

## The effect of different levels of heat stress on the behaviour of cows at different stage of lactation

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### ABSTRACT

Rising temperatures and humidity increasingly challenge the welfare and productivity of dairy cows worldwide. Behavioural changes are among the earliest responses to heat stress, reflecting animals' efforts to reduce heat load. This study investigated how heat stress and lactation stage interact to influence the behaviour of Holstein Friesian cows. Data were collected from 60 cows between 20 June and July 20, 2024, with 1680 records of eating, ruminating, and inactivity times. Cows were grouped by lactation stage: early (days in milk, (DIM)  $\leq 60$ ), mid (DIM 61–180), and late (DIM  $>180$ ). Behaviour was assessed under moderate (temperature–humidity index, (THI) 72–76) and severe heat stress (THI  $\geq 77$ ). A significant interaction was observed between the level of heat stress and lactation stage. Under severe heat stress, eating time declined significantly across all lactation groups, with the largest reduction observed in late-lactation cows. Ruminating time decreased significantly only in late-lactation cows, while remaining relatively stable in early and mid-lactation. Conversely, inactivity time increased in all groups, with the duration of inactivity rising as lactation advanced. These results suggest that behavioural responses to heat stress intensify with advancing lactation. In conclusion, late-lactation cows are more susceptible to heat stress than those in earlier stages, indicating a need for targeted mitigation strategies to support cow welfare and productivity.

### 1. Introduction

Climate change, along with its associated heat stress, is an undeniable and persistent challenge that continues to impact various ecosystems. As global temperatures rise and extreme temperature fluctuations become more frequent in temperate climates, heat stress has emerged as a significant and growing challenge in dairy farming, particularly affecting dairy cows (Henry et al., 2018; Polsky and von Keyserlingk, 2017). In fact, some projections suggest that global temperatures could increase by the year 2100 from 3.3 °C to 5.7 °C (Lee et al., 2023). This rise in temperature not only negatively impacts animal welfare and productivity but also weakens immune systems (Dahl et al., 2020). It can cause a decline in milk quality, reducing the levels of lactose, protein, and fat, including beta-casein, as well as a lower milk quantity (Chen et al., 2024; Cowley et al., 2015; Dunn et al., 2014). According to Khan et al. (2023) reproductive parameters are negatively impacted by low oocyte development and semen quality, resulting in a 20–30 % decrease

in conception rates due to heat stress. Additionally, it alters body metabolism and affects blood parameters; some hormones and enzymes, such as cortisol and insulin, increase as a reaction to heat stress, which further weakens the immune system (Aggarwal and Upadhyay, 2013; Lemal et al., 2023). The behaviour of cows during heat stress conditions was also studied by previous researchers. For example, according to Hoffmann et al. (2020) as a reaction to heat stress effect, cows show a variety of behavioural changes, such as changes in eating and activity. Further, Holinger et al. (2024) suggest that changes in behaviour could provide a valuable metric to evaluate heat stress effects. Several behaviours are considered signs of heat stress, such as prolonged standing, shade-seeking, and decreased activity, mobility, and lying period (Allen et al., 2015; Schütz et al., 2008). Cows employ two main strategies to improve respiratory efficiency and optimize body surface area; decreasing the duration of resting and extending the period of remaining upright. These actions help facilitate both sensible and insensible heat loss. This helps reduce heat transfer from warm surfaces when lying

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down (Anderson et al., 2013). Furthermore, the feeding behaviour of cows changes under heat stress, with reduced feed intake to limit heat produced from digestion by adjusting feeding time and the size and the length of the meal. At the same time, they increase water consumption and likely remain near the water trough (Ammer et al., 2018; Pontiggia et al., 2024). Another response to thermal stress observed in previous research is crowding behaviour, where cows gather closely and reduce the distance between one another (Polsky and von Keyserlingk, 2017; Pontiggia et al., 2024). Animals at various biological stages suffer as a result, potentially leading to substantial financial losses for farmers (Baccouri et al., 2023; Collier et al., 2019; Hansen, 2009; McManus et al., 2020). According to Key et al. (2014), for instance, estimates indicate that heat stress results in nearly \$1.5 billion in annual losses in lactating cows in the United States. Dairy cattle are particularly vulnerable to high levels of humidity and temperatures, especially given the production of considerable internal heat resulting from the energy demands of milk production (Coppock, 1985; Purwanto et al., 1990). Prolonged staying under high temperatures and humidity might lead to this internal heat to become excessive in cows. Heat stress develops as the combined impact of external warmth and the animal's internal activity surpasses its ability to maintain temperature balance, causing an elevation in internal body temperature as explained by Bernabucci et al. (2010). A composite metric known as the temperature-humidity index (THI) often evaluates humidity and temperature conditions (Chen et al., 2024). Berry et al. (1964) later subsequently modified the THI, which was used first by Thom (1959) to evaluate thermal comfort in humans, to measure thermal stress in dairy cows. THI effectively combines humidity and temperature to create a single indicator of climatic stress, particularly useful in understanding heat stress in productive animals. Previous researchers have mentioned a strong link between THI and bodily responses, such as elevated core body temperature and breathing rates in lactating cows under thermal stress (Thom, 1959; Yan et al., 2021). Thus, researchers frequently use THI to assess the incidence and severity of heat stress, as this index reflects the climatic conditions affecting cows' comfort and health (Armstrong, 1994; Dikmen and Hansen, 2009; Kadzere et al., 2002). Based on recent research, the critical THI thresholds are typically defined as 72–76 for the onset of moderate heat stress and  $\geq 77$  for the onset of severe heat stress (Ciliberti et al., 2024; Rodriguez-Venegas et al., 2023). As THI surpasses a cow's tolerance limit, thermal strain develops, negatively affecting its productivity and welfare (Azevedo et al., 2024; Shin et al., 2022; Stefanska et al., 2024). Thermal stress significantly impacted cows regardless of their lactation stage. Tao et al. (2018) observed that cattle subjected to thermal stress, compared to those kept in cooler conditions, produced less milk during early, mid, and late stages of lactation. Previous research indicates that mid-lactation cows experience greater adverse effect from hot weather than those in early and late lactation, as they exhibit lower milk production (Abeni et al., 2007; Basiricò et al., 2009; Bernabucci et al., 2010; Maust et al., 1972; Perera et al., 1986). Conversely, McDowell et al. (1976) mention that cows at the beginning of lactation experience a significantly greater reduction in gross efficiency when exposed to prolonged high temperatures (above 27 °C) compared to those in mid and late lactation. Furthermore, Abeni & Galli (2017) report that cows at the start of the lactation show a marked decrease in rumination time compared to other lactation stages under heat stress, which increase their vulnerability to thermal stress. In contrast, Müssner-Siemens et al. (2020) observed that a substantial reduction in the duration of the process of rumination can be caused by heat stress, approximately 65 min, in cows in advanced lactation phases ( $>150$  DIM) compared to other lactation stages. Moreover, Leliveld et al. (2023) reported that, at elevated THI levels, late-lactation cows exhibit increased respiratory effort relative to early- and mid-lactation cows, indicating heightened sensitivity towards heat during this phase. The debate about at which stage of lactation the cows are more vulnerable to heat stress conditions has always ensued among researchers. As a result, this study aims to, investigate the interactive effect of lactation stage and

