

Theses of Doctoral (PhD) Dissertation

**INVESTIGATION OF HEAT STRESS TOLERANCE OF
HOLSTEIN FRIESIAN COWS**

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1. INTRODUCTION

Climate change poses one of the most serious challenges to global agriculture, profoundly affecting animal production systems and farm sustainability. The increasing frequency of heat waves and prolonged periods of high temperature are particularly detrimental to dairy production, where heat stress has become a major concern for animal welfare and productivity (HENRY ET AL., 2018; THORNTON ET AL., 2022). Global temperatures are projected to rise by 3.3°C to 5.7°C by the end of the century (LEE ET AL., 2023), and even in temperate climates, dairy cows are increasingly exposed to environmental conditions that exceed their thermoregulatory capacity.

Heat stress represents a major challenge for dairy production due to the limited ability of cows to dissipate excess body heat. It reduces milk yield, alters metabolic homeostasis, impairs reproductive performance, and compromises animal welfare (BERNABUCCI ET AL., 2014; POLSKY AND VON KEYSERLINGK, 2017). While the effects of heat stress in arid and subtropical regions have been extensively studied, less is known about the impacts of short-term and seasonally fluctuating heat stress in temperate production systems. Coat colour may influence heat absorption, yet its interaction with heat stress on metabolic and milk traits remains largely unexplored. Similarly, seasonal temperature variation and its effects on blood metabolic indicators require further investigation to identify early markers of thermal stress. Reproductive performance is also highly sensitive to heat stress; however, the most critical periods relative to insemination and the associated thermal thresholds remain unclear. In addition, behavioural responses to heat stress, including changes in feeding, lying, and activity, vary across lactation stages, but it is not yet established which stage exhibits the greatest heat sensitivity. Finally, the long-term consequences of early-life heat stress on first-lactation performance and resilience have not been systematically evaluated.

This doctoral research aims to address these knowledge gaps in order to provide practical insights for managing heat stress in Holstein Friesian cows, improve animal welfare, and support productivity under increasingly variable climatic conditions. Accordingly, the specific objectives of this doctoral research are:

1. To investigate the interactive effects of coat colour and heat stress with short term sun exposure on milk traits and metabolic indicators in Holstein Friesian cows.
2. To assess the impact of seasonal temperature changes on blood metabolic indicators in lactating cows.

3. To determine the critical period of heat exposure around insemination that most affects conception success.
4. To evaluate the behavioural responses of cows to different levels of heat stress across stages of lactation.
5. To explore how heat stress history during early life (embryonic, calf stages) influences first-lactation performance.

2. MATERIALS AND METHODS

Studies 1 and 2 were part of the project 2018-1.3.1.-VKE-2018-00033, which was approved by the institutional animal welfare committee. All research activities complied with the Animal Experimentation Directive (Directive 2010/63/EU) of the European Union and the Hungarian regulation on animal experiments (40/2013. (II.14.)). No ethical approval was required for Studies 3, 4, and 5.

2.1. Description of study farms

This research was conducted on two commercial dairy farms located in southern Hungary, in Csongrád County. Farm A included 1,947 cows with an average milk yield of 42.32 kg/day, 4.8% fat, and 3.44% protein, and was used in Studies 1, 2, and 3. The farm consisted of six barns: two equipped with both ventilation and sprinkler cooling systems, three with ventilation only, and one without ventilation. Cows consumed on average 29.37 kg of dry matter per day. The total mixed ration (TMR) contained 50.35% dry matter and supplied 6.52 MJ NEL/kg DM, 16.9% crude protein, 33.1% neutral detergent fibre (NDF), 21.3% acid detergent fibre (ADF), 24.2% starch, and 4.5% ether extract. The ration was formulated by the farm nutritionist and met requirements for lactating dairy cows (NRC, 2001). Farm B included 1,105 cows with an average milk yield of 40.42 kg/day, 3.85% fat, and 3.46% protein, and was used in Studies 4 and 5. The farm consisted of three barns equipped with ventilation only. Cows consumed on average 28.12 kg of dry matter per day. The TMR contained 50.6% dry matter and supplied 6.35 MJ NEL/kg DM, 17.3% crude protein, 34.8% NDF, 22.8% ADF, 19.8% starch, and 3.9% ether extract. The ration was also formulated by the farm nutritionist and met requirements for lactating dairy cows (NRC, 2001).

2.2. Investigating the interaction effects of coat colour and heat-stress with short term sun exposure on milk traits and metabolic indicators in Holstein Friesian cows

2.2.1. Animals and experimental design

This experiment was conducted on a commercial dairy farm with a total of 660 records of blood parameters, including Insulin, Prolactin, and non-esterified fatty acids (NEFA), as well as milk quality traits such as protein, fat, lactose, and somatic cell count (SCC), were analysed. The data were collected from 55 healthy, multiparous Holstein-Friesian cows in their 2nd lactation, with an average body weight of 637 ± 29 kg, and varying black and white coat colour ratios, between April 2021 and September 2021. The cows were categorised into three groups according to their coat colour. 18 cows with $\geq 75\%$ white coats with days in milk (DIM) 98 ± 15 ,

18 cows with $\geq 75\%$ black coats DIM (101 ± 18), and 19 cows with an equal distribution of 50% black and 50% white coats with DIM (105 ± 21). Coat colour proportions were determined using standardised digital imaging and custom image analysis software to quantify black and white areas on the body surface.

All cows were housed together under identical management and environmental conditions. They were kept in a mechanically cooled barn with an insulated roof and lying stalls equipped with rubber mats, equipped with ventilators and sprinklers to mitigate summer heat stress. Ventilation speed and sprinkler activation increased automatically in response to rising ambient temperature and humidity, allowing dynamic adjustment of cooling intensity and maintenance of a controlled indoor environment. Water was provided *ad libitum* throughout the year. Cows were fed the same total mixed ration (TMR) *ad libitum* throughout the experiment, formulated to meet nutritional requirements and consisting of alfalfa silage, corn silage, alfalfa hay, molasses, and concentrate. Feed was distributed twice daily, typically at 09:00 and 16:00, to ensure consistent intake. To induce short-term heat exposure under natural conditions, cows were exposed daily to an outdoor yard without cooling systems for a fixed two-hour period between 12:00 and 14:00, corresponding to the hottest part of the day. The yard was unshaded, and during this period, a portion of the daily ration was also provided in the yard. Cows had *ad libitum* access to water and were managed as a single group during outdoor exposure. In 2021, a total of 78 days were classified as heat stress days ($BGHI \geq 68$) between June and September. Samples collected during these periods were considered to represent heat stress conditions.

2.2.2. Blood samples

During the experiment (1 April–30 September 2021), 55 cows were included. Blood samples (10 mL) were collected every 15 days via coccygeal venipuncture into serum separator tubes, yielding 12 samples per cow 6 under non-heat-stress (NHS) and 6 under heat-stress (HS) conditions. Sampling was performed between 6:00 and 6:30 a.m., before the first feeding, to minimise feeding effects. Periods were defined using the BGHI: NHS periods (1 April–10 June and 1–30 September) were characterised by $BGHI < 68$, while the HS period (11 June–31 August) was defined by $BGHI \geq 68$ sustained for at least three consecutive days. All procedures were standardised to ensure comparability and reduce variability.

2.2.3. Milk records

The cows were milked three times a day (at 8:00 AM, 3:00 PM, and 10:00 PM), and the milk yield of each cow was recorded automatically on a daily basis using DeLaval MM27BC milk meter in a herringbone DeLaval milking parlour system. A composite sample from three

milking times were measured twice per month on the same day as the blood samples were collected, and analysed for milk traits: protein, fat, lactose, and SCC. In summer the samples were taken after at least 3 days of consecutive heat stress ($BGHI \geq 68$).

2.3. The effect of seasonal changing temperature on blood metabolic indicators in Holstein Friesian cows

2.3.1. Animals and experimental design

This study was conducted on a commercial dairy farm in southeastern Hungary between December 2019 and December 2020. A total of 723 blood samples were analysed for insulin, prolactin, and non-esterified fatty acids (NEFA) from 382 multiparous Holstein–Friesian cows in their second lactation. Cows had an average milk yield of 42.5 ± 10 kg and a body weight of 632 ± 37 kg and were in early pregnancy (<90 days) to standardise metabolic status (ROCHE ET AL., 2009; SGUIZZATO ET AL., 2020). Only clinically healthy cows were included in the analysis. Cows were housed under identical barn conditions equipped with ventilators and sprinkler cooling systems, with water provided ad libitum. All cows received the same total mixed ration (TMR), consisting of alfalfa silage, corn silage, alfalfa hay, molasses, and concentrate, formulated to meet nutritional requirements. Feed was distributed twice daily (09:00 and 16:00) to maintain consistent intake. Data were collected across all four seasons: winter (166 samples; DIM 84 ± 15 and 172 ± 17), spring (191; 86 ± 19 and 179 ± 16), summer (220; 78 ± 21 and 175 ± 25), and autumn (145; 90 ± 11 and 168 ± 12) for the first and second samplings, respectively.

