



Cow individual activity response to the accumulation of heat load duration

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ABSTRACT

In the course of predicted climate change, the welfare of dairy cows and heat load to which they are exposed have become increasingly important even under moderate climate conditions. The objective of this study was to investigate the cow individual activity response to heat load in terms of the heat load duration and intensity in lactating, high-yielding Holstein-Friesian cows in a moderate climate zone.

The study was conducted from June 2015 to April 2017 in a naturally ventilated barn in Brandenburg, Germany. The determined temperature-humidity index (THI) inside the barn was used to define the heat load. The heat load was characterized by the average daily THI as well as the duration and intensity of the defined THI levels. In addition to the heat load on the measurement day, we studied the cow individual activity response to the heat load accumulated over the three days preceding the measurement day. The activity of the cows ($n = 196$) was measured by accelerometers and described the resting behavior and the number of steps per cow and day. The analysis models included autocorrelations in time series as well as individual cow factors.

An increase in the duration and intensity of heat load on the measurement day led to a decrease in the lying time and an increase in the number of steps. The cows showed a reduced activity response to heat load when there was additional heat load accumulation over the three days preceding the measurement day. The cows in an advanced stage of lactation were more sensitive to heat load than cows in the early lactation stage. Multiparous cows showed less pronounced activity responses than primiparous cows. Heat load accumulation and individual cow-related factors should be considered in prediction models for the sensitive animal-specific recognition of heat load on the basis of activity responses.

1. Introduction

In response to predicted climate change and the growing interest of consumers in livestock farming, the heat load experienced by dairy cows and their welfare have become one of the most important challenges facing the dairy industry. Cows are exposed to heat load conditions for several months of the year, even in the moderate climate of Central Europe (Gorniak et al., 2014; Heinicke et al., 2018; Lambertz et al., 2014). In the present study, the term “heat stress”, which has often been used in cited studies, was purposefully not used because of the absence of a clear definition for this term in the literature. “Heat load” describes the situation more clearly and precisely. The term refers to the influence of or exposure to heat from the environment. To reduce the adverse effects of heat load, it is important for farmers to know

when cows suffer from heat load and to correspondingly cool cows to help them effectively offload heat. Animal-based early warning systems could minimize the risk of heat load and optimize welfare by the automatic activation of cooling systems at an early stage of heat load. In addition to physiological thermoregulatory responses to heat load (de Andrade Ferrazza et al., 2017; Toušová et al., 2017), cows change their activity (Brzozowska et al., 2014; Endres and Barberg, 2007) to reduce the production of metabolic heat and to sustain their normal body temperature. Numerous studies have indicated that the activity of cows is a sensitive indicator of heat load. The studies found that the total daily lying time as well as the average duration of each lying bout decreased and the number of steps taken increased with increasing heat load (Brzozowska et al., 2014; Cook et al., 2007; De Palo et al., 2005; Endres and Barberg, 2007; Herbut and Angrecka, 2018b). The use of

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accelerometers for cows is already widespread, particularly because high peaks in activity are linked to estrus, and the data may help to determine the optimal time for insemination. Therefore, the use of additional activity data to detect possible signs of heat load is feasible and can be easily implemented on farms. In this respect, the absence of health disorders, such as lameness, must be ensured to avoid the misinterpretation of the measured results.

Currently, the temperature-humidity index (THI) at different thresholds is the standard method to define the intensity of heat load conditions. The most common are thresholds of 68 THI (Zimbelman and Collier, 2011) and 72 THI (Armstrong, 1994), which indicate the beginning of heat load based on an initial decrease in milk production. A study by Heinicke et al. (2018) determined that a similar heat load threshold (67 THI) led to changes in different activity traits in lactating, high-yielding dairy cows. Furthermore, this study revealed that the average daily THI, the heat load duration, and the accumulation of heat load on the measurement day should be considered to evaluate the heat load of cows. The heat load duration includes information on how long the cows are exposed to heat load per day. Furthermore, the daily heat load duration indirectly shows the variance in the average THI during the day, and conclusions can therefore be drawn about the heat load periods and recovery periods of the cows (Heinicke et al., 2018).

West et al. (2003) found that during a hot period, the heat loads two and three days preceding the measurement day had a greater impact on milk yield and dry matter intake than the values on the actual measurement day. In this context, it would be interesting to analyze the activity response following heat load accumulation over several days preceding the measurement day. The activity response to the accumulation of different intensities of heat load has not been studied thus far. Similar research was conducted only in the context of declining milk yields (Herbut et al., 2018).

Generally, the data obtained from activity monitoring systems should be compared with individual cow factors, such as days in milk and lactation number, because they influence the activity of individual cows (Bewley et al., 2010; Brzozowska et al., 2014; Maselyne et al., 2017). These factors also influence the metabolic heat production of the cows. Therefore, some cows might be more susceptible to heat load than others, and their activity changes in response to heat load may differ (Bernabucci et al., 2010; Das et al., 2016; Koch et al., 2016). Steensels et al. (2012) found various activity responses to heat load in dairy cows with different lactation numbers. Other studies concluded that milk production levels have an impact on the activity changes under heat load (Heinicke et al., 2018; Tapki and Şahin, 2006).

The objective of the present study was to investigate the activity response of lactating, high-yielding Holstein-Friesian cows in a moderate climate zone to heat load in terms of heat load duration and heat load intensity. Furthermore, we studied individual cow factors (e.g., milk production level, lactation number, and lactation stage) and

assumed that the duration and intensity of the heat load would influence the individual activity responses of the cows.

2. Materials and methods

2.1. Barn design and animals

The experimental farm was located in Brandenburg, Germany (approximately 56 km west of Berlin, coordinates: 52.4041666° N, 12.7791666° E, 32 m above sea level) and situated in a moderate climate zone between the maritime and continental climates (average annual temperature 9.9 ± 7.1 °C). The measurements were carried out in a naturally ventilated dairy barn with a loose housing system. The detailed barn design is described in the study by Heinicke et al. (2018). The herd consisted of 51 Holstein-Friesian cows (first to eighth lactation) that had an average daily milk yield of 40.7 ± 6.8 kg per cow. The average body weight per cow was 645 ± 28 kg.

2.2. Barn climate measurements

Climate data were recorded from June 2015 to April 2017. The ambient temperature and relative humidity within the barn were automatically measured every 10 min using EasyLog USB 2 + sensors (Lascar Electronics Inc., Erie, Pennsylvania, USA; temperature accuracy of ± 1 °C from -35 °C to +80 °C and relative humidity accuracy of ± 3.5% from 0% to 100% relative humidity in temperatures of -20 °C to +80 °C). The sensors were positioned at eight locations inside the barn (Heinicke et al., 2018) 3.4 m above the floor.