heat stress on the behaviours of Holstein Friesian cows. This will enhance the management of dairy farms in the contemporary climate change era.

## 2. Material and methods

### 2.1. Cows

A total of 60 cows were selected and divided into three groups: 20 cows at the beginning of lactation DIM ( $\geq 0$  and  $\leq 60$ ) ( $27.9 \pm 15.2$ ), 20 cows at mid-lactation DIM ( $<90$  and  $\leq 180$ ) ( $137.5 \pm 30$ ), and 20 cows at the end of lactation DIM  $180 >$  ( $269 \pm 30$ ). Cows were housed under the same condition in a free stall barn. The barn had no active cooling system during the experiment, cows were kept under naturally ventilated conditions, with open sides that allowed air circulation. This setup was chosen to reflect typical commercial farm conditions and to ensure that the behavioural responses were not influenced by artificial cooling interventions. This experiment took place over one month in the summer, from June 20 to July 20, 2024 with 1680 records of eating, ruminating, and inactivity times. The cows were provided with a total mixed ration (TMR) as their primary feed, with the same daily ration throughout the experiment, formulated to meet their nutritional needs. The TMR consisted of a combination of alfalfa silage, corn silage, alfalfa hay, molasses, and concentrate. The feed was distributed twice daily, typically at 4:00 am and 1:00 p.m., to ensure fresh availability and encourage consistent intake. The cows were milked 3 times per day at 4:00 a.m., 11:00 a.m. and 5:00 p.m.

### 2.2. Behaviour

The behaviour of cows was monitored using the Nedap SmartTag Neck (Nedap Livestock Management, Groenlo, the Netherlands; Velos software version 2024.1 LTS), recording each behavioural parameter in minutes at 15-min intervals. The data were aggregated to provide daily totals for each of the three behaviours, measured in minutes per day. This new technology, confirmed by previous researchers (Borchers et al., 2021; Quddus et al., 2022), for its accuracy in detecting feeding, rumination, and inactivity behaviour, has also been shown to be more precise than video recording and visual observation. The measured behaviours included the time cows spent eating, ruminating, and being inactive. Eating measurements were determined by the precise head motions of the cow while ingesting feed. The measurement of rumination was based on the subsequent mastication of the bolus and the rhythmic motion of the jaw during regurgitation, regardless of whether the cow was in a supine or upright posture. Inactive time was defined as intervals during which animals were standing or lying down still, without engaging in walking, ruminating, feeding, or other activities. Each tag transmitted the NT data to base stations within the barn vicinity every 15 min and then sent to Nedap servers, where the datasets were generated.

### 2.3. Meteorological data

Temperature and humidity were collected on a daily basis inside the barn by using sensors. The calculation of a temperature-humidity index was performed using the method reported by Dikmen et al. (2008):

$$THI = (1.8 \times T + 32) - (0.55 - 0.55 \times RH / 100) \times (1.8 \times T - 26)$$

Where:

T = air temperature (°C)

RH = relative humidity (%)

The THI thresholds adopted in this study were based on previous literature describing the responses of dairy cows under different heat loads. Moderate heat stress was defined as  $72 \leq THI < 77$ , and severe heat stress as  $THI \geq 77$ , following the classifications proposed by Rodriguez-Venegas et al. (2023) and Ciliberti et al. (2024). Under

naturally occurring environmental conditions, behavioural records were classified into heat stress categories according to the daily THI measurements. Days with THI values within the moderate heat stress range were assigned to the moderate heat stress group, whereas days exceeding this range were classified as severe heat stress. This classification resulted in 840 behavioural records in each group, reflecting the natural variation in thermal conditions during the study period, without any experimental manipulation of environmental factors.