2.3.2. Blood samples

A total of 723 blood samples were collected seasonally from 382 cows in winter (January), spring (April), summer (July), and autumn (October). Each cow was sampled twice in two different seasons during the experiment (COLLIER ET AL., 2017; COZZI ET AL., 2011), once during a cold season (winter or autumn) and once during a hot season (summer or spring). Samples were collected via coccygeal venipuncture into serum separator tubes at 06:00 h before feeding and processed to serum after centrifugation. During summer, sampling was performed during sustained heat stress (≥ 3 consecutive days). Insulin, prolactin, and NEFA concentrations were measured using commercial bovine ELISA kits.

2.4. The effect of heat stress around insemination period on the conception outcomes in dairy cows

2.4.1. Animals and experimental design

The research was carried out at in a single farm situated in the southern region of Hungary in continental climate. The study analysed 47,199 insemination events from 6,751 Holstein Freisen cows from 2002 to 2024, originated from 4121 dams and 329 sires. The average parity in our study was 1.61 ± 1.27 , with an average number of inseminations per cow of 2.88 ± 1.36 . The study periods were selected based on biological principles. The long pre-insemination period, spanning from 21 to 6 days before insemination (P1), next, the short pre-insemination heat stress period from 5 to 2 days before insemination (P2). The ovulation preparation and peri-insemination period from 1 before to 1 day after insemination (P3). Following this, the early post-insemination heat stress period from 2 to 7 days after insemination (P4). Finally, the long post-insemination period from 8 to 30 days after insemination (P5).

2.4.2. Conception

Only the artificial insemination data were used in the study success was defined as a binary outcome (1 = pregnancy, 0 = no pregnancy). All the insemination were performed after 12h from detecting the oestrus sings by using artificial inseminations. The cows underwent manual rectal palpation at least 40–45 days post-service during the next monthly herd visit by the veterinarian if no further oestrus or service was observed. Services were considered unsuccessful if a negative pregnancy diagnosis was received between 40- and 100-days post-service, or if at least one subsequent oestrus was documented within 100 days after service without a negative diagnosis.

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

2.5.1. Animals and experimental design

A total of 60 multiparous Holstein–Friesian cows in their second lactation were included, with an average milk yield of 36.12 ± 4.10 kg/day, milk fat of $3.50 \pm 0.10\%$, and protein of $3.34 \pm 0.07\%$. Cows were divided into three lactation groups with 20 cows per group: early (DIM 0–60; 27.9 ± 15.2), mid (DIM 90–180; 137.5 ± 30), and late lactation (DIM >180; 269 ± 30). Animals were housed in a free-stall barn under naturally ventilated conditions with open sides and no active cooling, representing typical commercial management without artificial heat mitigation. The experiment was conducted over one month in summer (20 June–20 July 2024), generating 1,680 behavioural records (eating, ruminating, inactivity). All cows received the

same TMR (alfalfa silage, corn silage, alfalfa hay, molasses, concentrate) formulated to meet nutritional requirements and fed twice daily (04:00 and 13:00). Milking was performed three times daily at 04:00, 11:00, and 17:00.

2.5.2. 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 feeding, rumination, and inactivity in minutes at 15-minute intervals, which were aggregated to daily totals. This technology has been validated for accuracy in detecting these behaviours (BORCHERS ET AL., 2021; QUDDUS ET AL., 2022), and has been shown to be more precise than video recording or visual observation. Feeding time was defined as periods of active ingestion, rumination as rhythmic chewing of the bolus, and inactive time as periods when cows were standing or lying still without engaging in feeding, rumination, or other activity.

2.6. Heat stress history effect on production and reproduction performance of dairy cows during first lactation

2.6.1. Animals and experimental design

The study was conducted in southern Hungary under continental climate conditions and included 3,182 Holstein–Friesian cows with complete first-lactation records. The cows were born between 2016 and 2022 from 2,396 dams and 184 sires, allowing linkage of maternal and individual heat stress exposure across development to performance traits. The production traits analysed were 305-day milk yield (kg) (Milk 305), 305-day fat yield (kg) (Fat 305), and 305-day protein yield (kg) (Protein 305). Reproductive traits included services per conception (SPC), first service conception rate (FSCR), age at first insemination (AFI), and age at first calving (AFC). Calves were housed in individual outdoor hutches until 3 months of age, with colostrum feeding shortly after birth followed by milk replacer, and ad libitum access to starter concentrate, hay, and water. After weaning, heifers were group-housed and gradually transitioned to TMR, and insemination was performed at ~60% of mature body weight. Lactating cows were housed in ventilated free-stall barns and fed a TMR ad libitum formulated for production. Early-life development was divided into prenatal trimesters (T1: 0–90 days, T2: 91–180 days, T3: 181–birth) and a postnatal period (0–3 months). Heat stress exposure differed across stages: first trimester (1,606 cows with 0 heat-stress days vs 1,576 with ≥ 1 day), second trimester (1,238 vs 1,944), third trimester (1,062 vs 2,120), and postnatal period (1,503 vs 1,679).

2.7. Meteorological data

Meteorological data (ambient temperature, T °C, and relative humidity, RH %) were recorded on a daily basis. In studies 1, 2, 3, and 5, data were obtained from the Hungarian Meteorological Service; the station was located 13 km from Farm A (studies 1–3) and 2 km from Farm B (study 5). In study 4, temperature and humidity were recorded inside the barn using sensors. For studies 2, 3, 4, and 5, the temperature–humidity index (THI) was calculated according to

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

Cows were classified as heat-stressed when $THI \geq 68$ (Studies 2, 3, and 5). In Study 4, heat stress was further divided into moderate ($72 \leq THI < 77$) and severe ($THI \geq 77$) categories. In Study 5, heat load was additionally quantified using the number of days with $THI \geq 68$ and cumulative THI (THI_c), calculated as the sum of daily exceedances above this threshold across developmental periods (first, second, and third trimesters of gestation and postnatal period).

In Study 1, heat load was assessed using the Black Globe–Humidity Index (BGHI), which incorporates solar radiation which was measured on daily basis also. BGHI was calculated following BUFFINGTON ET AL. (1981):

$$BGHI = T_{bg} + 0.36 \times T_{dp} + 41.5$$

Where: T_{bg} = black globe temperature (°C), T_{dp} = dew point temperature (°C),

Cows were considered under heat stress when $BGHI \geq 68$. BGHI was highly correlated with THI ($r = 0.99$) but was retained in Study 1 due to its improved representation of solar radiation.

2.8. Statistical analysis

All studies were analysed using mixed-effects modelling in R software (version 4.4.1; R Core) (TEAM, 2020), primarily with the lme4 package (BATES ET AL., 2015). The individual cow was generally considered the experimental unit, with repeated measurements (milk yield, composition, blood parameters, or behavioural traits) treated as observational units where applicable. Random effects were included to account for clustering and individual variability, commonly incorporating cow ID, parity, days in milk, year, month, and sire or inseminator, as appropriate. Fixed effects varied by study and included heat stress status, coat colour, lactation stage, season, or THI category.

In Studies 1, 2, 4, and 5, continuous outcomes were analysed using linear mixed-effects models. Post hoc comparisons were performed using Tukey's adjustment (LENTH, 2016), with

significance declared at $P < 0.05$. Model selection was based on the Akaike Information Criterion (AIC).

Study 3 used generalised linear mixed models with a binomial distribution and logit link to evaluate insemination success. THI at insemination was categorised into defined thresholds, with random intercepts for parity, year, cow, semen, and inseminator. Odds ratios with 95% confidence intervals were reported. Period-specific heat stress effects (P1–P5) were assessed, and for significant periods, Heritability of insemination success was estimated using a Bayesian animal model (MCMCglmm) (HADFIELD, 2010) with a threshold probit model on the liability scale, incorporating additive genetic effects from pedigree information. Heritability was calculated as:

$$h^2 = \frac{\sigma_a^2}{(\sigma_a^2 + \sigma^2_{Env} + R)}$$

Where:

h^2 = Heritability: Proportion of total phenotypic variance attributable to additive genetic variance; σ_a^2 = Additive genetic variance: Variance in insemination success due to genetic differences among cows; σ^2_{Env} = Variance due to year, semen, inseminator, parity; R = Residual variance, fixed at 1 for binary outcomes on the liability scale.

