on observation days based on the scan-sampling method in 10-min intervals (e.g. Pontiggia et al., 2023; Schütz et al., 2010; Vizzotto et al., 2015) during the time when cows were on pasture. Start and end time of the observations varied considerably among and within farms. The average start and end times for observations were 08:00 to 15:45 on Farm A, from 06:45 to 12:00 on Farm B, from 09:45 to 14:45 on Farm C and from 10:15 to 14:15 on Farm D. The observer was standing next to the fence in a position where she could oversee the pasture. If needed, the observer moved a few meters along the fence without disturbing the cows. The number of visible cows was recorded at the beginning of each scan because sometimes not all cows were visible at the same time due to topography. Subsequently, the number of cows showing the behaviours as described in Table 2 was noted. "Lying on sternum" and "lying on the side" were summed up as "lying" for all further analyses. Proximity to the drinker was assessed as either directly at the drinker or in a radius smaller than 10 m around the drinker. This distance was chosen due to good agreement among observers during training compared to smaller distances. The available shaded areas were visually estimated hourly in four categories: No shade, shade accessible for less than half of the herd, shade accessible for more than half of the herd or shade accessible for all cows. Shade was provided by trees or bushes, not by artificial shelters. The presence of insects (flies, horseflies) was assessed after each scan on three cows that were well visible, using binoculars if needed. A three-level score was applied to a 5-cm-diameter around the eye (alternating between left and right eye per scan): 0 = No insects visible, 1 = < 3 insects visible, 2 = > 3 insects visible.

Four observers were involved in data collection. Before the start of the study period the ethogram and the procedure during observations were extensively trained and simplified where necessary to reach a high agreement. During the study period, approximately 20 % of all observations were carried out simultaneously by two observers in order to assess inter-observer-agreement. Concordance correlation coefficients (CCC) were calculated for the inter-observer agreement for the variables recorded during direct observations.

Table 2: Ethogram of the recorded behaviours during direct observations

Behaviour	Description
Lying on sternum	Cow is lying on the sternum, all four legs are under the body or one/two front leg(s) is/are extended, no leg is loaded, head is turned back and rests on the body OR neck is extended and head rests on the floor OR head is raised (Krohn and Munksgaard, 1993)
Lying on the side	Cow is lying on the floor in the lateral position, no leg is loaded, the neck is extended and the head rests on the floor (Krohn and Munksgaard, 1993)
Standing / walking	Cow is upright, three or four feet on the ground
Feeding	Food in the mouth, chewing, or muzzle close to pasture (Krohn and Munksgaard, 1993)
Ruminating	Regurgitation, chewing and swallowing of previously eaten food (Krohn and Munksgaard, 1993)
Unclear feeding / ruminating	Head position/activity of the cow is not clearly recognizable (e.g. cow is facing away from the observer)
Low inter-individual distance	Cow standing with low inter-individual distance, less than one cow width (0.8 m) distance from other standing cows
Distance to drinker	Cow directly at the drinker or < 10 m from drinker
Position in the shade	Cow with at least the front half of its body in the shade of trees or bushes, including the head.

2.4. Activity measurements

Ten focal cows per herd were equipped with 3-dimensional accelerometers (MSR145 data logger, MSR Electronics GmbH, Seuzach, Switzerland), attached laterally to the metatarsus of the hind leg with velcro tape (specifically manufactured for use on dairy farms). The accelerometers had sufficient storing capacity to record around 12 days continuously. After 12 days, they were removed and during the next farm visit for the direct observations the same 10 focal cows per farm were equipped again with accelerometers. This procedure was repeated four to five times per farm, reaching on average 46 days of recordings per farm (Farm A: 44 days, Farm B: 50 days, Farm C: 38 days, Farm D: 52 days). The accelerometers have been validated for the recording of locomotor activity and lying behaviour in cows (Gygax et al., 2015; Johns et al., 2015) and the procedure has been described in Weigele et al.

(2018). The accelerometers recorded acceleration with a sampling rate of 1 Hz and a sensitivity of ±16 *g*. Raw data were extracted to CSV files (MSR software, version 6.06.14, MSR Electronics GmbH, Seuzach, Switzerland). The software R (R Core Team, 2022) was used to calculate lying (yes/no) and locomotor activity per cow and 10-min interval from raw data by employing the package "triact" (Simmler and Brouwers, 2023). Due to very strong skewing in the data, lying was calculated as binary variable with "yes" meaning the cow was lying at least once during the 10-interval and "no" meaning the cows was not lying during the respective interval. For locomotor activity the sum of measured acceleration in all three dimensions was summed up per 10-min interval. The unit used for this parameter was acceleration *g* / h. Only data recorded between 06:30 and 16:30 were included in analyses. The measurements in the afternoon (after 12:00) should be taken with caution as data on the time at which the cows were brought from the pasture into the barn were not available for all measurement days.