#### 2.4. Statistical analysis

Several models, including linear and linear mixed models, were tested to identify the best fit for the data. The model with the lowest Akaike Information Criterion (AIC) value was selected. This final model, meeting all assumptions, was implemented using the lmer function (Bates et al., 2015) from the lme4 package in R software (version 4.4.1, 2024) (Team, 2020). Each response variable (eating time, ruminating time, and inactive time) was modelled separately against the explanatory variable, Temperature-Humidity Index (THI), along with its interaction with lactation stage. To account for the effects of individual variability and parity on behaviour, cow ID and parity were included as random factors in the model. Day was not included as a repeated measure, as behavioural data were recorded as daily totals for each cow. The main objective was to evaluate the interactive effects of heat stress and lactation stage on cow behaviour. The single-factor effects of all parameters were assessed using the following equation:

$$Y_{ijk} = \beta_0 + \beta_1 HS_i + \beta_2 (HS_i \times CC_j) + u_{1k} + u_{2l} + \epsilon_{ijk}$$

Where:

$Y_{ijk}$  = Response variable for the observation of the heat stress status and stage of lactation (eating, ruminating, inactivity).

$\beta_0$  = Intercept

$\beta$  = Regression coefficients for the individual fixed effects ( $\beta_1$ : Coefficient for heat stress ( $HS_i$ ),  $\beta_2$ : Coefficient for the interaction term ( $HS_i \times CC_j$ ))

$HS_i$  = Fixed effect of the heat stress status

$CC_j$  = Fixed effect of the stage of lactation.

$(HS_i \times CC_j)$  = Fixed effect of the interaction between the heat stress

and the stage of lactation.

$u_{1k}$  = Random effect of the cow ID (to account for individual variations).

$u_{2l}$  = Random effect of the parity (to account for variations due to differences in parity).

$\epsilon_{ijk}$  = Residual error term.

A post hoc Tukey test was performed using the estimated marginal means (emmeans) package (Lenth, 2016) in R to assess the differences between the groups, and significance was declared at  $P < 0.05$ . In addition to the categorical analysis of heat stress, we performed a regression analysis treating THI as a continuous variable to examine the gradient effect of heat stress on cow behaviours.

### 3. Results

We observed significant ( $p < 0.001$ ) difference in cows' behaviour between severe and moderate heat stress conditions (Fig. 1). Under severe heat stress, cows spent significantly less time eating, with a reduction of 53.5 min/day (95 % CI [49.5, 57.5],  $p < 0.0001$ ) compared to moderate heat stress. A similar trend was observed in ruminating time, which decreased by 17.9 min/day (95 % CI [12.0, 23.8],  $p < 0.0001$ ), reflecting a significant reduction under more stressful conditions. In contrast, inactive time, defined as periods when cows were standing or lying down without movement, increased significantly by 77 min/day (95 % CI [69.7, 84.3],  $p < 0.0001$ ), indicating reduced activity levels under severe heat stress. To further explore the effect of heat stress as a continuous gradient, we analysed the change in behaviour per unit increase in THI. Each unit increase in THI was associated with a significant decrease in eating time of 6.72 min/THI unit (95 % CI [-7.24, -6.19],  $p < 0.0001$ ). Ruminating time also decreased by 1.84 min/THI unit (95 % CI [-2.63, -1.06],  $p < 0.0001$ ). In contrast, inactive time increased by 9.21 min/THI unit (95 % CI [8.23, 10.2],  $p < 0.0001$ ). These findings suggest that increasing heat stress consistently reduces time spent on feeding activities while increasing resting time across all lactation stages.

#### 3.1. Eating behaviour

Eating behaviour decreased significantly ( $p < 0.001$ ) with increasing severity of heat stress across all lactation stages (Fig. 2). Under moderate