In Study 5, early-life heat stress effects were analysed using linear and generalised linear mixed models. Exposure during gestation (first, second, and third trimesters) and postnatal period (0–3 months) was quantified using two metrics: Days ≥ 68 (number of heat-stress days with THI ≥ 68) and cumulative THI (THIc), calculated as the sum of exceedances above the threshold, reflecting both intensity and duration of exposure. These variables were included as continuous predictors, with zero exposure representing the baseline. Milk production traits (305-day milk yield, fat yield, and protein yield) and reproductive traits (age at first insemination, AFI, and age at first calving, AFC) were analysed using linear mixed-effects models. Services per conception (SPC) was analysed using a Poisson GLMM, and first service conception rate (FSCR) using logistic regression. Random effects included dam, sire, year, and month; for reproductive traits, inseminator and semen were also included. Effect sizes were scaled to represent 30-day increases in Days ≥ 68 and 30-unit increases in THIc for biological interpretability. Additionally, maternal and postnatal heat stress effects were evaluated simultaneously across developmental windows to identify sensitive periods of exposure.

3. RESULTS

3.1. Investigating the interaction effects of coat colour and heat-stress with short-term sun exposure on milk traits and metabolic indicators in Holstein Friesian cows

BGHI values were consistently below the heat-stress threshold ($BGHI < 68$) from 1 April to 10 June, corresponding to non-heat-stress (NHS) conditions (Figure 1). From 11 June to 31 August, THI values exceeded 68 continuously, indicating sustained heat stress with short-term sun exposure (HS). From 1 September to 30 September, BGHI values gradually decreased and returned to NHS levels. These fluctuations enabled the clear identification of HS and NHS days used in the subsequent analyses.

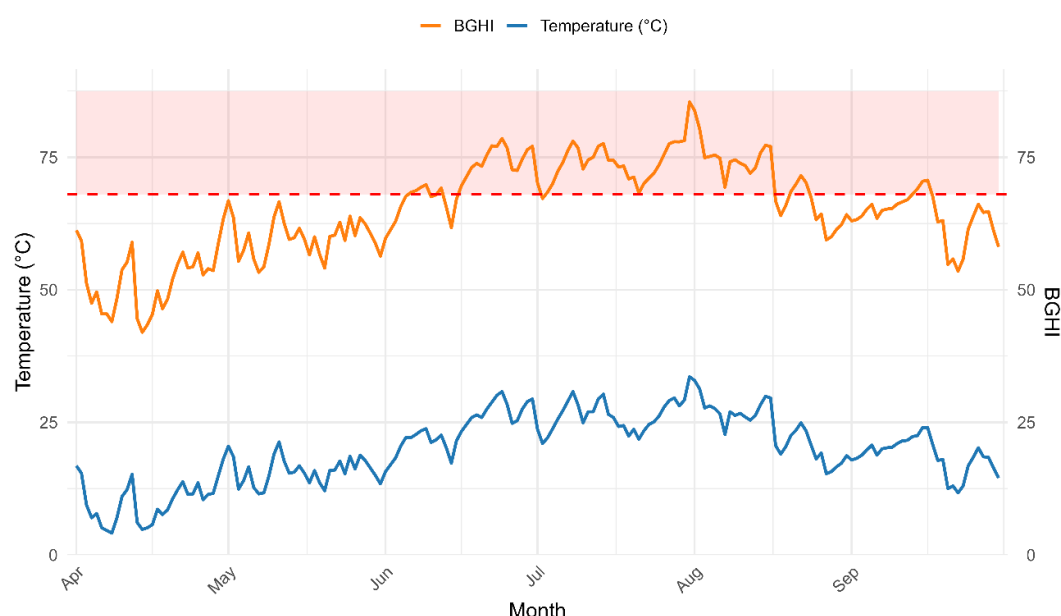


Figure 1. Daily ambient temperature and black globe humidity index (BGHI) in the open area during the study period

The shaded red area indicates values where $BGHI \geq 68$ (heat stress zone), and the dashed line represents the threshold for heat stress classification ($BGHI = 68$).

3.1.1. Effect of coat colour and its interaction with heat stress on milk traits and blood parameters

The present study demonstrated that coat colour did not significantly influence milk yield or milk composition under either HS and NHS conditions (Table 1). No significant differences were observed between black-predominant and white-predominant Holstein-Friesian cows for milk yield, milk fat, milk protein, lactose content, or somatic cell count. In addition, the interaction between coat colour and heat stress was not significant for any milk production traits (Table 2), indicating that the response to heat stress with short-term sun exposure was similar

in cows of both coat colour groups. Similarly, coat colour had no significant effect on blood biochemical parameters under either HS or NHS conditions (Table 3). Although black-predominant cows exhibited numerically higher concentrations of insulin and NEFA compared with white-predominant cows, these differences were not statistically significant. Furthermore, no significant interaction between coat colour and heat stress was detected for insulin, prolactin, or NEFA concentrations (Table 4), suggesting that coat colour did not modulate the metabolic response to heat stress with short-term sun exposure. The controlled housing conditions and relatively moderate climatic environment of the present study may therefore explain the absence of coat colour effects. Overall, the results suggest that under heat stress with short-term sun exposure and well-managed housing conditions, coat colour does not significantly influence productive or metabolic responses in Holstein-Friesian cows. These findings imply that coat colour alone may be of limited value as a selection criterion for improving heat tolerance in intensively managed dairy systems.

Table 1. Least square means ($\pm$ standard error) for the effect of coat colour on studied milk traits

Milk Traits (From April to September)	Coat colour			P value
	White (n = 18)	Black (n = 18)	Mixed (n = 19)	
	LS means $\pm$ SE	LS means $\pm$ SE	LS means $\pm$ SE	
Milk Yield (kg)	41.2 $\pm$ 1.19	42.8 $\pm$ 1.18	40.8 $\pm$ 1.18	0.523
Milk Fat (%)	3.73 $\pm$ 0.29	4.08 $\pm$ 0.28	4.04 $\pm$ 0.28	0.232
Milk Protein (%)	3.25 $\pm$ 0.09	3.17 $\pm$ 0.09	3.22 $\pm$ 0.09	0.365
Lactose (%)	5.04 $\pm$ 0.06	5.00 $\pm$ 0.05	5.04 $\pm$ 0.05	0.827
SCC 10 ³ cells/ml	219 $\pm$ 70.90	213 $\pm$ 0.06	167 $\pm$ 70.50	0.862

Values represent least square means (LS means) with their associated standard errors ($\pm$ SE) of different measured traits during the whole experiment period (from April 2021 to September 2021). n indicates the number of cows per coat colour group. P values indicate the statistical significance of coat colour effects on each trait.

Table 2. Least square means ($\pm$ standard error) for the interaction of coat colour and heat stress effect on milk yield and composition

Coat colour							
Milk Traits	White (n= 18)		Black (n = 18)		Mixed (n = 19)		P value
(From April to	NHS	HS	NHS	HS	NHS	HS	
September)	LS means ±	LS means ±	LS means ±	LS means ±	LS means ±	LS means ±	
	SE	SE	SE	SE	SE	SE	
Milk Yield (kg)	39.8±1.61	40.00 ± 1.28	42.00±1.44	41.7±1.32	39.1±1.62	39.9±1.23	0.996
Milk Fat (%)	3.70±0.34	3.77±0.29	4.20±0.31	3.96±0.30	4.13±0.34	3.95±0.29	0.658
Milk Protein (%)	3.32±0.10	3.19±0.10	3.23±0.10	3.11±0.10	3.33±0.10	3.12±0.10	0.316

Lactose (%)	5.07±0.06	5.00±0.06	5.02±0.06	4.99±0.06	5.08±0.06	5.01±0.55	0.762
SCC 10 ³ cells/ml	223.1±110.80	215.5±78.40	190.1±91.90	235.4±82.20	98.3±112	253.6±75.80	0.629

Values represent least square means (LS means) ± standard error (SE) of different measured traits during the heat-stressed period (from June 2021 to August 2021). n indicates the number of cows per coat colour group. P-values indicate the statistical significance of the coat colour and heat stress interaction.