The ambient temperature and relative humidity data were used to calculate the THI for each measurement location and each time point. In this study, the following formula from the National Research Council (1971) was used to define the THI:

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26),$$

where T is the dry bulb temperature in °C, and RH is the relative humidity in %.

The THI calculations at all eight measurement locations were averaged afterwards, so there was one average THI value for the barn per time point (every 10 min). This resulted in 144 THI values per day to describe the climate conditions inside the barn. In addition, the heat load intensity (THI level) for each time point was categorized as follows: THI < 68 (no heat load), 68 ≤ THI < 72 (mild heat load), 72 ≤ THI < 80 (moderate heat load), and 80 ≤ THI (severe heat load) (Armstrong, 1994; Zimbelman and Collier, 2011). The estimated climate effects on the average daily THI and the heat load duration (accumulation of time in hours in a particular THI level over the previous three day period under consideration) per heat load intensity are described in Table 1. Heat load effects were classified as contemporaneous

Table 1
Definition of the estimated climate effects.

contemporaneous heat load effects	
THI _t	average daily temperature-humidity index (THI) on the measurement day (t)
HLD _t ^{THI[68,72]}	mild heat load duration (number of time points with 68 ≤ THI < 72 on the measurement day (t))
	moderate heat load duration (number of time points with 72 ≤ THI < 80 on the measurement day (t))
HLD _t ^{THI > 80}	severe heat load duration (number of time points with THI ≥ 80 on the measurement day (t))
delayed heat load effects	
THI _{t-1}	average daily THI one day preceding the measurement day (t-1)
THI _{t-2}	average daily THI two days preceding the measurement day (t-2)
THI _{t-3}	average daily THI three days preceding the measurement day (t-3)
HLD _{t-1,t-2,t-3} ^{THI[68,72]}	mean mild heat load duration on all three days preceding the measurement day, given that 68 ≤ THI < 72 on at least one time point on each of the three days (t-1, t-2, t-3)
HLD _{t-1,t-2,t-3} ^{THI[72,80]}	mean moderate heat load duration on all three days preceding the measurement day, given that 72 ≤ THI < 80 on at least one time point on each of the three days (t-1, t-2, t-3)
HLD _{t-1,t-2,t-3} ^{THI ≥ 80}	mean severe heat load duration on all three days preceding the measurement day, given that THI ≥ 80 on at least one time point on each of the three days (t-1, t-2, t-3)

Table 2
Definitions, acronyms and units for the behavioral traits used in the analysis.

behavioral trait	acronym	unit	definition
total lying time	LT	min d ⁻¹	minutes per day the cow is lying (the activity sensor recorded a horizontal position)
number of lying bouts	LB	n d ⁻¹	average number of lying bouts per day based on a continuous lying time of a minimum of 4 min
lying bout duration	LBD	min d ⁻¹	average duration of all lying bouts per day based on the exact start and end times
number of steps	NS	n d ⁻¹	summation of the number of times per day the cow lifts her leg based on the amount of force used

(on the measurement day) or delayed (three days preceding the measurement day).

2.3. Activity measurements

The activity of the dairy cows was recorded from June 2015 to April 2017. Approximately 43 cows per day were each equipped with one IceTag3D™ activity sensor (IceRobotics, Edinburgh, UK). More details on the activity measurements are described in a study by Heinicke et al. (2018). Because of the permanent fluctuations in incoming and outgoing cows within the experimental herd, data on the activity values for a total of 196 different cows were collected during the entire experimental period.

The analyzed activity traits included the total lying time (LT), the number of lying bouts (LBs), the lying bout duration (LBD), and the number of steps (NS) per day (Table 2). Previous studies focused on the validation of the IceTag3D™ activity sensor. Standing and lying were identified with high accuracy (Mattachini et al., 2013; Trénel et al., 2009). Comparison of the NS counted from video recordings with the step count provided by the IceTag3D™ sensor indicated a strong correlation when the cows were walking. When the cows were standing, the lifting of a leg would occasionally lead to a single step, but not always. Thus, the use of the step count directly from the IceTag3D™ does not give an accurate estimate of the number of steps taken while walking (Nielsen et al., 2010).

2.4. Statistical data analysis

For each activity trait, a (generalized) linear mixed model was used to test the influence of the contemporaneous and delayed heat load effects on the activity of the cows. In addition, the following individual cow factors were included in the models as a single effect and in combination with the effects that describe the heat load duration: milk production level (G ; $Milk_t^{normal}$; $Milk_t^{high}$), lactation number (L_t^1 ; $L_t^{2,3}$; $L_t^{\geq 4}$), days in milk (DIM_t^{1-60} ; DIM_t^{61-150} ; $DIM_t^{>150}$), pregnancy status (G_t^0 ; G_t^{1-90} ; G_t^{91-180} ; $G_t^{>180}$), and estrus status ($I_{t,t-1}^{estrus}$). The reference group included cows that were classified with all of the following factors: DIM_t^{1-60} , $Milk_t^{normal}$, G_t^0 , not in estrus. The models incorporated the random effects of the individual cows, and the within-cow temporal correlation structure was modeled by autoregressive-moving average (ARMA) processes. The variables were chosen based on the model diagnostics. The model can be written as follows:

$$y_{ijklmnt} = \mu + a \cdot THI_t + b \cdot THI_{t-1} + c \cdot THI_{t-2} + d \cdot THI_{t-3} + Milk_i + L_j + DIM_k + G_l + I_m + f_{ijkl} \cdot HLD_t^{THI \in [68,72]} + g_{ijkl} \cdot HLD_t^{THI \in [72,80]} + h_{ijkl} \cdot HLD_t^{THI \geq 80} + i_{ijkl} \cdot HLD_{t-1,t-2,t-3}^{THI \in [68,72]} + j_{ijkl} \cdot HLD_{t-1,t-2,t-3}^{THI \in [72,80]} + k_{ijkl} \cdot HLD_{t-1,t-2,t-3}^{THI \geq 80} + cow_n + e_{ijklmnt}$$

where.

$y_{ijklmnt}$ is the observed value of the activity trait;
 μ is the general mean;
 a, b, c, d are the regression coefficients for the average daily temperature-humidity index (THI) on the measurement day (t) and one ($t-1$), two ($t-2$), and three ($t-3$) days preceding the measurement day;

$Milk_i$ is the fixed effect of the i -th level of milk production;
 L_j is the fixed effect of the j -th number of lactation;
 DIM_k is the fixed effect of the k -th stage of lactation;
 G_l is the fixed effect of the l -th trimester of pregnancy;
 I_m is the fixed effect of the m -th stage of estrus;
 $f_{ijkl}, g_{ijkl}, h_{ijkl}, i_{ijkl}, j_{ijkl}, k_{ijkl}$ are the regression coefficients for the interactions of the individual cow factors with the heat load duration (HLD) on the measurement day (t) and in the three days before ($t-1, t-2, t-3$) per heat load intensity;
 cow_n is the random effect of the n -th cow;
and $e_{ijklmnt}$ is the random residual.