2.5. Inter-individual distances from GPS measurements

In order to get more data on inter-individual distances on pasture and to validate the direct behavioural observations, we used GPS devices with continuous measurements on one farm. All 57 cows of farm B were equipped with GPS devices (Alptracker LoRaWAN device, Alptracker AG, Gamsen, Switzerland), attached to a collar, from July to September. Only data recorded during the day when cows were on pasture was used for further analyses. The accuracy of the position measurements ranged between 1.11 and 24.99 m. The accuracy of the GPS devices is depending on satellites in view, mountains and atmospheric disturbances. The devices were set up to try to find a position with an accuracy of below 5 m within 30 sec. All position measurements with an accuracy estimation greater than 4.99 m were excluded from the evaluation. Three devices had to be removed from all analyses due to erroneous measurements. Five cows left for alpine pastures at the beginning of the study and were therefore not considered. Due to battery issues most devices stopped recording within the last days of the study. Overall, we had measurements of 49 cows in total, during 69 days. The devices measured the cow’s position once every 10 min. It was not possible to set up these devices in a way that they recorded all at the same time. Therefore, for calculation of the inter-individual distances among cows, we rounded the time of every recording to the next five minutes. For every device that had a measurement within this 5-minute interval we calculated the distance (D) to all other devices in the same interval using the following formula:

$D = R * \text{sqrt}(x^2 + y^2)$

$R = 6371 * (\text{pi} / 180) * 1000$

x = latitude device1 – latitude device2

y = (longitude device1 - longitude device2) * cos(0.5 * (latitude device1 + latitude device2) * pi/180)

Resulting inter-individual distances between two cows larger than 200 m were removed from the data set due to implausibility. Thus, 29’699 assessed distances between two cows from in total 910’272 assessed distances had to be removed for this reason. We then calculated the average distance among all cows of the herd per 5-min interval.

2.6. Statistical analyses

All statistical analyses were conducted using the software R (R Core Team, 2022). Farm was our largest experimental unit, with repeated measurements on herd level within farm. A principal component analysis (PCA) was chosen as method for variable reduction of behavioural variables recorded in direct observations. The PCA approach was chosen to reduce the number of tests, to account for correlated variables, and to check whether there are underlying variables that explain the change in behaviour. To account for the repeated measurements per farm, a mixed model was calculated for each variable beforehand which only contained the date within farm as nested random effect but no explanatory variables. Models were calculated with the function “blmer” (package “blme”; Bates et al., 2015; Chung et al., 2013). Model assumptions were tested by visually inspecting residuals for deviances from normality or homogeneity of variance. Due to deviations all variables were transformed using the logit link function. The residuals of these models were then used for the PCA. The package “missMDA” (Josse and Husson, 2016) and a cross-validation approach was applied to deal with missing values that occurred due to missing shade on certain days or periods during observations (indicator “position in the shade”). Mixed effects models were calculated for the first three principal components of the PCA, as well as for proportion of lying cows and locomotor activity (from accelerometer data) and inter-individual distance (from GPS data). Explanatory variables were time of day (as continuous variable in 10-min intervals), maximum THI of the day (THI_{max}, one value per day) and their interaction. Random effect was date within farm. As the inter-individual distances were only measured with GPS on one farm, the random effect contained only date. P-values were calculated by comparing the full model to a model without the interaction or the main effect of interest. Calculations were done with either likelihood-ratio tests (function “anova”; accelerometer and GPS data) or parametric bootstrapping (function “PBmodcomp”; direct behavioural observation data). Model estimates were calculated using parametric bootstrapping for representative THI_{max} values and time of day. THI_{max} values for model estimation were selected based on the range of THI_{max} in the raw data: the lowest and highest THI_{max} values were rounded to the nearest value divisible by five. E.g. if the lowest THI_{max} in the raw data was 57.9, we calculated model estimates for a THI_{max} of 60. For visualization purposes, we also calculated estimates in steps of five between the lowest and highest rounded THI_{max}. Time of day (12:00 and 14:00) was chosen to represent the hottest period of the day where cows were still on pasture.

Additionally, for visualization purposes, continuous model estimates were calculated for the whole day from 06:30 to 16:30.

3. Results

3.1. Climatic conditions

Maximum THI (THI_{max}) per day on pasture during direct observations was on average (± standard deviation SD) 78.41 ± 3.5 (68.7–86.1; Minimum–Maximum). THI_{max} on pasture was reached in the early afternoon between 13:00 and 14:00. The average THI during direct observations was 73.5 ± 5.7. Average temperature during direct observations was 26.5 ± 5 °C, and average relative humidity was 53.7 ± 14.8 %. On the 30 days when direct observations where conducted, the minimum THI during 24 hours (measured close to the barn) ranged from 51.5 to 65.5. The maximum THI during 24 hours ranged from 66.2 to 86.3. Average SD of THI on these days was 5.8. THI_{max} per day as measured by the two loggers close to the barns during measurements with the accelerometers was on average 73.2 ± 5.2 (58.3-84.3; Minimum–Maximum). THI_{max} per day as measured by the logger close to the barn during GPS measurements on Farm B was on average 70.5 ± 3.7 (57.7-81.0; Minimum–Maximum). Shade for the whole herd was available during 10 % of all direct observation scans (82/958), while there was no shade during 43 % of all scans (346/958). Shade accessible for less than half of the herd was present during 28 % of all scans (219/958), and shade for more than half of the herd was present during 19 % of all scans (149/958).

3.2. Direct behavioural observations

Inter-observer-agreement was high for all observed variables. The concordance correlation coefficient ranged from 0.94 (ruminating) or 0.95 (directly at the drinker, close to drinker, low inter-individual distance) to > 0.99 (lying, feeding, in the shade).