Table 3. Least square means (± standard error) for effect of coat colour on blood parameters

Blood parameters (From April to September)	Coat colour			P value
	White (n= 18)	Black (n= 18)	Mixed (n= 19)	
	LS means ± SE	LS means ± SE	LS means ± SE	
Insulin (mIU/L)	10.21 ±2.83	11.12 ±2.55	5.31 ±3.09	0.325
Prolactin (ng/ml)	6.72 ±3.16	5.70 ±2.41	7.76 ±3.80	0.890
NEFA (µmol/L)	397 ±113	643 ±111	427 ±110	0.242

Values represent least square means (LS means) ± standard error (SE) for insulin, prolactin, and non-esterified fatty acids (NEFA) according to coat colour (white, black, and mixed) from June 2021 to August 2021. n indicates the number of cows per coat colour group. P-values indicate the statistical significance of coat colour effects on each blood parameter.

Table 4. Least square means (± standard error) for the interaction of coat colour and heat stress effect on blood parameters

		Coat colour					
Blood Parameters (From April to September)	White (n= 18)		Black (n= 18)		Mixed (n= 19)		P value
	NHS	HS	NHS	HS	NHS	HS	
	LS means ±	LS means ±	LS means ±	LS means ±	LS means ±	LS means ±	
	SE	SE	SE	SE	SE	SE	
Insulin (mIU/L)	8.11±3.84	12.32±2.50	7.13±2.94	15.12±2.63	2.99±2.69	7.72±2.41	0.584
Prolactin (ng/ml)	6.92 ±4.00	15.10±2.31	4.52±3.33	6.57±2.69	5.43±2.66	8.99±2.24	0.059
NEFA (µmol/L)	319±127	491±109	550±120	717±112	333±124	521±105	0.772

Values represent least square means (LS means) ± standard error (SE) for insulin, prolactin, and non-esterified fatty acids (NEFA) according to coat colour (white, black, and mixed) under heat stress (HS) and no heat stress (NHS) conditions from June 2021 to August 2021. n indicates the number of cows per coat colour group. P-values indicate the statistical significance of the coat colour × heat stress interaction.

3.1.2. Effect of heat stress with short term sun exposure on milk yield and composition

Milk production and composition were measured in cows subjected to HS and NHS conditions, as described in the Chapter 3.2. Since the study period extended from April to September, DIM was included in the mixed model to account for the natural progression of lactation and to minimise potential confounding between the lactation curve and heat stress effects. As illustrated in Figure 2, milk yield decreased across the study period. After adjustment for lactation-stage progression, cows under HS conditions produced a lower MY ($p = 0.011$) than cows in NHS conditions (40.52 kg vs 42.1 kg., respectively) a decrease of 1.5 kg was detected. Milk protein levels in cows under HS conditions were found to be lower ($p < 0.001$) compared to those in cows experiencing NHS conditions. The average milk protein content for cows under HS conditions was 3.14 %, while NHS conditions cows had higher average MP content of 3.29 %. Furthermore, there was a difference in the amount of lactose in the milk during HS and NHS periods ($p < 0.001$). This difference indicates a decrease, from 5.07 % during NHS to 5 % during HS conditions. However, the heat stress with short-term sun exposure did not have an effect on milk fat and somatic cells count (Table 5).

Table 5. Least square means ($\pm$ standard error) for the effect of heat stress on studied milk traits

Milk Traits (From April to September)	Stress status (n = 55)		P value
	NHS	HS	
	LS Means $\pm$ SE	LS Means $\pm$ SE	
Milk Yield (kg)	42.1 $\pm$ 1.42	40.52 $\pm$ 1.38	0.011*
Milk Fat (%)	4.01 $\pm$ 0.27	3.89 $\pm$ 0.25	0.416
Milk Protein (%)	3.29 $\pm$ 0.09	3.14 $\pm$ 0.09	0.001*
Lactose (%)	5.07 $\pm$ 0.06	5.00 $\pm$ 0.06	0.001*
SCC 10 ³ cells/ml	171 $\pm$ 67.40	235 $\pm$ 53.40	0.357

Values represent least square means (LS means) $\pm$ standard error (SE) for milk yield, milk fat, milk protein, lactose, and somatic cell count (SCC) under heat stress (HS) and no heat stress (NHS) conditions from June 2021 to August 2021. n indicates the number of cows. P-values indicate the statistical significance of heat stress effects. An asterisk (*) denotes statistically significant differences ($p < 0.05$).

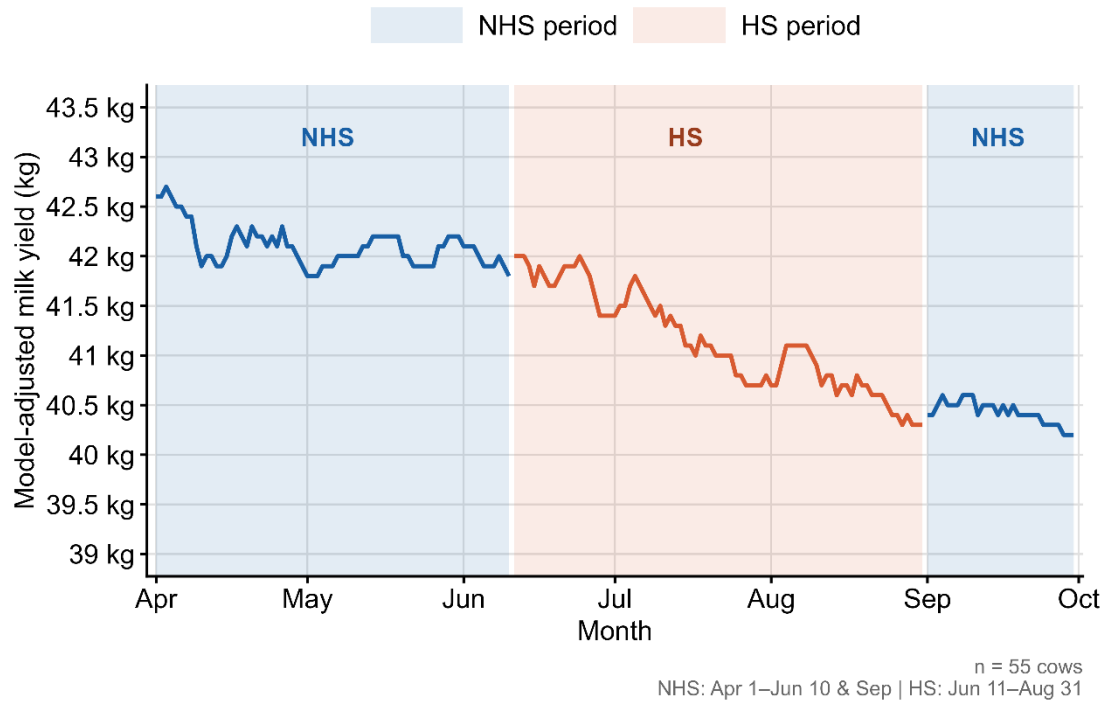


Figure 2. Model-adjusted daily milk yield during NHS and HS periods

Adjusted daily milk yield trends in cows during non-heat stress (NHS) and heat stress (HS) periods. Values represent model-adjusted milk yield obtained from a mixed-effects model accounting for days in milk (DIM) and cow ID. Shaded regions indicate NHS (blue) and HS (orange) periods. n= 55 cows.

3.1.3. Effect of heat stress with short-term sun exposure on blood parameters

At the level of blood parameters, heat stress with short-term sun exposure had a marked effect on the metabolic and endocrine profile of the cows. Blood insulin concentration increased by 194% under heat stress compared with thermoneutral conditions (Table 6). Similarly, prolactin levels were significantly affected by heat stress with short-term sun exposure ($p < 0.0028$), increasing from 3.21 ng/ml under non-heat stress conditions to 10.24 ng/ml under heat stress, corresponding to an increase of 319%. Non-esterified fatty acids (NEFA) were also significantly affected by heat stress, increasing from 382 $\mu\text{mol/L}$ under non-heat stress conditions to 571 $\mu\text{mol/L}$ under heat stress conditions, representing an increase of 67%. Despite the blood samples being collected early in the morning on the day following heat stress with short-term sun exposure, insulin, prolactin, and NEFA levels remained significantly higher compared to non-heat stress conditions, confirming the cumulative effect of heat stress on the physiological state of the cows (ECKHARDT ET AL., 2025; PINTO ET AL., 2025; RHOADS ET AL., 2013). Overall, these results indicate that heat stress with short-term sun exposure significantly impairs metabolic and endocrine function in dairy cows.