The null hypotheses for all tested traits were defined as the contemporaneous and delayed heat load effects as well as the individual cow factors having no effect on the activity traits of the dairy cows. The significance level for the (generalized) linear mixed model was 0.05. All analyses were performed using the free statistical software R version 3.4.2 (R Development Core Team, 2017). The linear mixed models were estimated using the lme function from the nlme package (Pinheiro et al., 2014). The generalized linear mixed models were estimated with the glmmPQL function from the MASS package (Ripley et al., 2018).

3. Results

3.1. Total daily lying time

The significant results of the linear mixed model for LT are presented in Table 3. The table indicates the strength of the impact of heat

Table 3

The significant results of the linear mixed model for the total daily lying time in seconds per day depending on the average daily temperature-humidity index (THI), daily heat load duration (HLD) per heat load level, milk production level (Milk), days in milk (DIM), lactation number (L), gestation status (G) and estrus days (I) with t = measurement day. Standard errors for the predicted coefficients are in parentheses.

Contemporaneous heat load effects and interactions	Individual cow factors
THI_t	$Milk_t^{low}$ 1141.4** (540.1)
$HLD_t^{THI \in [68,72]}$	DIM_t^{61-150} 133.7 (290.5)
$HLD_t^{THI \in [72,80]}$	$DIM_t^{>150}$ 1142.3*** (432.2)
$HLD_t^{THI \geq 80}$	$L_t^{\geq 4}$ 3115.0*** (1102.6)
$\times DIM_t^{61-150}$	1417.7*** (342.4)
$\times DIM_t^{>150}$	G_t^{91-180} 1088.8** (553.7)
Delayed heat load effects and interactions	$I_{t,t-1}^{estrus}$ -5506.7*** (282.9)
THI_{t-1}	109.1*** (13.4)
THI_{t-3}	19.1** (9.7)
$HLD_{t-1,t-2,t-3}^{THI \in [72,80]}$	30.2*** (6.9)
$\times L_t^{\geq 4}$	-25.7*** (9.6)
$HLD_{t-1,t-2,t-3}^{THI \geq 80}$	67.7** (27.4)
$\times L_t^{\geq 4}$	-80.5** (36.3)
	Intercept 41797.4*** (1016.5)

***p < 0.01, **p < 0.05 THI values: 99648 activity values: 22221

load effects and individual cow factors. Within the following sub-chapters, the impact of the individual cow factors, the contemporaneous and delayed heat load effects and the interaction effects between the heat load effects and individual cow factors are described.

3.1.1. Individual cow factors

Under conditions without heat load on the measurement day and the days before the measurement day,² the LT of the cows in the reference group (L_t^1 , $Milk_t^{normal}$, G_t^0 , not in estrus) was approximately 647 min (Fig. 1). If the characteristics of the cow differed from those of the cows in the reference group, the significant individual cow factors must also be taken into account to determine the estimated LT (Table 3). Low-producing cows were found to lie down more than cows with a normal or high level of milk production. The LT of $L_t^{\geq 4}$ cows increased by 52 min compared with the LT of cows in an earlier stage of lactation. Cows with $DIM_t^{\geq 150}$ laid down approximately 19 min longer than cows with less than 150 DIM (Fig. 1). This was also reflected by the increasing LT with increasing gestation status. Cows in estrus laid down significantly less than cows that were not in estrus.

3.1.2. Contemporaneous heat load effects

The average daily THI and the tested effects of heat load duration on the measurement day were negatively correlated with LT (Table 3). In addition to the general negative effect of THI, the LT in the herd decreased with the increasing intensity of the heat load. The longer the heat load duration on the measurement day was, the stronger this correlation was. The LT was 479 min (Fig. 1) for cows in the reference group with heat load on the measurement day and without heat load on the days before.³

3.1.3. Delayed heat load effects

In contrast to the contemporaneous heat load effects, the delayed heat load effects were positively related to LT. The LT of cows in the reference group increased to 568 min (Fig. 1) when there was heat load on the measurement day and additional heat load over the three days preceding the measurement day.⁴

3.1.4. Interaction effects between the heat load effects and individual cow factors

The contemporaneous heat load effects of mild and moderate heat load duration were independent of any individual cow factors, whereas the reduction in LT in response to severe heat load duration was dependent on the DIM. The predicted reduction in LT when the severe heat load duration was increased by 10 min was higher in cows with and $DIM_t^{\geq 150}$ than in cows with DIM_t^{1-60} (Table 3; Fig. 2(a)). This resulted in a reduction in the LT for cows with $DIM_t^{\geq 150}$ under heat load on the measurement day of approximately 194 min (reduction in the LT of cows in the reference group: 168 min) (Fig. 1).

The delayed heat load effects of moderate and severe heat load duration were dependent on the lactation number. The cows with $L_t^{\geq 4}$ reacted less strongly to increasing moderate heat load duration than the cows in earlier stages of lactation (Fig. 2(b)). In response to the delayed heat load effect of severe heat load duration, the cows with $L_t^{\geq 4}$ reduced their LT, whereas the other cows showed an increase in LT (Table 3, Fig. 2(c)). Consequently, LT increased only by approximately 51 min (increase in LT for cows in the reference group: 90 min) when there was an additional day with heat load over the three days preceding the measurement day (Fig. 1).

² $THI_t = 46$; $THI_{t-1} = 46$; $THI_{t-3} = 46$.

³ $THI_t = 73$; $THI_{t-1} = 46$; $THI_{t-3} = 46$; $HLD_t^{THI \in [68,72]} = 26$; $HLD_t^{THI \in [72,80]} = 56$; $HLD_t^{THI \geq 80} = 27$.

⁴ $THI_t = 73$; $THI_{t-1} = 70$; $THI_{t-3} = 69$; $HLD_t^{THI \in [68,72]} = 26$; $HLD_t^{THI \in [72,80]} = 56$; $HLD_t^{THI \geq 80} = 27$; $HLD_{t-1,t-2,t-3}^{THI \in [68,72]} = 43$; $HLD_{t-1,t-2,t-3}^{THI \geq 80} = 15$.

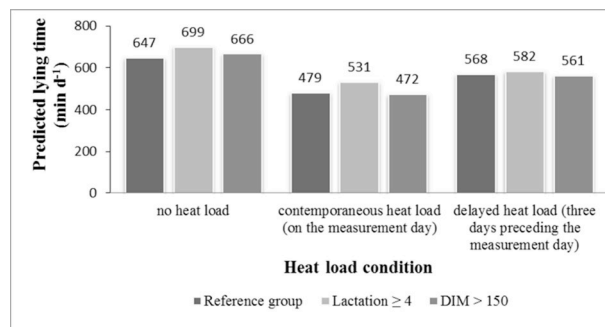


Fig. 1. Predicted total daily lying time (min d^{-1}) of cows in the reference group (L_t^1 , $Milk_t^{normal}$, G_t^0 , not in estrus) in lactation ≥ 4 and with DIM > 150 under different heat load conditions.