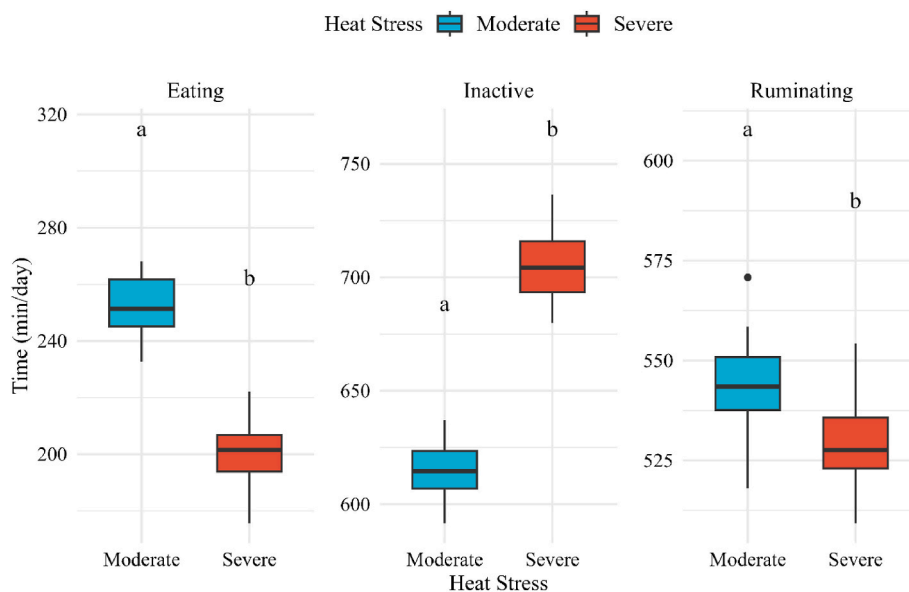
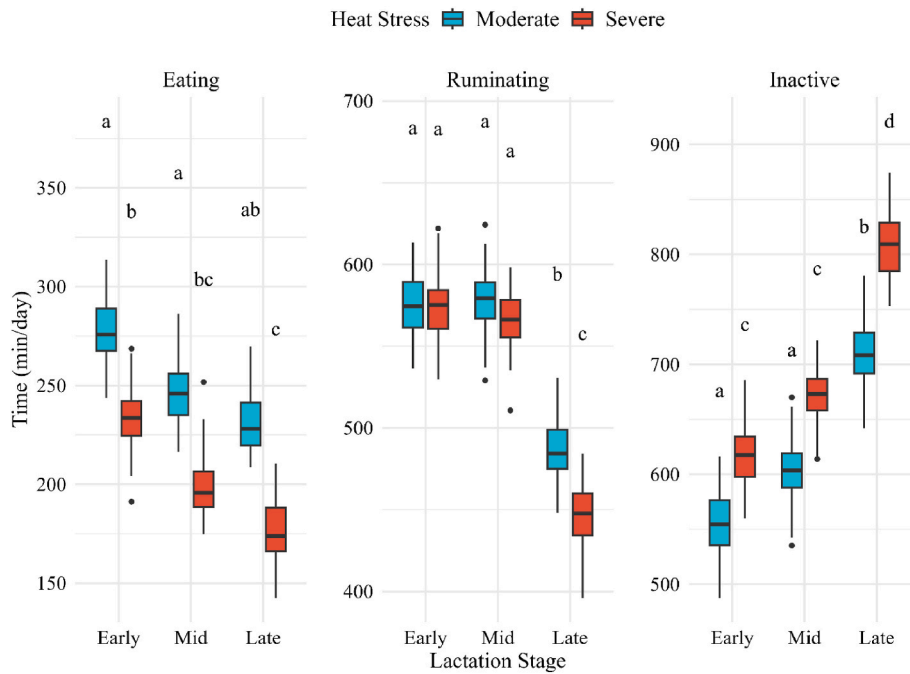


Fig. 1. Box plots illustrating the effect of heat stress on the daily total time spent eating, ruminating, and inactive behaviours, expressed in minutes per day (min/day). The horizontal line within each box represents the median, and the box represents the interquartile range (25th–75th percentiles). Different superscripts (a, b) within each behaviour indicate significant differences ( $p < 0.05$ ).



**Fig. 2.** Box plots illustrating the interaction between heat stress and lactation stage on the daily total time spent eating, ruminating, and inactive behaviours, expressed in minutes per day (min/day). The horizontal line within each box represents the median, and the box represents the interquartile range (25th–75th percentiles). Different superscripts (a, b, c, d) within each behaviour indicate significant differences ( $p < 0.05$ ). Each lactation stage included 20 cows (sample size  $n = 20$ ).

heat stress, eating time was 277 min/day for cows in early lactation, 251 min/day for mid-lactation, and 232 min/day for late lactation. Under severe heat stress, these values decreased to 231 min/day, 197 min/day, and 172 min/day, respectively with no significant difference between early and mid-lactation, nor between mid and late lactation. The reduction in eating time under severe heat stress was 45.8 min/day for early lactation (95 % CI [52.7, 38.9],  $p < 0.0001$ ), 54.7 min/day for mid-lactation (95 % CI [61.6, 47.8],  $p < 0.0001$ ), and 59.9 min/day for late lactation (95 % CI [66.8, 53.0],  $p < 0.0001$ ).

### 3.2. Ruminating behaviour

We observed variation in ruminating behaviour in response to heat stress across lactation stages (Fig. 2). Under moderate heat stress, ruminating time was 575 min/day for early-lactation cows, 579 min/day for mid-lactation cows, and 486 min/day for late-lactation cows. Under severe heat stress, ruminating time remained unchanged for early-lactation cows at 575 min/day, but decreased to 561 min/day for mid-lactation cows and to 449 min/day for late-lactation cows. Early-lactation cows showed no significant change (0.3 min/day, 95 % CI [9.9, 10.5],  $p = 0.9995$ ), mid-lactation cows had a non-significant reduction of 17.9 min/day (95 % CI [28.1, 7.7],  $p = 0.051$ ), and late-lactation cows exhibited the largest and significant reduction of 37.4 min/day (95 % CI [-47.6, -27.2],  $p < 0.0001$ ).