Table 6. Least square means ($\pm$ standard error) for effect of heat stress on blood parameters

Blood parameters	Stress status (n= 55)		P value
	HS	NHS	
	LS Means $\pm$ SE	LS Means $\pm$ SE	
Insulin (mIU/L)	11.52 $\pm$ 1.45	5.93 $\pm$ 2.25	0.004*
Prolactin (ng/ml)	10.24 $\pm$ 1.40	3.21 $\pm$ 3.11	0.028*
NEFA (μ mol/L)	571 $\pm$ 63.20	382 $\pm$ 86.40	0.010*

Values represent least square means (LS means) $\pm$ standard error (SE) for insulin, prolactin, and non-esterified fatty acids (NEFA) under heat stress (HS) and no heat stress (NHS) conditions from June 2021 to August 2021. n indicates the number of cows. P-values indicate the statistical significance of heat stress effects. An asterisk (*) denotes statistically significant differences ($p < 0.05$).

3.1.4. The effect of seasonal changing temperature on blood metabolic indicators in Holstein Friesian cows

3.1.5. Effects of season on insulin level in the blood

The analysis revealed a clear seasonal variation in the average THI, which serves as an indicator of environmental heat stress. The THI was highest during the summer months (77.3), followed by spring (69.5), autumn (48.1), and winter (43.7) (Figure 3). Based on these findings, we categorized spring and summer as hot seasons, with an average THI ≥ 68 , whereas autumn and winter were classified as cold seasons (THI < 68).

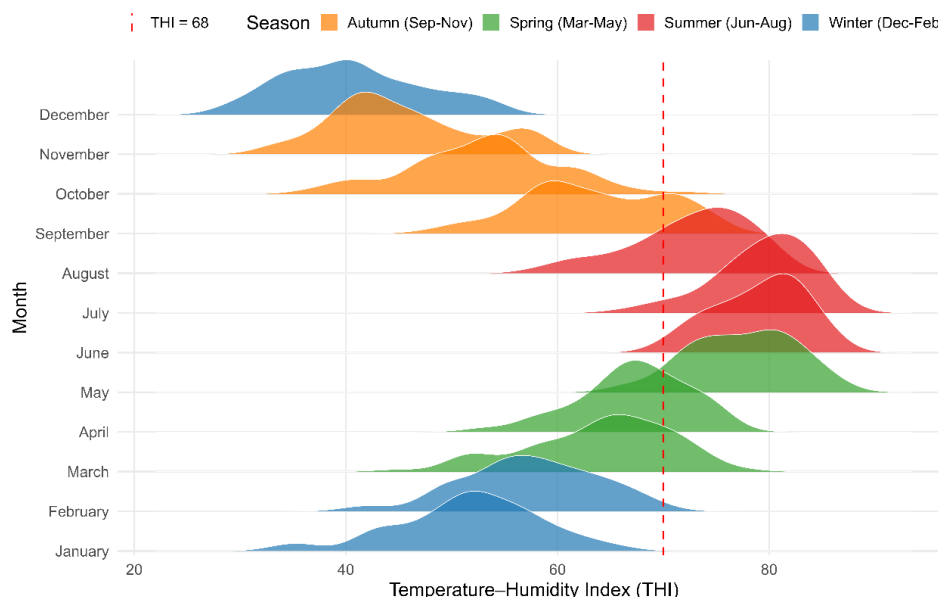


Figure 1. Changes in THI across seasons, December 2019-2020 in southeast Hungary.

Monthly distribution of daily temperature-humidity index (THI) values throughout the year. Density curves illustrate the variation in THI across months, highlighting seasonal patterns with higher THI values during summer months and lower values during winter months.

The seasonal effect on blood insulin levels was statistically significant ($p < 0.01$), (Table 7). The results showed that insulin levels varied considerably across seasons, with significant differences noted between hotter (spring and summer) and colder (autumn and winter) periods. In spring, the mean insulin concentration was 9.86 mIU/L, which was significantly ($p < 0.01$) higher than that in both winter (6.30 mIU/L) and autumn (5.92 mIU/L). Similarly, during summer, insulin levels were elevated, reaching 8.80 mIU/L, which was again significantly ($p < 0.01$) higher than the levels recorded in the colder seasons (winter and autumn). The absence of a significant difference in insulin levels between spring and summer, as well as between winter and autumn, suggests that insulin secretion is particularly sensitive to temperature fluctuations associated with the transition between the cold and hot seasons, rather than the absolute difference between the two hot and two cold seasons. These findings indicate that, under Hungarian climatic conditions, spring has increasingly become a thermally challenging period for dairy cows. The average spring THI of 69.5 exceeded the commonly accepted threshold for heat stress in dairy cattle $\text{THI} \geq 68$; (COLLIER ET AL., 2006), likely reflecting recent climate change trends. Importantly, previous research has shown that photoperiod alone does not significantly influence insulin levels in dairy cows (ESPINOZA AND OBA, 2017; TUCKER ET AL., 1984), reinforcing the conclusion that the observed seasonal variation in insulin in the present study is primarily driven by heat stress.

3.1.6. Effects of season on prolactin level in the blood

Analysis of prolactin levels across seasons revealed a significant seasonal effect ($p < 0.01$) (Table 7). Prolactin levels were lowest in autumn (3.78 ng/mL), increased slightly in winter (6.49 ng/mL), and continued to rise in spring (8.58 ng/mL) and summer (11.22 ng/mL). The difference between autumn and summer was particularly striking, with prolactin levels being significantly higher in summer than in autumn ($p < 0.01$). However, there were no significant differences in prolactin levels between autumn and winter or between autumn and spring. This indicates that while prolactin levels increased with the transition to warmer seasons, the changes between the colder seasons were less pronounced. Moreover, these findings suggest that prolactin secretion is strongly influenced by increasing temperatures. The gradual increase in prolactin levels as temperatures increase, especially in summer, could indicate that the secretion of this hormone is closely linked to heat stress, potentially aiding the physiological adaptation of cows to elevated temperatures.

3.1.7. Effects of season on NEFA level in the blood

Analysis of NEFA blood levels across seasons indicated no significant seasonal effects ($p > 0.226$) (Table 7). NEFA levels were 596 $\mu\text{mol/L}$ in autumn, 508 $\mu\text{mol/L}$ in winter, 529 $\mu\text{mol/L}$ in spring, and 556 $\mu\text{mol/L}$ in summer. Despite these variations, the differences in NEFA levels between the seasons were not statistically significant. This lack of significant difference suggests that NEFA concentrations remain relatively stable throughout the seasons, indicating that factors influencing NEFA levels may be less affected by seasonal temperature changes than other hormones.

Table 7. Least square means ($\pm$ standard error) for the effect of season on blood parameters

Blood parameters	Seasons (n = 382)				P value
	Autumn	Winter	Spring	Summer	
	LS means $\pm$ SE	LS means $\pm$ SE	LS means $\pm$ SE	LS means $\pm$ SE	
Insulin (mIU/L)	5.92 ^a $\pm$ 0.62	6.30 ^a $\pm$ 0.59	9.86 ^b $\pm$ 0.50	8.80 ^b $\pm$ 0.51	<0.001
Prolactin (ng/mL)	3.78 ^a $\pm$ 0.81	6.49 ^{ab} $\pm$ 0.78	8.58 ^b $\pm$ 0.66	11.22 ^c $\pm$ 0.67	<0.001
NEFA ($\mu\text{mol/L}$)	596 $\pm$ 41.00	508 $\pm$ 40.20	529 $\pm$ 35.60	556 $\pm$ 35.90	0.226

Values represent least square means (LS means) $\pm$ standard error (SE) for insulin, prolactin, and non-esterified fatty acids (NEFA) across seasons (autumn, winter, spring, and summer). n indicates the total number of cows. P-values indicate the statistical significance of seasonal effects. Within a row, values with different superscript letters (a, b, c) differ significantly ($p < 0.05$).