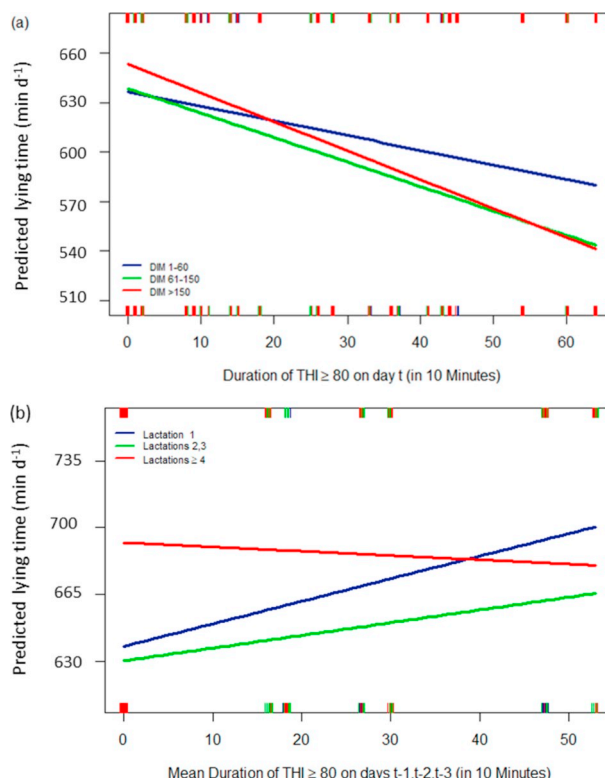


Fig. 2. Significant interactions in the linear mixed model for the daily lying time. Figure (a) shows the effects of the duration of severe heat load exposure for each lactation stage category. Figure (b) shows the effect of the mean duration of severe heat load exposure on all three days preceding the measurement day depending on the lactation number. The blue lines correspond to the respective reference group in the model. The colored lines at the x-axis indicate where measurement data are actually contained to model the estimated values.

3.2. Number of lying bouts

The significant results of the generalized linear mixed model for LBs are presented in Table 4. The model was estimated using a log link function. The table indicates the strength of the impact of the heat load effects and the individual cow factors. Within the following sub-chapters, the impact of the individual cow factors, the contemporaneous and delayed heat load effects and the interaction effects between the heat load effects and individual cow factors are described.

3.2.1. Individual cow factors

The cows in the reference group had a predicted number of LBs of approximately 15 bouts under conditions without heat load on the

Table 4

The significant results of the generalized linear mixed model for the logarithmized number of lying bouts per day depending on the average daily temperature-humidity index (THI), daily heat load duration (HLD) per heat load level, days in milk (DIM), lactation number (L) and estrus days (I) with t = measurement day. Standard errors for the predicted coefficients are in parentheses.

Contemporaneous heat load effects and interactions		Individual cow factors	
$HLD_t^{THI \in [68,72]}$	-0.0003** (0.0001)	DIM_t^{61-150}	-0.0670*** (0.0124)
$HLD_t^{THI \in [72,80]}$	-0.0004*** (0.0001)	$DIM_t^{>150}$	-0.0798*** (0.0182)
Delayed heat load effects and interactions		$L_t^{2,3}$	-0.2885*** (0.0437)
THI_{t-2}	0.0022*** (0.0004)	$L_t^{\geq 4}$	-0.1569*** (0.0530)
$HLD_{t-1,t-2,t-3}^{THI \in [68,72]}$	0.0004** (0.0002)	$I_{t,t-1}^{estrus}$	-0.0618*** (0.0106)
$HLD_{t-1,t-2,t-3}^{THI \geq 80}$	0.0033*** (0.0008)	Intercept	2.5991*** (0.0443)
$x L_t^{\geq 4}$	-0.0024** (0.0011)		

***p < 0.01, **p < 0.05 THI values: 99648 activity values: 22227

measurement day and on the days before.⁵ The DIM and lactation number individual cow factors had a significant effect on the number of LBs. Cows with and $DIM_t^{>150}$ laid down approximately 6.7% and 7.98% less often, respectively, than cows with DIM_t^{1-60} . In cows in $L_t^{2,3}$ and $L_t^{\geq 4}$, the number of LBs decreased by 28.85% and 15.69%, respectively, compared with the number of LBs in cows in L_t^1 . The cows in estrus laid down less frequently than the cows that were not in estrus (Table 4).

3.2.2. Contemporaneous heat load effects

The average daily THI had no significant effect on the number of LBs. However, the number of LBs was negatively affected by the heat load duration on the measurement day (Table 4). Each additional 10 min of mild and moderate heat load duration reduced the number of LBs by a small percentage. As a consequence, the number of LBs decreased to 14.4 bouts for cows in the reference group experiencing heat load on the measurement day but not on the days before.⁶

3.2.3. Delayed heat load effects

The delayed heat load effects of THI_{t-2} and mild and the severe heat load duration were positively related with the number of LBs (Table 4). This resulted in a predicted increase in the number of LBs to 16 bouts for cows in the reference group with heat load on the measurement day and additional heat load over the three days before the measurement day.⁷

3.2.4. Interaction effects between the heat load effects and individual cow factors

The contemporaneous heat load effects due to mild and moderate heat load were independent of any individual cow factors.

The delayed heat load effect of severe heat load duration depended on the lactation number. Cows in $L_t^{\geq 4}$ reacted less to increasingly severe heat load duration over the three days before the measurement day than cows in earlier stages of lactation (Fig. 3).

⁵ $THI_{t-2} = 46$.

⁶ $THI_{t-2} = 46$; $HLD_t^{THI \in [68,72]} = 26$; $HLD_t^{THI \in [72,80]} = 56$.

⁷ $THI_{t-2} = 71$; $HLD_t^{THI \in [68,72]} = 26$; $HLD_t^{THI \in [72,80]} = 56$; $HLD_{t-1,t-2,t-3}^{THI \in [68,72]} = 12$; $HLD_{t-1,t-2,t-3}^{THI \geq 80} = 15$.