### 3.3. Inactive behaviour

Inactive behaviour increased significantly ( $p < 0.05$ ) with the progression of heat stress severity across all lactation stages (Fig. 2). Under moderate heat stress conditions, inactive time was 559 min/day for cows in early lactation, 596 min/day for those in mid-lactation, and 709 min/day for those in late lactation. With the onset of severe heat stress, these values rose significantly ( $p < 0.05$ ) to 620 min/day, 670 min/day and 804 min/day respectively. Inactivity time increased significantly under severe heat stress, particularly in late-lactation cows. Early-lactation cows showed a significant increase of 61.1 min/day (95 % CI

[48.4, 73.8],  $p < 0.0001$ ), while mid-lactation cows also showed a significant increase of 74.1 min/day (95 % CI [61.4, 86.8],  $p < 0.0001$ ). The largest increase was observed in late-lactation cows, with an increase of 94.7 min/day (95 % CI [82.0, 107.4],  $p < 0.0001$ ).

To further explore the effect of heat stress as a continuous gradient, we analysed the change in behaviour per unit increase in THI across lactation stages (Table 1). For eating behaviour, THI had a significant negative effect across all lactation stages, with the reduction becoming more pronounced as lactation progressed: a decrease of 5.9 min/THI unit in early lactation, 6.7 min/THI unit in mid-lactation, and 7.5 min/THI unit in late lactation. Ruminating behaviour showed a significant decrease only in late lactation by 4.6 min/THI unit, with no significant effect in early and mid-lactation. Inactive behaviour increased significantly with THI across all stages, with the largest effect in late lactation 12.1 min/THI unit, followed by mid-lactation 8.4 min/THI unit and early lactation 7.2 min/THI unit. These findings complement the categorical analysis by revealing a progressive impact of heat stress, particularly on late-lactation cows.

**Table 1**

Changes in cow behaviour (minutes per unit increase in temperature–humidity index, (min/THI unit)) across different lactation stages. Values represent estimated changes (min/THI unit) with 95 % confidence intervals (CI) and associated p-values. Negative values indicate a decrease in behaviour duration with increasing THI. Each lactation stage included 20 cows (sample size  $n = 20$ ).

Behaviour	Lactation Stage	(min/THI unit)	95 % CI	p-value
Eating	Early lactation	-5.9	[-6.8, -5.0]	<0.001
	Mid lactation	-6.7	[-7.6, -5.8]	<0.001
	Late lactation	-7.5	[-8.4, -6.6]	<0.001
Ruminating	Early lactation	0.3	[-1.0, 1.7]	0.636
	Mid lactation	-1.2	[-2.6, 0.1]	0.076
	Late lactation	-4.6	[-6.0, -3.3]	<0.001
Inactive	Early lactation	7.2	[5.5, 8.9]	<0.001
	Mid lactation	8.4	[6.7, 10.0]	<0.001
	Late lactation	12.1	[10.4, 13.8]	<0.001

#### 4. Discussion

In this study, we aimed to investigate how heat stress impacts behaviour of cows at different stages of lactation. With global temperatures projected to rise due to climate change, particularly in regions with a continental climate such as Hungary, understanding these effects is essential. The insights gained from this research could guide approaches to alleviating heat stress in dairy cows, thereby improving both animal welfare and productivity in heat-affected environments. Moreover, identifying specific behavioural changes in cows under heat stress can enable farmers to implement targeted interventions to alleviate discomfort and maintain optimal health. This research has the potential to provide valuable contributions to the agricultural industry, supporting the well-being of dairy cows in a changing climate.

The present findings indicate that heat stress significantly reduces eating time in dairy cows, particularly as its severity increases (THI  $\geq 77$ ), which aligns with previous studies (Antanaitis et al., 2024; Cook et al., 2007; Holinger et al., 2024; Leliveld et al., 2025; Lovarelli et al., 2024), that reported reductions in ruminating, eating, and walking times under severe heat stress compared to moderate conditions. This behavioural adaptation, manifested as reduced eating time, may be explained, on one hand, by the need to limit metabolic heat production and thermogenesis by minimising internal heat generated during eating, serving as a protective mechanism to maintain thermal balance (Qu et al., 2015). On the other hand, they may reflect physiological alterations induced by heat stress. Specifically, heat stress modifies the endocrine profile, including elevated levels of leptin, a hormone that acts on the hypothalamus to suppress appetite and promote hypermetabolism, both of which negatively impact feed intake (Bernabucci et al., 2006; Caruso et al., 2023; Morera et al., 2012; Skoracka et al., 2025). Concurrently, heat stress has been associated with decreased circulating levels of ghrelin, a hormone known to stimulate appetite by activating orexigenic neurons in the hypothalamic arcuate nucleus (Ioannis et al., 2021). Leptin and ghrelin thus exert opposing effects on energy intake regulation, with heat stress tipping the balance toward appetite suppression.

The present findings show that the rumination time decreases with increasing intensity of heat stress. These results are consistent with previous research showing a decrease in rumination duration as the THI increases (Acatincai et al., 2009; Lovarelli et al., 2024; Moallem et al., 2010; Ramón-Moragues et al., 2021). This reduction likely reflects an effort to lower internal heat production from rumen fermentation (Ando et al., 1997). This is supported by Mader and Davis (2004), who noted that a significant portion of metabolic heat is generated during the fermentation of roughage in the rumen, which raises the body temperature of cows. Physiologically, rumination is a key driver of saliva production, which introduces buffering agents into the rumen. Heat-stressed cows often have reduced feed intake and diminished rumination activity, leading to a lower influx of these critical buffering substances (Ramón-Moragues et al., 2021; Vermunt, 2021). This reduction in rumination time could also be associated with decreased eating time, based on our results and also as noted by Moretti et al. (2017). Additionally, an inverse relationship exists between respiratory rate and rumination duration, as heat-stressed cows often exhibit increased panting as a thermoregulatory response, which further contributes to reduced rumination (Paudyal, 2021; Soriani et al., 2013). Furthermore Heat-stressed cows exhibit diminished blood flow to the digestive tract (Rfús, 2019), altered ruminal microbial populations (Correia Sales et al., 2021), reduced ruminal motility (Abeni and Galli, 2017), and decreased digesta fractional passage rates, thereby increasing the risk of ruminal acidosis (Meneses et al., 2021). Consequently, in response to these physiological changes, animals reduce their roughage intake, which further diminishes rumen motility and rumination (Nardone et al., 2010).