3.2. The effect of heat stress around insemination period on the conception outcomes in dairy cows

3.2.1. Effect of THI on insemination success on the day of insemination

THI on the day of insemination had a significant ($p < 0.001$) effect on insemination success in dairy cows. Table 12 shows the effect of THI on insemination success in dairy cows on the day of insemination. At lower THI values (≤ 50), there was no significant ($p > 0.05$) effect on the probability of insemination success, with odds ratios (OR) close to 1. From THI 59 onwards, a general declining trend in insemination success was observed. A significant reduction was first detected at THI = 59 ($B = -0.14$, $OR = 0.87$, $p = 0.03$), followed by THI = 60 ($B = -0.18$, $OR = 0.84$, $p = 0.007$). However, intermediate THI values (61 and 63) did not reach statistical significance, though their odds ratios of 0.90 and 0.88 remained consistent with the overall declining trend, reflecting natural sampling variability across thresholds rather than a true biological recovery. Despite this local variability, further significant reductions were observed at higher THI levels, including THI = 62 ($OR = 0.73$, $p < 0.001$), THI = 64 ($OR = 0.71$, $p < 0.001$), and THI ≥ 68 , where OR values consistently indicated a marked decline in insemination

success. The most severe impact was observed at THI = 76 (B = -1.21, OR = 0.30, $p < 0.001$), corresponding to a 70% reduction in odds of success compared to the reference category.

Table 8. Effect of temperature–humidity index (THI) on insemination success in dairy cows on the day of insemination

THI threshold	B slope	Odds ratio	95% Confidence interval	N $\geq$ threshold	P	Success rate [%]
≤ 44	Referent					
45	0.02	1.02	[0.89, 1.18]	1053	0.750	34.9
46	0	1	[0.88, 1.15]	1110	0.952	34
47	0.02	1.02	[0.9, 1.15]	1377	0.761	33.8
48	0.08	1.09	[0.94, 1.26]	912	0.282	34.6
49	0.03	1.03	[0.9, 1.19]	1067	0.637	32.6
50	0.01	1.02	[0.88, 1.17]	1119	0.832	32.8
51	-0.07	0.93	[0.81, 1.07]	1132	0.326	31
52	0.06	1.06	[0.92, 1.23]	970	0.435	33.3
53	-0.09	0.91	[0.79, 1.05]	1047	0.200	32.7
54	-0.06	0.94	[0.81, 1.09]	1018	0.386	29.8
55	-0.1	0.91	[0.79, 1.05]	1121	0.181	31.6
56	-0.05	0.95	[0.82, 1.09]	1118	0.458	30.9
57	-0.12	0.89	[0.77, 1.02]	1170	0.089	29.9
58	-0.03	0.98	[0.84, 1.14]	903	0.750	31.3
59	-0.14	0.87	[0.76, 0.99]	1315	0.030*	29.8
60	-0.18	0.84	[0.73, 0.95]	1346	0.007*	28
61	-0.11	0.9	[0.78, 1.04]	1057	0.145	29
62	-0.31	0.73	[0.64, 0.84]	1252	$<0.001^*$	26
63	-0.12	0.88	[0.77, 1.02]	1108	0.083	30.4
64	-0.34	0.71	[0.61, 0.82]	1156	$<0.001^*$	26
65	-0.29	0.75	[0.65, 0.87]	1053	$<0.001^*$	26.6
66	-0.44	0.65	[0.56, 0.75]	1143	$<0.001^*$	23.8
67	-0.45	0.64	[0.55, 0.74]	1149	$<0.001^*$	23.6
68	-0.42	0.65	[0.58, 0.74]	1541	$<0.001^*$	24.3
69	-0.52	0.59	[0.51, 0.69]	1205	$<0.001^*$	22.5
70	-0.56	0.57	[0.5, 0.66]	1261	$<0.001^*$	23.5
71	-0.53	0.59	[0.51, 0.69]	1082	$<0.001^*$	22.7
72	-0.63	0.53	[0.45, 0.62]	1080	$<0.001^*$	20.3
73	-0.64	0.53	[0.44, 0.63]	878	$<0.001^*$	21.5
74	-0.85	0.43	[0.36, 0.51]	844	$<0.001^*$	19.7
75	-0.64	0.53	[0.44, 0.63]	765	$<0.001^*$	21.8

76	-1.21	0.3	[0.23, 0.39]	463	<0.001*	14.7
77	-0.79	0.46	[0.37, 0.55]	697	<0.001*	20.9

Values represent logistic regression coefficients (B slope), odds ratios (OR) with 95% confidence intervals (CI), number of inseminations at or above each THI threshold ($N \geq \text{threshold}$), associated p-values, and observed insemination success rates (%). Odds ratios are expressed relative to the referent category ($\text{THI} \leq 44$). Odds ratios < 1 indicate a reduced probability of insemination success with increasing THI. Asterisks (*) denote statistically significant effects ($p < 0.05$). Each row represents an independent comparison at the specified THI threshold; the $N \geq \text{threshold}$ in each row indicates the number of insemination records meeting that threshold.

3.2.2. Period specific effects of heat stress on insemination success

The impact of heat stress on insemination success was analysed across five distinct time periods relative to insemination: P1, P2, P3, P4, P5. Based on previous results, the cows were categorised into four heat stress levels: No Heat Stress, Small Heat Stress, Medium Heat Stress, and Severe Heat Stress. Heat stress experienced during P1 had a significant ($p < 0.001$) negative impact on the odds of successful insemination. Cows exposed to small, medium, and severe heat stress had significantly lower odds of success compared to those under no heat stress conditions, with medium heat stress showing the most substantial effect, a 30% reduction in the odds of success. The odds ratios for the three heat stress levels were as follows: Small Heat Stress (OR = 0.823, 95% CI: 0.759–0.891), Medium Heat Stress (OR = 0.697, 95% CI: 0.625–0.777), and Severe Heat Stress (OR = 0.773, 95% CI: 0.675–0.885). In contrast, heat stress in P2 did not have a significant effect on insemination success ($p > 0.05$), with no statistically significant differences observed between small, medium, and severe heat stress categories. During the peri-insemination period P3, small and medium heat stress did not affect significantly ($p > 0.05$) the odds of success compared to no heat stress conditions. However severe heat stress was the only category to show a significant ($p < 0.001$) negative effect. Cows under severe heat stress had significantly lower odds of successful insemination compared to those without heat stress (OR = 0.693, 95% CI: 0.565–0.850). In the short period after insemination P4, heat stress did not show any significant ($p > 0.05$) effects on insemination success. The odds ratios for all levels of heat stress were close to 1. In the P5, severe heat stress again showed a significant negative effect on insemination success. Cows under severe heat stress had a 15% reduction in the odds of successful insemination. Severe Heat Stress (OR = 0.854, 95% CI: 0.743–0.981). However, no significant effects were found for small and medium heat stress in this period (Table 9). These patterns suggest that the timing and severity of heat stress are critical determinants of reproductive success. This study shows that the period P1 appears to be the most susceptible to heat stress, as even small levels of heat stress can significantly affect insemination outcomes compared to other timeframes.

Table 1. Odds ratios for insemination success under different levels of heat stress across periods surrounding insemination

Time Period	Heat Stress Category	Odds Ratio	95% CI [Odds Ratio]	N	p-value
P1	No HS	Referent		29028	
	Small Heat Stress	0.823	[0.759, 0.891]	8381	<0.001***
	Medium Heat Stress	0.697	[0.625, 0.777]	6339	<0.001***
	Severe Heat Stress	0.773	[0.675, 0.885]	3451	<0.001***
P2	No Hs	Referent		28924	
	Small Heat Stress	0.973	[0.886, 1.069]	9343	0.574
	Medium Heat Stress	0.913	[0.792, 1.053]	5369	0.212
	Severe Heat Stress	1.005	[0.844, 1.197]	3536	0.952
P3	No Hs	Referent		28892	
	Small Heat Stress	0.926	[0.835, 1.027]	9343	0.147
	Medium Heat Stress	0.887	[0.757, 1.039]	5396	0.137
	Severe Heat Stress	0.693	[0.565, 0.850]	3536	<0.001***
P4	No Hs	Referent		29028	
	Small Heat Stress	0.941	[0.858, 1.032]	9247	0.195
	Medium Heat Stress	0.935	[0.814, 1.075]	5318	0.345
	Severe Heat Stress	0.922	[0.776, 1.096]	3606	0.353
P5	No Hs	Referent		29827	
	Small Heat Stress	1.058	[0.978, 1.145]	8600	0.165
	Medium Heat Stress	0.941	[0.840, 1.054]	5815	0.300
	Severe Heat Stress	0.854	[0.743, 0.981]	2957	0.026*

Values represent odds ratios (OR) with corresponding 95% confidence intervals (CI), sample size (N), and associated p-values. For each period, the No Hs “No heat stress” category was used as the reference. Heat stress categories were defined as: No Heat Stress ($THI < 60$), Small Heat Stress ($60 \leq THI < 68$), Medium Heat Stress ($68 \leq THI < 72$), and Severe Heat Stress ($THI \geq 72$). Odds ratios below 1 indicate a reduced probability of insemination success relative to the reference category. Asterisks denote levels of statistical significance ($p < 0.05^*$, $p < 0.001^{***}$).