The first three components of the PCA explained 77.1 % of the total variance in the data (Table 3). Lying (negative loading), feeding (positive) and ruminating (negative) loaded strongly on the first principal component (PC1), which was thus named “feeding and standing”. The two variables related to the distance to the drinker loaded on PC2, which was named “proximity to drinker”. PC3 contained strong loadings from the variable “low inter-individual distance” and “in the shade”. This PC was thus named “standing in close proximity and seeking shade”.

Results from the mixed models showed that the THI_{max} per day did not affect the three PCs per se (Table 4). However, all three PCs demonstrated a characteristic daily pattern (effect of time of day) which differed depending on THI_{max} (one value per day) as indicated by interactions between time of day and THI_{max} (Fig. 1; Table 4). “Feeding and standing” decreased over the course of the day. On days with higher THI_{max} “feeding and standing” was more pronounced in the morning (between around 09:00 and 11:00) compared to days with lower THI_{max}, as indicated by the interaction. On days with higher THI_{max} “proximity to drinker” was more pronounced in the morning and less at noon. “Standing in close proximity and seeking shade” was observed more often in the afternoon on days with higher THI_{max}, while it was rarely seen on days with lower THI_{max} (Fig.1).

Insect infestation increased with THI on pasture. Mean THI associated with the three insect infestation scores was: 66.8 (± 7.4) for score 0, 72.3 (± 6.2) for score 1 and 74.9 (± 5.2) for score 2.

Table 3: Results of principal component analysis of behavioural variables recorded in direct observations. Loadings > 0.4 or < -0.4 are highlighted in bold.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Lying	**-0.452**	-0.269	-0.445	-0.107	-0.015	**-0.710**	-0.099
Feeding	**0.569**	-0.132	-0.033	-0.034	0.042	-0.386	**0.711**
Ruminating	**-0.545**	-0.160	-0.227	0.017	0.027	**0.458**	**0.644**
Low inter-individual distance	-0.316	0.288	**0.489**	**0.558**	-0.351	-0.319	0.203
Directly at drinker	-0.025	**0.629**	-0.195	**-0.543**	**-0.506**	-0.024	0.119
Close to drinker	-0.037	**0.637**	-0.293	0.246	**0.659**	-0.093	0.056
In the shade	-0.269	0.012	**0.622**	**-0.565**	**0.429**	-0.162	0.106

Proportion of variance	0.375	0.219	0.177	0.103	0.073	0.027	0.026
Cumulative proportion of variance	0.375	0.594	0.771	0.873	0.947	0.974	1.000

Table 4: Model estimates, 95% confidence intervals and p-values for the analysed behavioural variables. Model estimates are presented for representative low and high THI_{max} values as well as for 12:00 and 14:00

Outcome variable		Model estimates [95% confidence interval]		p-value		
	THI_{max} 1)	12:00	14:00	Interaction time of day x THI_{max}	Time of day	THI_{max}
PC1 “Feeding and standing” [PC score]	70	-0.66 [(-1.14) – (-0.07)]	-0.90 [(-1.53) – (-0.11)]	0.001	0.004	0.272
	85	-0.43 [(-1.04) – 0.07]	-1.26 [(-1.90) – (-0.61)]			
PC2 “Proximity to drinker” [PC score]	70	0.66 [0.31 – 1.05]	-0.16 [(-0.51) – 0.21]	<0.001	0.002	0.304
	85	-0.66 [(-0.94) – (-0.30)]	-0.47 [(-0.91) – (-0.08)]			
PC3 “Low inter-individual distance and shade” [PC score]	70	-0.38 [(-0.73) – (-0.05)]	-0.37 [(-0.77) – 0.05]	<0.001	<0.001	0.171
	85	0.31 [0.03 – 0.58]	0.72 [0.39 – 1.11]			
Lying [proportion per 10-min-interval]	60	0.43 [0.38 – 0.58]	-2)	<0.001	<0.001	<0.001
	85	0.11 [0.08 – 0.18]	-			
Activity [log(*g*/h)]3)	60	5.41 [5.13 – 5.73]	-	<0.001	<0.001	<0.001
	85	6.22 [5.95 – 6.51]	-			
Inter-individual distance [m]	60	69.04 [64.94 –74.45]	64.17 [59.95 – 69.53]	<0.001	<0.001	<0.001
	80	12.92 [8.42 – 17.20]	11.07 [6.38 – 15.19]			

1) THI_{max} refers to the maximum THI per day measured on pasture during direct observation (PC1, PC2, PC3) or close to the barn (lying proportion, activity, inter-individual distance).

Model estimates for two representative $\mathrm{THI}_{\max}$ values (low and high) have been calculated which best represent the range of THI values within each data set.

[2] Activity data from the afternoon are not presented due to uncertainties about the position of the cows (barn or pasture)

[3] The unit *g* stands for gravitiy acceleration (9.81 m/s^2).