In the current findings, the duration of inactivity significantly increased with the increase of the severity of heat stress. Antanaitis et al.

(2024), Herbut and Angrecka (2018), Lovarelli et al. (2024), Leliveld et al. (2025) reported similar findings, observing increased inactivity under heat stress conditions. Increased energy cost related to movement explains this increase in inactivity. For example, dairy cows expend approximately 55 J per kg<sup>0.75</sup> per meter during movement, which, for a 600-kg cow walking 1 km, equates to around 7 MJ of net energy (Shephard and Maloney, 2023). Since a large portion of this metabolised energy is converted into heat, physical activity significantly impacts heat balance and, consequently, the cows' well-being (Sørensen, 2011). Furthermore, according to Coulon et al. (1998), walking alone can raise by up to +1 °C the temperature of the body, further demonstrating the thermal impact of exercise on ruminants. As a result of these physiological challenges, cattle respond to elevated temperatures by altering their behaviour. Specifically, they reduce walking time, extend the time spent upright and reduce the resting period (Polsky and von Keyserlingk, 2017). This behavioural shift can be seen as a natural strategy to mitigate heat stress. Moreover, standing plays a particularly important role in this adaptation, as it serves a dual purpose. Not only does it minimize heat production by avoiding physical exertion, but it also enhances heat dissipation. By increasing skin surface exposure, standing facilitates evaporative, convective, and radiant heat transfer, helping cows maintain their comfort in hot conditions (Allen et al., 2015; Tapkı and Şahin, 2006; West, 2003).

In terms of behaviour of cows at various lactation phases, we found a significant interaction between the stage of lactation and THI categories for all studied behaviours. with late-lactation cows showing greater reductions in eating time under severe heat stress (THI  $\geq 77$ ) compared to early and mid-lactation cows no significant difference between early and mid-lactation, nor between mid and late lactation. This aligns with Leliveld et al. (2023) and Chen et al. (2024) who noted similar vulnerabilities linked to reduced dry matter intake and milk yield in late lactation. Early- and mid-lactation cows allocate more time to feeding and less to resting compared to late-lactation cows, driven by their high energy demands for milk production (Churakov et al., 2021; Gross, 2023). This physiological drive mitigates the behavioural impact of heat stress, resulting in smaller reductions in feeding time, as these cows prioritize nutrient intake to sustain milk yield (Kanjanaputhipong et al., 2015; Zhao et al., 2019). In contrast, late-lactation cows produce less milk and, under normal conditions, maintain stable feed intake to support body condition restoration (Baumgard and Rhoads Jr, 2013). Under severe heat stress, however, they reduce feeding time and chewing activity more markedly to minimize ruminal heat production, as their milk production is low (Collier et al., 2006). Furthermore, late-lactation cows exhibit higher respiratory rates under heat stress compared to early- and mid-lactation cows (Leliveld et al., 2023; Tresoldi et al., 2025), which may further reduce feeding time, as increased panting competes with eating behaviour (Soriani et al., 2013). In early lactation, cows have lower leptin and higher ghrelin levels, which promote increased feed intake to meet the energy demands of milk production (Ketaby and Mohammad-Sadegh, 2023; Nowroozi-Asi et al., 2016). In late lactation, leptin levels are higher, and ghrelin levels are lower, reflecting reduced energy needs. Under heat stress, these hormonal profiles are altered, potentially increasing leptin or decreasing ghrelin, leading to reduced appetite (Ioannis et al., 2021; Turk et al., 2024). This effect may be more pronounced in late lactation cows, where the natural drive to eat is weaker, possibly contributing to greater reductions in feeding time compared to early lactation cows. Mid-lactation cows, characterised by moderate energy requirements, exhibit intermediate feeding times, reflecting a balance between competing physiological demands (Allen, 2023). This intermediate pattern arises from their transitional physiological state; although their milk yield remains substantial, it is in decline, requiring more feeding effort than late-lactation cows to meet residual energy needs (Gross and Bruckmaier, 2019). However, they retain greater flexibility than early-lactation cows to reduce feed intake for thermoregulation without severely compromising production. Their hormonal profile, marked by a moderate rise in leptin and a partial

decline in ghrelin, leads to less appetite suppression under heat stress compared to cows in late lactation (Nowroozi-Asi et al., 2016; Turk et al., 2024). Additionally, mid-lactation cows tend to experience less respiratory stress than those in late lactation (Leliveld et al., 2023), reducing behavioural conflict between thermoregulation and feeding. This combination of moderate metabolic drive, transitional hormonal regulation, and lower respiratory competition places mid-lactation cows in a physiological middle ground, which might explain why their feeding time under severe heat stress did not differ significantly from that of cows in early or late lactation.