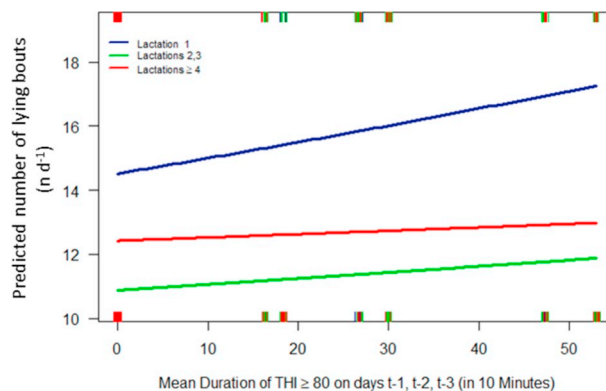


Fig. 3. Significant interactions in the generalized linear mixed model for the number of lying bouts. Figure shows the effects of the mean duration of severe heat load on all three days preceding the measurement day depending on the lactation number. The blue line shows the slopes for the respective reference group in the model. The colored lines at the x-axis indicate where measurement data are actually contained to model the estimated values.

3.3. Lying bout duration

The significant results of the linear mixed model for LBD are presented in Table 5. The model was estimated using a log link function. The table indicates the strength of the impact of the heat load effects and the individual cow factors. Within the following subchapters, the impact of the individual cow factors, the contemporaneous and delayed heat load effects and the interaction effects between the heat load effects and individual cow factors are described.

3.3.1. Individual cow factors

The predicted LBD of cows in the reference group without heat load on the measurement day and three days before⁸ was approximately 48.07 min (Fig. 4). Independent of the heat load, the individual cow factors had significant effects on the LBD (Table 5). Cows with more DIM had a longer LBD than those with fewer DIM. The LBD of cows with $DIM_t^{>150}$ increased by 5.28 min compared with the LBD of cows in the reference group (Fig. 4). Cows in $L_t^{2,3}$ and $L_t^{\geq 4}$ had a longer LBD compared with cows in L_t^1 . This was also reflected in the increasing LBD within the gestation status. During estrus days, LBD was shorter than when the cows were not in estrus.

3.3.2. Contemporaneous heat load effects

The average daily THI and the heat load duration on the measurement day were negatively correlated with LBD (Table 5). A longer duration of mild, moderate and severe heat loads resulted in a shorter LBD, in addition to the general negative effect of THI_t . The effect of heat load duration depended on the intensity of the heat load. The cows in the reference group had a reduced LBD of 39.31 min (Fig. 4) under heat load on the measurement day and without heat load the days before.⁹

3.3.3. Delayed heat load effects

The delayed heat load effects of THI_{t-1} and moderate heat load duration had a positive effect on LBD. In contrast, THI_{t-2} was negatively related to LBD (Table 5). The LBD of cows in the reference group increased slightly to approximately 40.32 min (Fig. 4) under heat load on the measurement day and additional heat load over the three days before.¹⁰

⁸ $THI_t = 46$; $THI_{t-1} = 46$; $THI_{t-2} = 46$.

⁹ $THI_t = 73$; $THI_{t-1} = 46$; $THI_{t-2} = 46$; $HLD_t^{THI \in [68,72]} = 26$; $HLD_t^{THI \in [72,80]} = 56$; $HLD_{t-1,t-2,t-3}^{THI \geq 80} = 27$.

¹⁰ $THI_t = 73$; $THI_{t-1} = 70$; $THI_{t-2} = 71$; $HLD_t^{THI \in [68,72]} = 26$; $HLD_t^{THI \in [72,80]} = 56$; $HLD_{t-1,t-2,t-3}^{THI \geq 80} = 27$; $HLD_{t-1,t-2,t-3}^{THI \in [72,80]} = 43$.

Table 5

The significant results of the linear mixed model for the logarithmized lying bout duration measured in seconds per day depending on the average daily temperature-humidity index (THI), daily heat load duration (HLD) per heat load level, milk production level (Milk), days in milk (DIM), lactation number (L), gestation status (G) and estrus days (I) with t = measurement day. Standard errors for the predicted coefficients are in parentheses.

Contemporaneous heat load effects and interactions		Individual cow factors	
THI_t	-0.0041*** (0.0005)	$Milk_t^{low}$	0.0100 (0.0206)
$HLD_t^{THI \in [68,72]}$	-0.0004*** (0.0001)	DIM_t^{61-150}	0.0682*** (0.0110)
$HLD_t^{THI \in [72,80]}$	-0.0009*** (0.0001)	$DIM_t^{> 150}$	0.1042*** (0.0164)
$HLD_t^{THI \geq 80}$	-0.0011** (0.0005)	$L_t^{2,3}$	0.2466*** (0.0346)
$x DIM_t^{61-150}$	-0.0016*** (0.0006)	L_t^{4}	0.2273*** (0.0424)
$x DIM_t^{> 150}$	-0.0017*** (0.0006)	G_t^{1-90}	0.0492*** (0.0130)
Delayed heat load effects and interactions		G_t^{91-180}	0.0579*** (0.0210)
THI_{t-1}	0.0025*** (0.0006)	$I_{t,t-1}^{estrus}$	-0.0805*** (0.0102)
THI_{t-2}	-0.0019*** (0.0005)		
$HLD_{t-1,t-2,t-3}^{THI \in [72,80]}$	0.0003** (0.0001)		
$HLD_{t-1,t-2,t-3}^{THI \geq 80}$	-0.0003 (0.0006)		
$x Milk_t^{low}$	-0.0027** (0.0012)		
		Intercept	8.1280*** (0.0377)

***p < 0.01, **p < 0.05 THI values: 99648 activity values: 22221

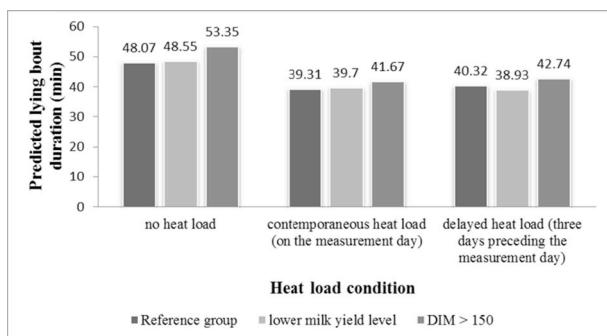


Fig. 4. Predicted lying bout duration (min) for cows in the reference group (L_t^1 , DIM_t^{1-60} , $Milk_t^{normal}$, G_t^0 , not in estrus) with a lower milk yield level and with $DIM > 150$ under different heat load conditions.

3.3.4. Interaction effects between the heat load effects and individual cow factors

The contemporaneous heat load effects of mild and moderate heat load duration were independent of any individual cow factors, whereas the reduction in LBD in response to severe heat load duration was dependent on DIM. The predicted reduction in LBD with increasing severe heat load duration by 10 min was higher in cows with DIM_t^{61-150} and $DIM_t^{> 150}$ compared to cows with DIM_t^{1-60} (Table 5; Fig. 5(a)). This resulted, for example, in a reduction of LBD for cows with by approximately 11.68 min (reduction of LBD for cows in the reference group: 8.76 min) under heat load on the measurement day (Fig. 4).