3.3. Activity measurements

Proportion of lying cows and general activity were affected by time of day, $\mathrm{THI}_{\max}$ as well as their interaction (Table 4; Fig. 2). In the morning until around 09:00 the proportion of lying cows increased on all days. On hot days it then decreased towards noon, while it remained high on cooler days. The daily pattern of locomotor activity was accordingly exactly the opposite. Activity was highest shortly before noon on hotter days. Measurements in the afternoon have to be treated with caution as data on the time at which the cows were brought from the pasture into the barn were not available for every measurement day.

3.4. Inter-individual distance (GPS data)

The average inter-individual distance among cows on pasture was affected by time of day, $\mathrm{THI}_{\max}$ as well as their interaction (Table 4; Fig. 3). While the average distance remained relatively high on cooler days with a $\mathrm{THI}_{\max}$ of around 60, it clearly decreased towards noon on warmer days. In the afternoon of days with a $\mathrm{THI}_{\max}$ of 80 the average inter-individual distance of the herd was found to be as low as around 10 m, indicating that the cows were standing in close proximity to each other.

4. Discussion

The results of our explorative study show that there are some distinct differences in daily behavioural patterns of grazing dairy cows on days with relatively high versus days with relatively low $\mathrm{THI}_{\max}$. The most distinct change of patterns over the course of the day (interaction between $\mathrm{THI}_{\max}$ and time of day) occurred for lying behaviour, activity, proximity to the drinker and inter-individual distances. With increasing daily $\mathrm{THI}_{\max}$, cows reduced their lying time, were more active, and displayed more standing in close proximity to each other, especially in the afternoon. In the morning they were observed in proximity to the drinker, while they sought shade, if available, in the afternoon. Thus, a set of variables seems to be indicative of heat stress.

A reduction of inter-individual distances over the course of the day on days with higher THI_{max} was found both in the direct observations and with the temporally much higher resolution of the GPS-measurements. Inter-observer-agreement was high for all applied indicators during direct observations, including inter-individual distance. We can thus resume that visually assessing the number of cows on pasture with low inter-individual distances is a reliable and valid indicator for heat stress, which can be used in practice. Similarly, we observed a changed lying pattern over the course of the day depending on THI_{max} in direct observations as well as with accelerometer data. However, the daily pattern for lying was intertwined with feeding in the PCA and thus not as clearly visible as with accelerometer data. Proximity to the drinker was another indicator, whose daily pattern changed with THI_{max}. Water is a limited resource and the competition at the water trough increases with increasing ambient temperature (McDonald et al., 2020). In a previous study with cows housed indoors, it has been shown that competition at the water trough and also water intake peaked between 08:00 and 12:00 and between 16:00. and 20:00 (McDonald et al., 2020). This is similar to our findings, in which on hot days more cows were seen close to the drinker in the early morning, but not in the early afternoon. Assumingly, with increasing THI closer to noon, cows moved away from the drinker because they progressively started to move together (reduce their inter-individual distances), to move into the shade and to increase their activity. An increase in activity during the same time has been confirmed by the accelerometer data, although being counterintuitive per se in a situation of potential heat stress. One explanation for the increased activity might be an increase in insect avoidance behaviour. Another explanation could be the motivation to get into close proximity to other cows.

The phenomenon of cows reducing their inter-individual distances could also be described as grouping or crowding behaviour. A similar behavioural pattern has been described anecdotally in cows inside the stables during summer months (Erbez et al., 2012; Javorová et al., 2014; van Schaik et al., 2021). In both Czech studies grouping behaviour in dairy cows occurred from 10:00 to 19:00 and it was associated with increasing heat load as it appeared when air temperature exceeded 19 °C (Javorová et al., 2014) or when daily average temperatures reached 20 °C (Erbez et al., 2012). A Dutch study in 2021 investigated various causes for the appearance of the crowding behaviour in dairy cattle farms during summer (van Schaik et al., 2021). This study was initiated by 50 preceded reports of farmers on such grouping behaviour of their dairy herds inside the barn. However, although the grouping behaviour could be confirmed in the field study, climatic conditions could not be identified as single reason for it. Rather multiple reasons including insects were discussed (van Schaik et al., 2021). Although at first glance it seems contra-intuitive to stand close to each other and thereby inhibit air circulation, there are some possible explanations for this behaviour. (1) Firstly, it could be related to shade seeking. When sheep e.g. show grouping behaviour in the heat, they stand in a circle with the head pointing towards the centre. By lowering their heads, their bodies can provide some shade for each other. (2) Secondly, grouping behaviour is a reaction associated with external threats in general that induce distress responses (Polsky and von Keyserlingk, 2017), e.g. in several prey animals during the attack of a predator. Grouping and hiding inside the herd reduces the risk for the individual animal and has been described as the “Hamilton’s selfish herd hypothesis” (Hamilton, 1971). If this explanation holds true, it would mean that the observed cows were in a situation of serious stress and countermeasures are highly welfare relevant. (3) Finally, a third explanation could be that the grouping behaviour is a reaction to an infestation with stinging insects in cows (El Ashmawy et al., 2019). El Ashmawy et al. (2019) observed that cows in barns with high insect infestation moved close together and pointed the head towards the center of the group. They called this behaviour "bunching". For horses it has similarly been described that they stand in close proximity and show social grooming to reduce insect load (Christensen et al., 2022). In our study, THI and insect infestation were correlated and their effects cannot be separated. However, no grouping behaviour in the sense of standing still for social grooming or pointing the head towards the centre of the group was observed among the cows in our study. The grouping behaviour was rather associated with restlessness and the whole herd moved around the grazing area. This was also underlined by an increase in locomotor activity and a reduction in the proportion of lying cows. Boland et al. (2008) confirmed in their study that cattle show rising avoidance behaviour such as tail flicks, leg stomps, skin twitches and head throws with increasing insect infestation which could also influence the overall activity of the animals. Future studies should further investigate the causes of grouping behaviour in cows in relation to heat stress and insect infestation. The disentanglement of these two stressors will help to apply tailored measures and to improve welfare of grazed dairy cows.