Ruminating time was lower for late lactation cows under moderate and with high reduction under severe heat stress compared to other lactation stages. These results match the observations of Müschner-Siemens et al. (2020), who reported a reduction in ruminating time for late lactation cows as THI increased. Our results may be explained by the fact that late-lactation cows exhibit higher respiratory rates under heat stress compared to early- and mid-lactation cows (Leliveld et al., 2023; Tresoldi et al., 2025). An inverse relationship has been observed between respiratory rate and rumination duration, as heat-stressed cows often display increased panting as a thermoregulatory response. This leads to significant saliva loss due to open mouth breathing and drooling, which further contributes to reduced rumination (Paudyal, 2021; Soriani et al., 2013; Vermunt, 2021). This physiological change is less pronounced in early- and mid-lactation cows, where the drive to ruminate remains strong due to higher energy demands. Furthermore, as highlighted by Moretti et al. (2017), a reduction in eating time is closely associated with a decline in ruminating activity, given the interdependence of these behaviours in maintaining rumen function and overall digestive health. In our study, cows in late lactation exhibited the most significant decrease in eating time compared to those in early and mid-lactation. This marked reduction in feeding behaviour may, in turn, have contributed to a greater decline in ruminating time. Additionally, rumination is positively associated with milk production, as higher-yielding cows require greater fibre intake and rumen activity to support their metabolic demands (Gross, 2023). Late-lactation cows typically produce less milk than those in early or mid-lactation, and this decline in productivity is often accompanied by a natural reduction in rumination time (Krpalkova et al., 2022). Under heat stress, this relationship may be further amplified, contributing to a more pronounced behavioural response in late-lactation cows.

Inactivity time was higher for cows in late lactation compared to those in early and mid-lactation. Our outcomes align with Heinicke et al. (2019), that late-lactation cows are more sensitive to heat load based on their changes in activity. Under severe heat stress, late lactation cows exhibit lower activity compared to early and mid-lactation cows, likely as a strategy to conserve energy and minimize metabolic heat production. This behavioural adaptation can be attributed to several physiological factors. Consistent with our findings of greater inactivity time in late-lactation cows under severe heat stress, these cows typically exhibit start to build body reserve, which, while beneficial for maintaining energy reserves, poses a challenge under heat stress due to the insulating effect of excess adipose tissue (Cincović et al., 2011). This fat layer impedes effective heat dissipation from the body core to the skin surface, making late-lactation cows particularly vulnerable to severe heat stress (Shephard and Maloney, 2023). In contrast, early- and mid-lactation cows, with lower body reserve and higher metabolic demands to sustain peak milk production, tend to remain more active to support feeding and other behaviours despite heat stress (Roche et al., 2007). Furthermore, severe heat stress requires a major redirection of blood flow to the skin to support heat dissipation and an increased respiratory rate through panting (Krishnan et al., 2023). These physiological responses place enormous strain on the cardiovascular system (Habeeb et al., 2018). This strain may be even greater in cows with higher reserve, such as those in late lactation, compared to early and mid-lactation. Their larger body size and fat insulation can lead to underlying cardiovascular stress (Ünal and Uztimur, 2024). Prolonged heat exposure also

contributes to dehydration and electrolyte imbalances, which further burden the cardiovascular system (Mohammadifard et al., 2019). In this context, standing or moving places additional demands on circulation. In contrast, increased inactivity, such as lying down, reduces the cardiovascular load associated with posture and movement (Frondelius et al., 2015). This allows the body to redirect limited resources toward core thermoregulation, including panting and vasodilation. Additionally, late-lactation cows show the greatest reduction in eating time under heat stress. Since a major driver of cow activity is feeding behaviour, including walking to the feed bunk, eating, ruminating, and social competition at the bunk, their significantly reduced intake directly translates into less time spent on feeding-related activities, contributing substantially to overall inactivity. For late-lactation cows, however, increasing inactivity becomes a critical behavioural strategy to minimize internal heat production, as their reduced capacity for heat dissipation limits active thermoregulatory behaviours.