The delayed heat load effect of severe heat load duration was dependent on the milk production level. The most pronounced decrease in LBD with increasing severe heat load duration occurred for the low-producing cows (Table 5; Fig. 5(b)). Consequently, the LBD of low-producing cows only slightly decreased, whereas the LBD of the other cow groups increased when there was heat load on the measurement day and additional heat load over the three days preceding the measurement day (Fig. 4).

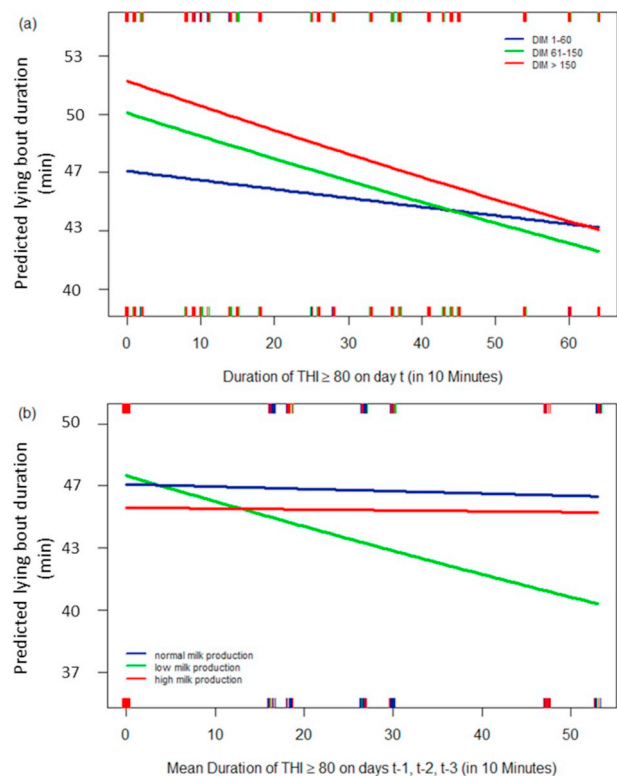


Fig. 5. Significant interactions in the linear mixed model for the lying bout duration. Figure (a) shows the effects of the duration of severe heat load exposure for each lactation stage category. Figure (b) shows the effect of the mean duration of severe heat load exposure on all three days preceding the measurement day depending on the milk production level. The blue lines show the slopes for the respective reference group in the model. The colored lines at the x-axis indicate where measurement data are actually contained to model the estimated values.

3.4. Number of steps

The significant results of the linear mixed model for NS are presented in Table 6. The model was estimated using a log link function. The table indicates the strength of the impact of the heat load effects and the individual cow factors. Within the following subchapters, the impact of the individual cow factors, the contemporaneous and delayed heat load effects and the interaction effects between the heat load effects and individual cow factors are described.

3.4.1. Individual cow factors

The cows in the reference group had an NS of approximately 2062 steps under conditions without heat load on the measurement day and on the days before¹¹ (Fig. 6). Independent of the climatic conditions, the individual cow factors had significant effects on the predicted NS (Table 6). The NS decreased with increasing DIM, increasing lactation number, and increasing gestational status. For example, the cows in $L_t^{2,3}$ took approximately fewer 665 steps than the cows in the reference group (Fig. 6). In contrast, the cows in estrus took significantly more steps than the cows not in estrus.

3.4.2. Contemporaneous heat load effects

In general, an increase in the average daily THI and the heat load duration on the measurement day led to an increase in the NS (Table 6). As a result, the NS increased to 2482 steps when the cows in the reference group were exposed to heat load on the measurement day

¹¹ THI_t = 46; THI_{t-1} = 46; THI_{t-2} = 46.

Table 6

The significant results of the linear mixed model for the logarithmized number of steps per day depending on the average daily temperature-humidity index (THI), daily heat load duration (HLD) per heat load level, milk production level (Milk), days in milk (DIM), lactation number (L), gestation status (G) and estrus days (I) with t = measurement day. Standard errors for the predicted coefficients are in parentheses.

Contemporaneous heat load effects and interactions		Individual cow factors	
THI _t	0.0013** (0.0006)	Milk _t ^{low}	-0.0218 (0.0216)
HLD _t ^{THIε[68,72]}	0.0014*** (0.0003)	DIM _t ⁶¹⁻¹⁵⁰	-0.0453*** (0.0112)
× L _t ^{2,3}	-0.0007** (0.0004)	DIM _t ^{>150}	-0.0609*** (0.0169)
× L _t ^{≥4}	-0.0012*** (0.0004)	L _t ^{2,3}	-0.1164*** (0.0365)
HLD _t ^{THIε[72,80]}	0.0015*** (0.0003)	L _t ^{≥4}	-0.3892*** (0.0448)
× G _t ^{>180}	0.0024** (0.0012)	G _t ¹⁻⁹⁰	-0.0509*** (0.0134)
× L _t ^{2,3}	-0.0006** (0.0003)	G _t ⁹¹⁻¹⁸⁰	-0.0532** (0.0219)
× L _t ^{≥4}	-0.0009** (0.0004)	G _t ^{>180}	-0.0928* (0.0498)
HLD _t ^{THI≥80}	0.0011*** (0.0003)	I _{t,t-1} ^{estrus}	0.5204*** (0.0118)
Delayed heat load effects and interactions			
THI _{t-1}	-0.0022*** (0.0007)	HLD _{t-1,t-2,t-3} ^{THIε[72,80]}	-0.0019*** (0.0003)
THI _{t-2}	0.0016** (0.0007)	× L _{t-3} ^{2,3}	0.0009*** (0.0003)
HLD _{t-1,t-2,t-3} ^{THIε[68,72]}	0.0001 (0.0003)	× L _{t-3} ^{≥4}	0.0020*** (0.0004)
× Milk _t ^{low}	0.0014*** (0.0005)	HLD _{t-1,t-2,t-3} ^{THI≥80}	-0.0013** (0.0005)
		Intercept	7.5993*** (0.0410)

***p < 0.01, **p < 0.05 THI values: 99648 activity values: 22221

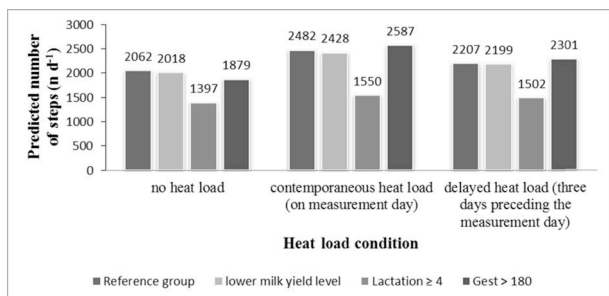


Fig. 6. Predicted daily number of steps (n d⁻¹) taken by cows in the reference group (L_t¹, DIM_t¹⁻⁶⁰, Milk_t^{normal}, G_t⁰, not in estrus) with lower milk yield level in lactation ≥ 4 and with Gest > 180 under different heat load conditions.

without heat load on the days before¹² (Fig. 6).