3.2.3. Heat stress dependent heritability of insemination success

The heritability of the insemination results on the liability scale increased with the severity of heat stress during period P1, rising from 0.048 under no heat stress conditions ($THI < 60$) to 0.129 under severe heat stress ($THI \geq 72$). Similar trends were observed for periods P3 and P5, with heritability increasing from 0.048 and 0.040, respectively, under no heat stress conditions to 0.124 and 0.140, respectively, under severe heat stress (Table 10). However, the higher heritability observed under severe stress indicates a greater potential for genetic selection in increasingly prevalent heat-stressed environments, aligning with climate change projections.

Table 2. Liability-scale heritability estimates of insemination success across different periods (P1, P3, and P5) and levels of heat stress

Period	Heat stress category	Heritability	N	CrI_2.5	CrI_97.5
P1	No Heat Stress	0.048	29028	0.022	0.100
	Small Heat Stress	0.081	8381	0.033	0.173
	Medium Heat Stress	0.112	6339	0.044	0.241
	Severe Heat Stress	0.129	3451	0.047	0.275
P3	No Heat Stress	0.048	28892	0.022	0.099
	Small Heat Stress	0.079	9343	0.032	0.17
	Medium Heat Stress	0.107	5396	0.039	0.231
	Severe Heat Stress	0.124	3536	0.043	0.265
P5	No Heat Stress	0.040	29827	0.020	0.095
	Small Heat Stress	0.082	8600	0.031	0.176
	Medium Heat Stress	0.093	5815	0.035	0.197
	Severe Heat Stress	0.140	2957	0.047	0.295

Values represent posterior mean heritability estimates with corresponding 95% Bayesian credible intervals (CrI), sample size (N). Heat stress categories were defined as: No Heat Stress ($THI < 60$), Small Heat Stress ($60 \leq THI < 68$), Medium Heat Stress ($68 \leq THI < 72$), and Severe Heat Stress ($THI \geq 72$).

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

3.3.1. Effect of different levels of heat stress on dairy cow behaviour

We observed significant ($p < 0.001$) difference in cows' behaviour between severe and moderate heat stress conditions (Figure). 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.

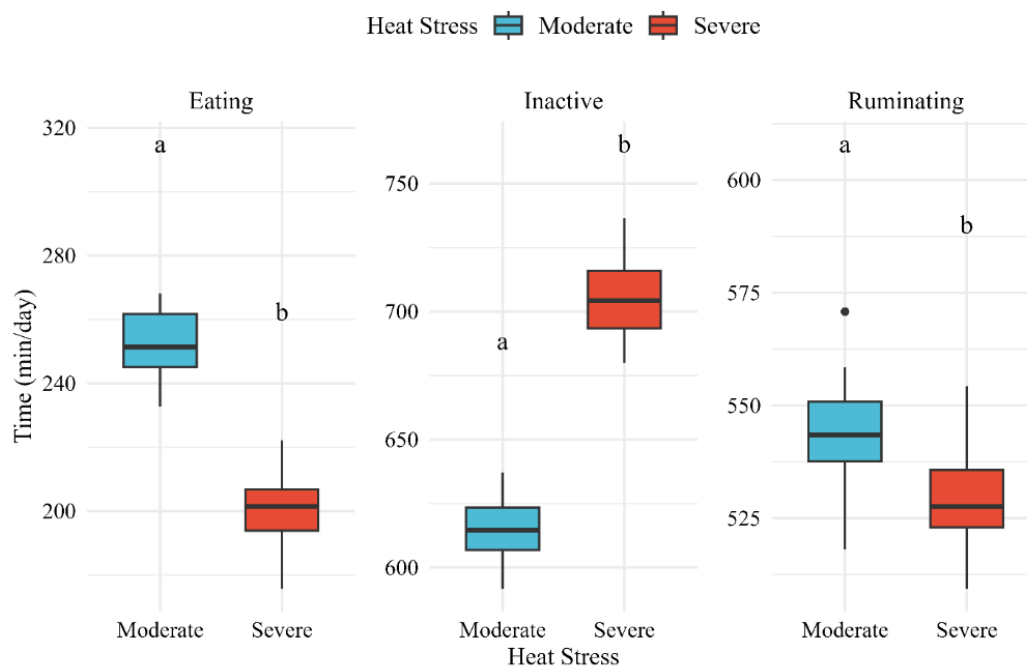


Figure 4. Box plots illustrating the effect of heat stress on the daily total time spent eating, ruminating, and inactive behaviours, expressed in minutes per day (min/day)

The horizontal line within each box represents the median, and the box edges correspond to the 25th and 75th percentiles (interquartile range). Different superscripts (a, b) within each behaviour indicate significant differences ($p < 0.05$).

3.3.2. Interaction between heat stress and lactation stage on dairy cow behaviour

Eating behaviour decreased significantly ($p < 0.001$) with increasing severity of heat stress across all lactation stages (Figure 5). Under moderate 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], $p < 0.0001$).

We observed variation in ruminating behaviour in response to heat stress across lactation stages (Figure 5). 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], $p < 0.0001$).

Inactive behaviour increased significantly ($p < 0.05$) with the progression of heat stress severity across all lactation stages (Figure 5). 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 11). 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. Overall, these results indicate that late-lactation cows are more behaviourally vulnerable to severe heat stress than early- and mid-lactation cows.

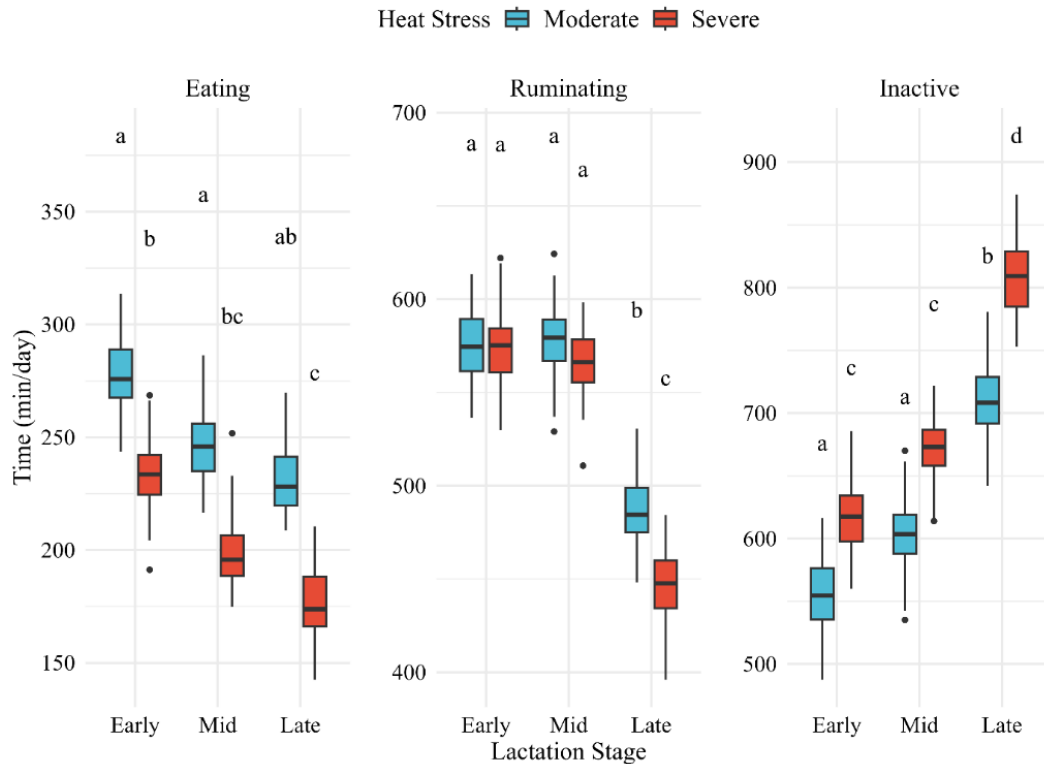


Figure 5. Box plots illustrating the interaction between heat stress and lactation stage on the daily total time spent eating, ruminating, and inactive behaviours, expressed in minutes per day (min/day)

The horizontal line within each box represents the median, and the box edges correspond to the 25th and 75th percentiles (interquartile range). Different superscripts (a, b, c, d) within each behaviour indicate significant differences ($p < 0.05$). Each lactation stage included 20 cows (sample size $n = 20$).