Cows in late lactation were more vulnerable to heat stress compared to those in early or mid-lactation under severe heat stress, showing the greatest decrease in eating time, ruminating time, and activity time. These results align with the meta-analysis conducted by (Chen et al., 2024; Heinicke et al., 2019). This susceptibility may be explained by the fact that cows in late lactation often have higher body reserve compared to those in early or mid-lactation (Arote and Siddiqui, 2020; Friggens and Badsberg, 2007; Roche et al., 2007). The increased body fat in that stage of lactation can make these cows more vulnerable to heat stress, as adipose tissue acts as an insulator, reducing heat dissipation efficiency and potentially leading to higher internal body temperatures during periods of heat stress (Brown-Brandl et al., 2006; Cincovic et al., 2011). Piccioli-Cappelli et al. (2014) observed that late-lactation cows prioritised replenishing body tissues over milk production when exposed to thermal stress. This finding aligns with Moe (1981) observation that the effectiveness of dietary energy for fat accumulation in the body is higher during late lactation (approximately 75 %) compared to the dry period (60 %). While a significant proportion of dietary energy is allocated to milk production during earlier lactation stages, relatively more energy is directed towards body tissue deposition during late lactation (Zhang et al., 2016). This redistribution of energy reflects the changing physiological priorities of cows as they progress through their lactation cycle, driving them to find alternative solutions, such as behavioural changes, to reduce internal heat. Additionally, this result could also be influenced by hormonal changes across lactation stages. Prolactin, which has a vital part in thermoregulation by enhancing peripheral vasodilation and sweat gland activity (Ladyman et al., 2025; Langan, 2024), decreases as lactation advances (Lacasse et al., 2019). Prolactin also regulates the transport of electrolytes and water across various fluid compartments, including the kidney, stomach, and mammary gland. Similarly, cortisol, a key stress hormone, tends to decline in late lactation (Cotticelli et al., 2024; Saqib et al., 2022), potentially reducing the cow's capacity to manage heat stress, as cortisol specifically promotes gluconeogenesis, the metabolic process that converts non-carbohydrate precursors, such as amino acids, into glucose, providing an immediate energy source and increasing blood glucose levels (Brenner et al., 1998; Ma et al., 2023; Thau et al., 2019). Although hormonal mechanisms such as increased prolactin and cortisol may contribute to these behavioural changes, these variables were not directly measured in the present experiment. Furthermore, this could be influenced through the increased generation of internal heat connected to the progression of gestation (Casarotto et al., 2025; Ferrell et al., 1976). Sguizzato et al. (2020) demonstrated that the rise in heat production may be less significant from the mammary gland than from the gravid uterus, which produces more heat, particularly as the foetus uses substantial amounts of amino acids for energy (Bowman et al., 2021), which treis linked to increased heat generation linked to the metabolism of carbohydrates (Musharaf and Latshaw, 1999; Popson et al., 2023). According to Ouellet et al. (2021) the temperature of the foetus is largely influenced by that of the mother, as the foetus has limited self-regulation capacity. Foetal temperature is

usually slightly higher than maternal temperature, exceeding it by less than 1 °C in most mammalian species, such as humans (Asakura, 2004; Lubkowska et al., 2019; Mota-Rojas et al., 2023). Calculations estimate 3 W kg<sup>-1</sup> as the foetal heat production, approximately double the resting heat output of an adult (Edwards et al., 2003). Heat transfer occurs via the umbilical circulation, as well as through the uterine wall, amniotic fluid and foetal skin. The umbilical circulation typically responsible for dispersing around 85 % of the heat with only 10–20 % dissipated via amniotic fluid (Kasiteropoulou et al., 2020; Schroder and Power, 1997) This could drive cows to adopt alternative strategies to reduce heat stress, such as lowering eating, ruminating, and activity levels.

Our findings, which indicate that late-lactation cows exhibit greater reductions in eating, ruminating, and activity times under severe heat stress (THI ≥77) compared to early- and mid-lactation cows, contrast with previous studies that reported higher susceptibility in early- and mid-lactation stages (Abeni et al., 2007; McDowell et al., 1976; Bernabucci et al., 2010). These earlier studies primarily focused on declines in milk production, whereas our study emphasises behavioural responses. Further research that simultaneously evaluates both behavioural and production parameters is needed to provide a more comprehensive understanding of how heat stress affects cows at different lactation stages.

The possible limitations of the study include that data collection was restricted to one month, whereas a longer period would better capture behavioural variations. Furthermore, direct physiological measurements, such as dry matter intake, body temperature, or hormone levels, were not included, limiting interpretation of the underlying physiological mechanisms. Additionally, while THI was primarily analysed categorically for interpretability, a continuous regression was performed to explore gradient effects, providing a more nuanced view of heat stress impacts. Despite these possible limitations, the findings offer important insights into the behavioural responses of lactating cows under naturally occurring heat stress conditions.

## 5. Conclusion

The current study showed that heat stress affects the behaviour of cows. As the severity of heat stress increases (THI ≥77), eating time, ruminating time, and activity time decrease in response to elevated heat stress levels. The effect of heat stress interacts significantly with the stage of lactation. Under moderate heat stress, there is no significant difference in eating time between lactation stages. However, under severe heat stress, late-lactation cows show significantly lower eating time compared to early-lactation cows. Ruminating time and activity time are significantly lower for late-lactation cows under both moderate and severe heat stress compared to cows in early and mid-lactation. Interestingly, as lactation advances, cows exhibit a greater reduction in eating, ruminating, and activity times under severe heat stress compared to other lactation stages. In conclusion, cows in late lactation are more susceptible to heat stress than those in early and mid-lactation, highlighting their need for additional support to mitigate heat stress. These results may contribute to refining management strategies and environmental modifications to mitigate heat stress in dairy barns located in hot climates. Although the late lactation stage is often considered less critical than earlier stages, implementing enhanced management practices, particularly heat stress mitigation strategies such as providing energy-dense feed or shifting feeding to cooler parts of the day could help maintain nutritional intake. Additionally, combine feeding strategies with cooling measures shade, ventilation, fans, sprinklers to further reduce heat stress and its impact on appetite, could improve future cow performance and reduce health complications. Further research involving more samples and an expanded range of behavioural parameters are necessary to develop a clearer insight into how heat stress interacts with cow behaviour.

## CRedit authorship contribution statement

**Wissem Baccouri:** Writing – original draft, Software, Conceptualization. **George Wanjala:** Validation, Software, Formal analysis. **Violetta Tóth:** Investigation, Data curation. **István Komlósi:** Writing – review & editing, Validation. **Edit Mikó:** Validation, Supervision, Resources, Methodology.

## Data availability statement

The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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