To analyse the indicators collected by direct behavioural observations, we applied a PCA. This approach was chosen, among other things, to reduce complexity and to assess whether rather a combination of (partially correlated) variables might be indicative of heat stress. The results have shown that three principal components explain 77.1% of the whole variance in the data set. The three components included behavioural variables of different behavioural aspects (feeding and standing, proximity to drinker and low inter-individual distance and shade). All three components were found to develop differently over the course of the day depending on $THI_{max.}$ These findings therefore show that the cows adapted their behaviour on hot days in three dimensions: 1) they reduced lying, feeding and ruminating, 2) they moved closer to the drinker (in the morning, but not around noon) and 3) they changed their position by either moving closer to other cows or by seeking shade (in the afternoon, but not in the morning). Thus, it seems to be rather a complex of behavioural changes that is indicative of heat stress. Especially regarding heat stress assessment in practice, monitoring of cows on pasture with respect to heat stress should thus include all three observed dimensions of behavioural changes.

The differences in daily behavioural patterns associated with different $\mathrm{THI}_{\mathrm{max}}$ values indicate that these changes were caused by increasing heat load. Due to the on-farm setting of our study, it was not possible to collect physiological data such as vaginal or rumen temperature or respiration rate. We can therefore only capture associations between THI and behaviour, with an uncertainty remaining if the ambient climatic conditions actually caused heat stress in the observed cows. However, the chosen behavioural indicators have been found to be correlated with physiological indicators of heat stress before (Pontiggia et al., 2023). Particularly concerning the assessment of seeking shade on pasture the direct record of solar radiation beside air temperature and humidity could have provided additional information to the present study. Studies, like the one reported by Tucker et al. (2008) showed, that the intensity and amount of solar radiation on pasture influenced the use of shaded areas by dairy cattle. However, the THI and the heat-load-index, a thermal index that also includes the solar radiation, are at least moderately up to highly positively correlated (e.g. r = 0,83; Islam et al., 2023). Thus, we think we could use the THI as representative index.

Behavioural changes associated with heat stress were detected although there was a high variation among farms with respect to breed, horn status, milk yield, pasture size, vegetation on pasture and topography. The chosen multi-centre on-farm approach entails a high external validity and allows the transfer of results to other situations. In the future, applicable thresholds for the identified behavioural changes need to be developed that consider the individual farm and herd preconditions.

5. Conclusions

Dairy cows on pasture are particularly exposed to direct solar radiation during summer periods combined with limited provision of cooling measurements compared to inside the barn. Our results were obtained under commercial farm conditions, with different cattle breeds under moderate heat load. We could show that dairy cows change daily behavioural patterns on pasture in relation to the environmental heat load such as grouping behaviour, increased activity or prolonged standing consistently across study farms even under temperate climatic conditions and for non-high-yielding cows. These behavioural changes might be adaptive to a certain extent, but also indicative of a stressful situation. This could additionally be reinforced by an increasing insect infestation that is associated with higher THI_{max} values, which might lead to increased insect avoidance behaviour by the cows.. All evaluated variables were affected either by an interaction between THI_{max} and time of day (changed behavioural pattern), or just by THI_{max}. Thus, a set of variables seems suitable to monitor heat stress of dairy cows on pasture.

CRediT authorship contribution statement

Mirjam Holinger: Conceptualization, Methodology, Investigation, Formal analyses, Visualization, Writing – original draft, Writing – review & editing, Supervision. Verena Bühl: Investigation, Data curation, Writing – review & editing. Manuela Helbing: Formal analyses. Lena Pieper: Investigation. Sabine Kürmann: Investigation. Alice Pontiggia: Conceptualization, Methodology. Frigga Dohme-Meier: Funding acquisition, Conceptualization. Nina Keil: Funding acquisition, Conceptualization, Writing – review & editing. Stefanie Ammer: Funding acquisition, Conceptualization, Methodology, Writing – review & editing.