3.4.3. Delayed heat load effects

The delayed effects of THI_t and moderate and severe heat load duration were negatively correlated with the NS. However, THI_{t-2} was positively correlated with to the NS (Table 6). Consequently, the predicted NS for the cows in the reference group decreased to 2207 steps under heat load on the measurement day with additional heat load over the three days preceding the measurement day.¹³

3.4.4. Interaction effects between the heat load effects and individual cow factors

The contemporaneous heat load effects of mild and moderate heat load duration were dependent on the lactation number. The cows in and L_t^{≥4} showed a smaller increase in the NS with increasing heat load duration on the measurement day (Table 6) than primiparous cows. In contrast, cows in G_t^{>180} reacted more strongly to moderate heat load duration; the cows in L_t^{≥4} increased their NS by approximately 153 steps, whereas the cows in G_t^{>180} increased their NS by 708 steps under heat load on the measurement day (increase in the NS taken by cows in the reference group: 420) (Fig. 6).

¹² THI_t = 73; THI_{t-1} = 46; THI_{t-2} = 46; HLD_t^{THIε[68,72]} = 26; HLD_t^{THIε[72,80]} = 56; HLD_t^{THI≥80} = 27.

¹³ THI_t = 73; THI_{t-1} = 70; THI_{t-2} = 71; HLD_t^{THIε[68,72]} = 26; HLD_t^{THIε[72,80]} = 56; HLD_t^{THI≥80} = 27; HLD_{t-1,t-2,t-3}^{THIε[72,80]} = 43; HLD_{t-1,t-2,t-3}^{THI≥80} = 15.

The delayed heat load effect of mild heat load duration was dependent on the milk production level, and the effect of moderate heat load duration was dependent on the lactation number. When the mild heat load duration increased over the three days preceding the measurement day, only the low-producing cows responded and increased their NS (Table 6; Fig. 7 (a)). In response to the delayed heat load effect of moderate heat load duration, the cows in L_t^{2,3} reacted less strongly and exhibited a lower NS than the cows in the reference group. Under the same conditions, the cows in L_t^{≥4} even showed a slight increase in the NS (Fig. 7 (b)).

4. Discussion

In the present study, cow individual activity responses to heat load in terms of the heat load duration and intensity were investigated. A large number of climate measurements inside the barn were collected to represent the climate conditions over a long period of almost two years. The wide range of climate differences inside a naturally ventilated barn necessitates several measurement points in the barn (Hempel et al., 2018; Schüller and Heuwieser, 2016). For long-term measurement, we collected data on a large number of heat load days (number of days with THIε[68,72], 202 d; THIε[72,80], 116 d; THI≥80, 6 d). Therefore, the heat load conditions were modeled accurately. The corresponding activity data of the cows were recorded for an average of seven months per cow. The management of the dairy barn unfortunately made it impossible to record longer data sets per cow.

4.1. Individual cow factors

The results of the present study indicated that individual production-related cow factors influence the activity of cows independent of barn climatic conditions.

The LT increases with increasing days in milk (Bewley et al., 2010; Endres and Barberg, 2007; Gomez and Cook, 2010; Maselyne et al., 2017) and increasing lactation number (Stensels et al., 2012). Bewley et al. (2010) recognized a trend of decreasing LT with increasing milk production. This finding was confirmed by the significantly higher LT of cows with lower milk production levels than that of cows with higher milk production levels in the present study. An increase in the lactation number resulted in decreased LB and increased LBD, which was confirmed by the results of Brzozowska et al. (2014). Moreover, it has been

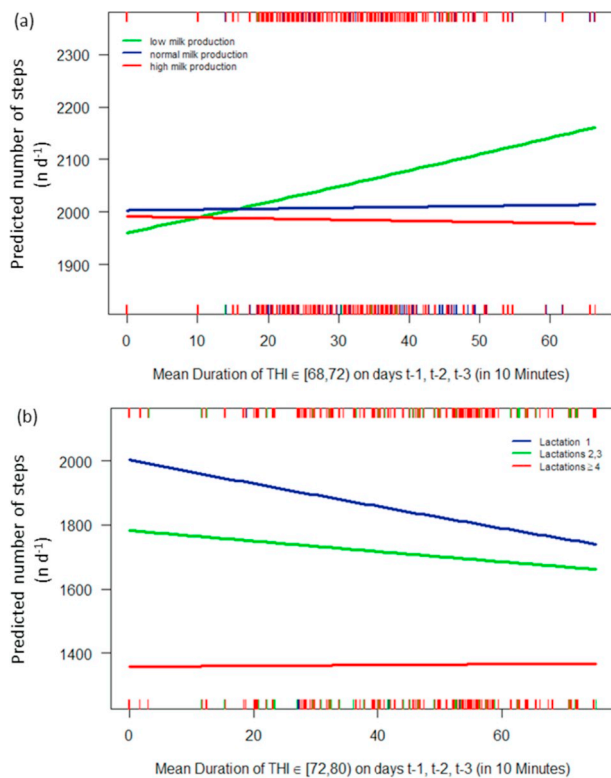


Fig. 7. Significant interactions in the linear mixed model for the number of steps. Figure (a) shows the effect of the mean duration of mild heat load exposure on all three days preceding the measurement day depending on the milk production level. Figure (b) shows the effect of the mean duration of moderate heat load exposure on all three days preceding the measurement day for each lactation number. The blue lines show the slopes for the respective reference group in the model. The colored lines at the x-axis indicate where measurement data are actually contained to model the estimated values.

verified that the LB and NS decrease, but LBD increases with an increasing number of days in milk (Brzowska et al., 2014; Steensels et al., 2012). Furthermore, it was already known that cows react by increasing their NS and decreasing their LT during their days in estrus (Arney et al., 1994; Firk et al., 2002). It is particularly important to take this into account when changes in activity are interpreted.

Our results once again confirmed how important it is to evaluate the activity of individual groups of cows on the basis of different cow factors. In future studies, it would be conceivable to analyze the activity response of each individual cow with machine learning algorithms or image recognition procedures to monitor the daily activity pattern of the cows.

4.2. Contemporaneous heat load effects

The present study investigated the activity of dairy cows and the influence of the average daily THI and the effects of heat load duration and intensity on the measurement day. The main advantage of this study is the novel inclusion of how long the cows had been exposed to heat load per day and how strong that heat load was.