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References

Allen, J.D., Hall, L.W., Collier, R.J., Smith, J.F., 2015. Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. J. Dairy Sci. 98, 118–127. https://doi.org/10.3168/JDS.2013-7704

Ammer, S., Lambertz, C., Gauly, M., 2016. Is reticular temperature a useful indicator of heat stress in dairy cattle? J. Dairy Sci. 99, 10067–10076. https://doi.org/10.3168/JDS.2016-11282

Ammer, S., Lambertz, C., von Soosten, D., Zimmer, K., Meyer, U., Dänicke, S., Gauly, M., 2018. Impact of diet composition and temperature-humidity index on water and dry matter intake of high-yielding dairy cows. J. Anim. Physiol. Anim. Nutr. (Berl). 102, 103–113. https://doi.org/10.1111/JPN.12664

Aubé, L., Mialon, M.M., Mollaret, E., Mounier, L., Veissier, I., de Boyer des Roches, A., 2022. Review: Assessment of dairy cow welfare at pasture: measures available, gaps to address, and pathways to development of ad-hoc protocols. Animal 16, 100597. https://doi.org/10.1016/j.animal.2022.100597

Bates, D., Machler, M., Bolker, B.M., Walker, S.C., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01

Bernabucci, U., Lacetera, N., Baumgard, L.H., Rhoads, R.P., Ronchi, B., Nardone, A., 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 4, 1167–1183. https://doi.org/10.1017/S175173111000090X

Bohmanova, J., Misztal, I., Cole, J.B., 2007. Temperature-Humidity Indices as Indicators of Milk Production Losses due to Heat Stress. J. Dairy Sci. 90, 1947–1956.

https://doi.org/10.3168/jds.2006-513

Boland, H.T., Scaglia, G., Umemura, K., 2008. Impact of Horn Flies, Haematobia irritans (L.) (Diptera: Muscidae), on the Behavior of Beef Steers. Prof. Anim. Sci. 24, 656–660. https://doi.org/https://doi.org/10.15232/S1080-7446(15)30918-9

Brügemann, K., Gernand, E., von Borstel, U., König, S., 2012. Defining and evaluating heat stress thresholds in different dairy cow production systems. Arch. Anim. Breed. 55, 13–24. https://doi.org/10.5194/aab-55-13-2012

CH2018, 2018. CH2018 – Climate Scenarios for Switzerland, Technical Report, National Centre for Climate Services. Zurich.

Christensen, J.W., Strøm, C.G., Nicová, K., de Gaillard, C.L., Sandøe, P., Skovgård, H., 2022. Insect-repelling behaviour in horses in relation to insect prevalence and access to shelters. Appl. Anim. Behav. Sci. 247. https://doi.org/10.1016/j.applanim.2022.105560

Chung, Y., Rabe-Hesketh, S., Dorie, V., Gelman, A., Liu, J., 2013. A nondegenerate likelihood estimator for variance parameters in multilevel models. Psychometrika 78, 685–709. https://doi.org/10.1007/S11336-013-9328-2

El Ashmawy, W.R., Williams, D.R., Gerry, A.C., Champagne, J.D., Lehenbauer, T.W., Aly, S.S., 2019. Risk factors affecting dairy cattle protective grouping behavior, commonly known as bunching, against Stomoxys calcitrans (L.) on California dairies. PLoS One 14, e0224987. https://doi.org/10.1371/JOURNAL.PONE.0224987

Erbez, M., Bøe, K.E., Falta, D., Chládek, G., 2012. Crowding of dairy cows in a cubicle barn during the hot summer months. Arch. Anim. Breed. 55, 325–331. https://doi.org/10.5194/AAB-55-325-2012

Eslamizad, M., Lamp, O., Derno, M., Kuhla, B., 2015. The control of short-term feed intake by metabolic oxidation in late-pregnant and early lactating dairy cows exposed to high ambient temperatures. Physiol. Behav. 145. https://doi.org/10.1016/j.physbeh.2015.03.044

Gaughan, J.B., Mader, T.L., Holt, S.M., Hahn, G.L., Young, B., 2002. Review of current assessment of cattle and microclimate during periods of high heat load. Am. Soc. Anim. Sci. - Annu. Meet. 24, 77–80.

Hamilton, W.D., 1971. Geometry for the selfish herd. J. Theor. Biol. 31, 295–311. https://doi.org/10.1016/0022-5193(71)90189-5

Heinicke, J., Hoffmann, G., Ammon, C., Amon, B., Amon, T., 2018. Effects of the daily heat load duration exceeding determined heat load thresholds on activity traits of lactating dairy cows. J. Therm. Biol. 77, 67–74. https://doi.org/10.1016/J.JTHERBIO.2018.08.012

Hoffmann, G., Herbut, P., Pinto, S., Heinicke, J., Kuhla, B., Amon, T., 2020. Animal-related, non-invasive indicators for determining heat stress in dairy cows. Biosyst. Eng. 199, 83–96. https://doi.org/10.1016/j.biosystemseng.2019.10.017

Islam, M.A., Lomax, S., Doughty, A.K., Islam, M.R., Thomson, P.C., Clark, C.E.F., 2023. Revealing the diversity of internal body temperature and panting response for feedlot cattle under environmental thermal stress. Sci. Rep. 13, 4879. https://doi.org/10.1038/s41598-023-31801-7

Javorová, J., Velecká, M., Falta, D., Večeřa, M., Andrýsek, J., Studený, S., Chládek, G., 2014. The effect of air temperature and time of day on distribution of Czech fleckvieh cows within the barn. Acta Univ. Agric. Silvic. Mendelianae Brun. 62, 12. https://doi.org/10.11118/actaun201462010117

Josse, J., Husson, F., 2016. missMDA: A Package for Handling Missing Values in Multivariate Data Analysis. J. Stat. Softw. 70, 1–31. https://doi.org/10.18637/JSS.V070.I01

Kadzere, C.T., Murphy, M.R., Silanikove, N., Maltz, E., 2002. Heat stress in lactating dairy cows: a review. Livest. Prod. Sci. 77, 59–91. https://doi.org/10.1016/S0301-6226(01)00330-X

Krohn, C.C., Munksgaard, L., 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments II. Lying and lying-down behaviour. Appl. Anim. Behav. Sci. 37, 1–16. https://doi.org/https://doi.org/10.1016/0168-1591(93)90066-X

McDonald, P. V., von Keyserlingk, M.A.G., Weary, D.M., 2020. Hot weather increases competition between dairy cows at the drinker. J. Dairy Sci. 103. https://doi.org/10.3168/jds.2019-17456

NRC, 1971. A Guide to Environmental Research on Animals: Committee on Physiological Effects of Environmental Factors on Animals. National Academy of Sciences, Washington.

Polsky, L., von Keyserlingk, M.A.G., 2017. Invited review: Effects of heat stress on dairy cattle welfare. J. Dairy Sci. 100, 8645–8657. https://doi.org/10.3168/JDS.2017-12651

Pontiggia, A., Münger, A., Eggerschwiler, L., Holinger, M., Stucki, D., Ammer, S., Bruckmaier, R., Dohme-Meier, F., Keil, N.M., 2023. Behavioural responses to moderate heat stress in grazing dairy cows in temperate climate. Manuskript submitted for publication.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Renaudeau, D., Collin, A., Yahav, S., De Basilio, V., Gourdine, J.L., Collier, R.J., 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6, 707–728. https://doi.org/10.1017/S1751731111002448

Schütz, K.E., Rogers, A.R., Poulouin, Y.A., Cox, N.R., Tucker, C.B., 2010. The amount of shade influences the behavior and physiology of dairy cattle. J. Dairy Sci. 93, 125–133. https://doi.org/10.3168/jds.2009-2416

Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest. Prod. Sci. 67, 1–18. https://doi.org/https://doi.org/10.1016/S0301-6226(00)00162-7

Simmler M., Brouwers S. P., 2023. triact package for R: Analyzing the lying behavior of cows from accelerometer data. Preprint available at SSRN: https://ssrn.com/abstract=4560833 (under review)

Tucker, C.B., Rogers, A.R., Schütz, K.E., 2008. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. Appl. Anim. Behav. Sci. 109, 141–154. https://doi.org/https://doi.org/10.1016/j.applanim.2007.03.015

van Schaik, G., Seinen, P., Muskens, J., van Erp, T., Keurentjes, J., Huss, A., Kromhout, H., 2021. Possible causes of aberrations in adverse grouping behavior of dairy cows: A field study. J. Dairy Sci. 104, 7000–7007. https://doi.org/10.3168/JDS.2020-19269

Vizzotto, E.F., Fischer, V., Thaler Neto, A., Abreu, A.S., Stumpf, M.T., Werncke, D., Schmidt, F.A., McManus, C.M., 2015. Access to shade changes behavioral and physiological attributes of dairy cows during the hot season in the subtropics. Animal 9, 1559–1566. https://doi.org/https://doi.org/10.1017/S1751731115000877

Weigele, H.C., Gygax, L., Steiner, A., Wechsler, B., Burla, J.B., 2018. Moderate lameness leads to marked behavioral changes in dairy cows. J. Dairy Sci. 101, 2370–2382. https://doi.org/10.3168/JDS.2017-13120

Figure captions:

Fig. 1.

The daily behavioural pattern of the first three principal components from grazing dairy cows depending on the $\mathrm{THI}_{\mathrm{max}}$ per day. Principal components (PC1, PC2, PC3) were calculated from the behavioural variables assessed in direct observations. Lines represent model estimates; shaded areas represent 95% confidence intervals. Dots show raw data. Four $\mathrm{THI}_{\mathrm{max}}$ values were selected for model estimates and visualization.

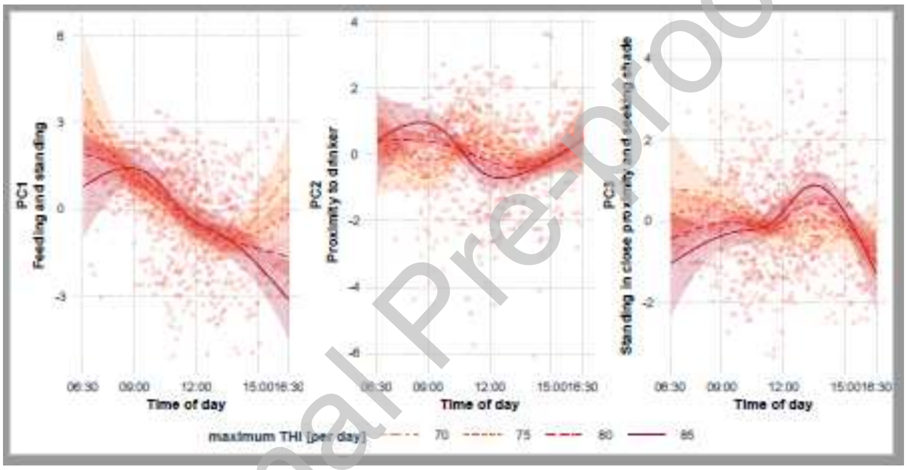


Fig. 2.