The effect of the average daily THI indicated activity responses of the cows similar to those described in previous studies. The decrease in LT observed during heat load conditions agrees with findings from the literature (Cook et al., 2007; Endres and Barberg, 2007; Herbut and Angrecka, 2018a). The same result was observed by Brzowska et al. (2014) and Steensels et al. (2012) and decreased LT was reported during the summer months. A decrease in LT with increasing heat load duration on the measurement day was observed. This observation confirmed our previous results (Heinicke et al., 2018), in which the

activity responses increased with increasing daily THI, and the longer the heat load duration on the measurement day lasted, the stronger this correlation became. This study was based on the same dataset but included other models with different tested effects. Heinicke et al. (2018) determined heat load thresholds of the average daily THI that led to changes in different activity traits and examined the influence of increases in the daily heat load duration on the activity traits. The present study analyzed the activity in more detail with the help of auto-correlations in time series, different heat load intensities, heat load accumulation over several days, and individual cow factors. The reason for the decrease in LT under heat load is that a standing posture exposes a larger surface area of the skin to the surrounding air than a lying posture does, and, consequently, a greater heat dissipation rate can be achieved (Bouraoui et al., 2002; Hillman et al., 2005).

The less pronounced activity response of the number of LBs to heat load agreed with the findings reported by Endres and Barberg (2007) and by Zähler et al. (2004). According to Brzowska et al. (2014) and Steensels et al. (2012), the number of LBs was not associated with the season.

As a result of the decreased LT and constant number of LBs during heat load, the LBD decreased as the heat load increased (Brzowska et al., 2014; De Palo et al., 2005; Endres and Barberg, 2007).

The increase in the NS with increasing heat load, which is perhaps an indication of restlessness and stress, agrees with the findings of Endres and Barberg (2007). Similar results were observed in studies by Brzowska et al. (2014) and Steensels et al. (2012), who found that the NS was higher in summer than in the winter months. Further studies indicated that increased activity during heat load increased body temperature (i.e., increased the heat load), which led to an increase in standing time (Allen et al., 2015), and according to the results of Cook et al. (2007), the time spent standing in the alley increased. A more recent paper by Abeni and Galli (2017) also noted an increase in the activity index during heat load conditions. An increase in the time spent standing or walking might be an important behavioral thermoregulation strategy that allows the cows to increase the movement of air around their body. Since cows lose a considerable amount of their body heat from their underside, they may reduce heat load by standing or walking (Tucker and Schütz, 2009).

The contemporaneous heat load had a large direct effect on the resting behavior and activity since cows seek immediate relief from unpleasant environmental conditions. The activity traits influenced by the different heat load durations and intensities on the measurement day showed the same correlations as the effect of the average daily THI on the measurement day. However, it would be possible to make more precise predictions concerning the activity under heat load if additional information on the heat load duration at different intensities was available.

4.3. Delayed heat load effects

Our approach to analyze the activity response after heat load accumulation over several days preceding the measurement day is innovative and has not been performed before. The results therefore cannot be compared to data from the literature. An exception is a study by Herbut et al. (2018), who analyzed long-term heat waves in the context of milk performance. Our findings show that the accumulation of heat load over the three days preceding the measurement day led to less pronounced activity responses on the measurement day than conditions with heat load only on the measurement day. This indicates that with the accumulation of persistent heat load, the cows become exhausted and exhibit signs of tiring, which implies a compensatory effect on the initial activity response of cows to heat load, even if they are still exposed to the heat load. We assume that when the cows were exposed to heat load on all three days preceding the measurement day, they could not further increase their activity response beyond a limit and had adapted on the measurement day.

Previous studies that analyzed the reactions of cows subjected to lying deprivation illustrated the motivation for increased lying and the compensatory reactions (Cooper et al., 2007; Norring and Valros, 2016). They recognized that the cows recovered some of their lost lying time by rescheduling feeding and standing times. These new results confirm that the contemporaneous climate conditions as well as the delayed climate conditions significantly affected the activity response of dairy cows.

4.4. Interaction effects between the heat load effects and individual cow factors

The lactating, high-yielding Holstein-Friesian cows examined in the present study individually reacted with a more or less pronounced activity response to the heat load conditions inside the barn. The study carried out by Purwanto et al. (1990) demonstrated that heat production in cows with high milk productivity is greater than that in other groups. For this reason, differences in metabolic activity and hormonal control are mentioned.

The results of the present study suggested that the cows in an advanced stage of lactation reacted more strongly by decreasing LT and the LBD than the cows in an early stage of lactation. Generally, cows in an advanced stage of lactation have a longer LT and a longer LBD than other cows under climate conditions without heat load, but the differences decrease as the exposure time to heat load increases. Another study found similar results concerning activity and milk yield, in which cows in the early phase of lactation were less susceptible to heat load than cows in a more advanced phase. This is probably because the former partially rely on body energy mobilization for milk yield, where the latter rely only on dry matter intake, which is particularly depressed during heat stress (Abeni and Galli, 2017).

The multiparous cows showed a smaller increase in the NS with contemporaneous heat load effects compared with the primiparous cows. It is possible that a higher body weight, larger number of claw horn lesions, and higher ranking could explain the decreased activity of multiparous cows compared to that of primiparous cows. Steensels et al. (2012) also included the effect of lactation number in their analysis and found that the number of LBs was significantly higher in summer (11.2 n d^{-1}) than in winter (10.8 n d^{-1}) for dairy cows in their second lactation. However, the number of LBs was significantly lower in summer (11 n d^{-1}) than in winter (11.4 n d^{-1}) for cows with three or more lactations. These findings could not be confirmed in the present study but still emphasize the individual cow activity responses to heat load.

In a previous study, we found that cows with higher milk production levels reacted most sensitively by decreasing the LT to increasing heat load to a greater extent than cows with normal and lower milk production levels (Heinicke et al., 2018). This is consistent with the conclusions of Tapkı and Şahin (2006), who found that high-producing dairy cows were more sensitive to resting activity than low-producing dairy cows.

Individual cow activity responses to heat load must be investigated more intensively in further studies to identify the most suitable indicator animals that show early and clear activity responses to heat load. Coat color and genetic parentage could possibly be included in further studies.

5. Conclusions

Our study confirmed that an increase in the duration and intensity of heat load led to a decrease in the LT and an increase in the NS. In addition to earlier studies, we included a novel assessment of the influence of delayed heat load effects and individual cow factors under heat load on dairy cow activity. The cows showed a reduced activity response to heat load when there was additional heat load accumulation over the three days preceding the measurement day. Our study found cow individual activity responses to heat load. Primiparous cows

and cows in an advanced stage of lactation were the most sensitive to heat load. Heat load accumulation as well as individual cow factors should be considered in predictive models for the sensitive animal-specific recognition of heat load based on activity responses.

Conflicts of interest

The authors declare no conflict of interest.

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