The daily pattern of proportion of lying dairy cows and locomotor activity on pasture depending on the $\mathrm{THI}_{\mathrm{max}}$ per day. Lying and activity were measured with accelerometers. Lines represent model estimates, shaded areas represent 95% confidence intervals. Six representative $\mathrm{THI}_{\mathrm{max}}$ values were selected for model estimates and visualization. Measurements in the afternoon have to be treated with caution as data on the time at which the cows were brought from the pasture into the barn were not available for every measurement day. It is therefore unclear whether the measurements have been made on pasture or in the barn. The unit *g* stands for gravitiy acceleration (9.81 m/s^2).

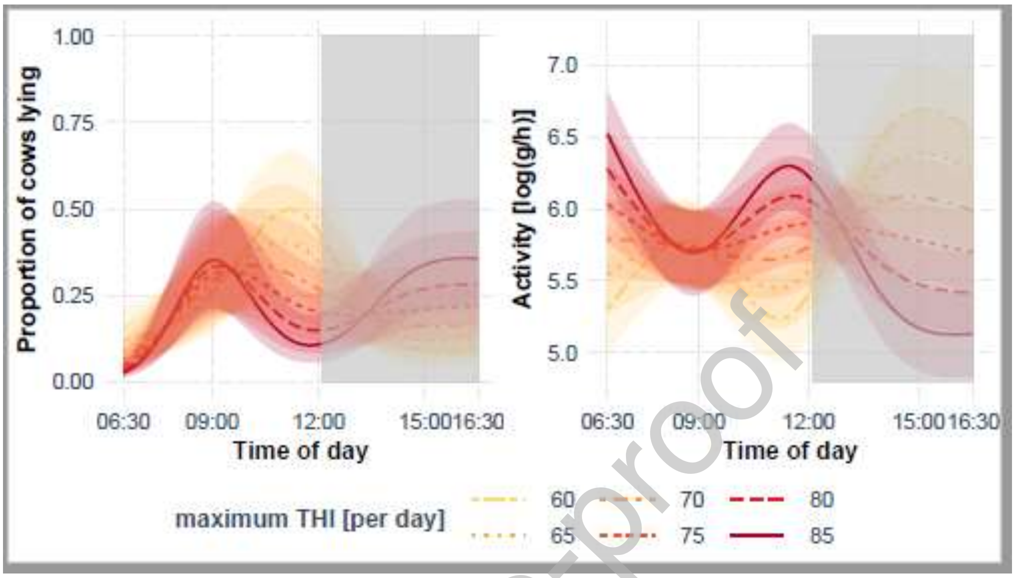
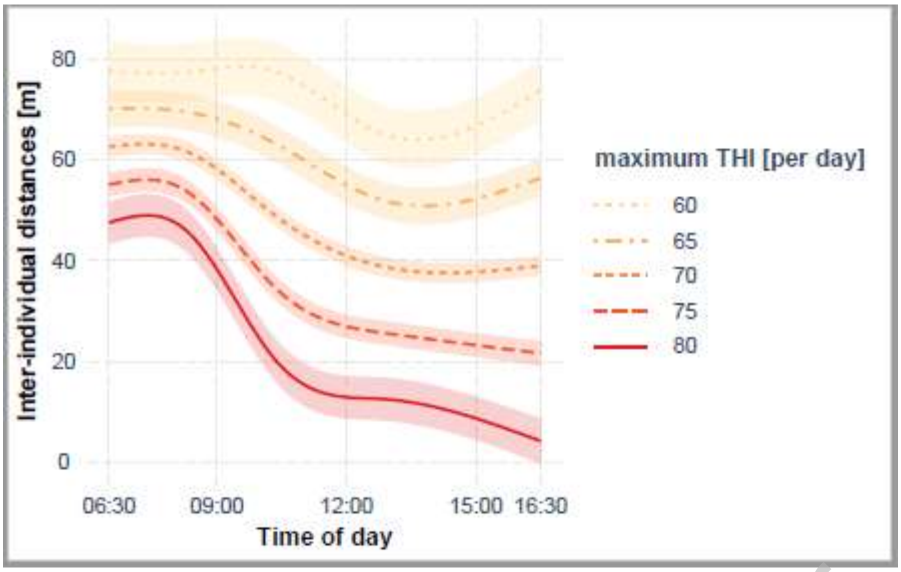


Fig. 3.

The daily pattern of inter-individual distances of grazing dairy cows depending on the THI$_{max}$ per day. Inter-individual distances were calculated from GPS coordinates (data from 49 cows, on one farm). Lines represent model estimates; shaded areas represent 95% confidence intervals. Five representative THI$_{max}$ values were chosen for model estimations and visualization.


Inter-individual distances [m]
80
60
40
20
0
06:30
09:00
12:00
15:00
16:30
Time of day
maximum THI [per day]
60
65
70
75
80

Statement regarding the experiment conducted for the publication “Behavioural changes to moderate heat load in grazing dairy cows under on-farm conditions” to be published in Livestock Science:

The experiment was approved by the Cantonal Veterinary Office (Granges-Paccot, Fribourg, Switzerland; approval no. 2018_04_FR). All measures complied with the national welfare legislation at all times.