Table 11. Changes in cow behaviour (minutes per unit increase in temperature–humidity index, (min/THI unit)) across different lactation stages

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

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 $n = 20$).

3.4. Heat stress history effect on production and reproduction performance of dairy cows during first lactation

3.4.1. Effects of early-life heat stress on first-lactation milk production

A total of 3182 Holstein Friesian cows with complete first lactation records were included in the present study. The analysis of first-lactation 305-day milk yield revealed a significant negative impact of heat stress exposure during early foetal development. Specifically, each additional day with THI ≥ 68 during the first trimester was associated with a reduction of 6.65 kg/305-day yield (95% CI [-10.44, -2.87], $p < 0.001$), which corresponds to a decrease of 199.63 kg of milk over a 30-day exposure period (95% CI [-313.18, -86.07] kg). In contrast, heat stress during the second trimester did not significantly affect milk yield (0.246 kg/305-day yield; 95% CI [-2.60, 3.09], $p = 0.865$), nor did exposure during the third trimester (-1.940 kg/305-day yield; 95% CI [-5.43, 1.55], $p = 0.277$). Postnatal heat stress was also not significantly associated with milk yield (-7.97 kg/305-day yield; 95% CI [-15.97, 0.04], $p = 0.083$) (Figure 6).

The analysis of first-lactation 305-day milk protein yield indicated that heat stress exposure during early foetal development (T1) had a significant negative effect. Specifically, each additional day with THI ≥ 68 during T1 was associated with a reduction of 0.22 kg/305-day yield (95% CI [-0.34, -0.10], $p < 0.001$), which corresponds to a decrease of 6.63 kg of protein over a 30-day exposure period (95% CI [-10.18, -3.08] kg). In contrast, heat stress during the second (T2) and third (T3) trimesters of gestation, as well as postnatal heat stress, did not significantly affect milk protein yield (T2: -0.04 kg/305-day yield, 95% CI [-0.13, 0.05], $p = 0.34$; T3: -0.02 kg/305-day yield, 95% CI [-0.13, 0.09], $p = 0.73$; postnatal: -0.13 kg/305-day yield, 95% CI [-0.30, 0.04], $p = 0.370$) (Figure 6).

The analysis of first-lactation 305-day milk fat yield indicated that heat stress exposure during early foetal development (T1) had a significant negative effect. Specifically, each additional day with THI ≥ 68 during T1 was associated with a reduction of 0.16 kg/305-day yield (95% CI [-0.31, -0.01], $p = 0.040$), which corresponds to a decrease of 4.73 kg of fat over a 30-day exposure period (95% CI [-9.23, -0.23] kg). In contrast, heat stress during the second (T2) and third (T3) trimesters of gestation, as well as postnatal heat stress, did not significantly affect milk fat yield (T2: 0.06 kg/305-day yield, 95% CI [-0.05, 0.17], $p = 0.307$; T3: 0.15 kg/305-day yield, 95% CI [0.01, 0.29], $p = 0.35$; postnatal: 0.08 kg/305-day yield, 95% CI [-0.03, 0.19], $p = 0.622$) (Figure 6).

Consistent with these findings, analyses based on cumulative heat stress load confirmed that only first-trimester foetal exposure was significantly associated with reduced 305-day milk yield, 305-day milk protein, 305-day milk fat, whereas cumulative heat stress during second- and third-trimester gestation and the postnatal period showed no significant effects (Table 13). Taken together, these results suggest that the first trimester of gestation represents a particularly sensitive developmental window during which heat stress can program long-term reductions in lactational performance.

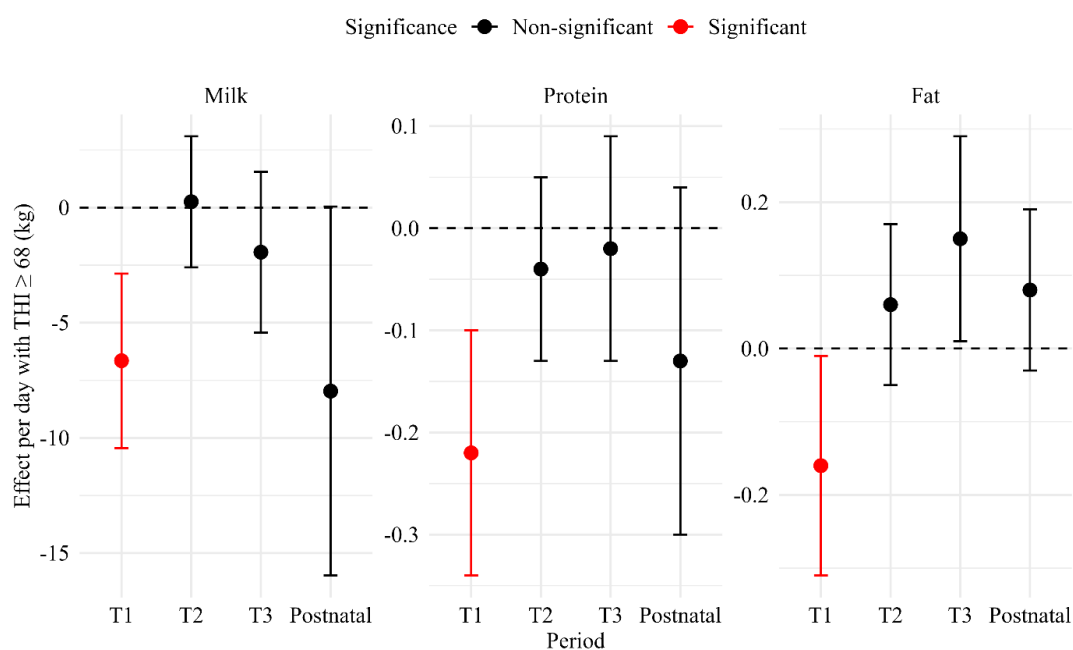


Figure 6. Early-Life Heat Stress Days and 305-Day Milk, Fat, and Protein Yield

Estimated effects of heat stress exposure, expressed as the number of days with a temperature–humidity index (THI) ≥ 68 during distinct early-life periods, first trimester of pregnancy (T1), second trimester (T2), third trimester (T3), and postnatal period (0–3 months), on 305-day milk, fat, and protein yield in Holstein Friesian cows. Effects are presented as the change in total 305-day yield (kg) per additional day with THI ≥ 68 , along with corresponding 95% confidence intervals (CI).

Table 3. Early-Life Cumulative Heat Stress (THIc) and 305-Day Milk, Fat, and Protein Yield

Parameters (n = 3,182)	Period	Estimate per unit increase (kg)	95% CI (kg)	Estimate per 30- unit increase (kg)	95% CI (kg)	P-value
Milk 305	Foetal T1	-1.63	[-2.41, -0.84]	-48.7	[-72.4, -25.1]	<0.001
	Foetal T2	0.01	[-0.65, 0.67]	0.4	[-19.4, 20.2]	0.967
	Foetal T3	-0.35	[-1.06, 0.36]	-10.6	[-31.9, 10.8]	0.332
	Postnatal	-1.23	[-2.72, 0.26]	-37.0	[-81.6, 7.7]	0.105
Protein 305	Foetal T1	-0.06	[-0.07, -0.03]	-1.66	[-2.38, -0.94]	<0.001
	Foetal T2	-0.01	[-0.02, 0.01]	-0.26	[-0.86, 0.34]	0.397
	Foetal T3	-0.01	[-0.02, 0.01]	-0.14	[-0.78, 0.51]	0.682
	Postnatal	-0.02	[-0.07, 0.02]	-0.73	[-2.12, 0.65]	0.299
Fat 305	Foetal T1	-0.04	[-0.07, -0.01]	-1.29	[-2.21, -0.38]	<0.001
	Foetal T2	0.01	[-0.02, 0.02]	0.12	[-0.64, 0.87]	0.765
	Foetal T3	0.03	[-0.01, 0.05]	0.81	[-0.02, 1.63]	0.056
	Postnatal	0.01	[-0.04, 0.05]	0.14	[-1.45, 1.73]	0.859

Estimated effects of cumulative heat stress exposure (THIc), defined as the sum of daily temperature–humidity index values exceeding the threshold ($\text{THI} \geq 68$), during distinct early-life periods on 305-day milk, fat, and protein yield in Holstein Friesian cows. Effects are expressed as the change in total 305-day yield (kg) per unit increase and per 30-unit increase in cumulative THI above the threshold, together with corresponding 95% confidence intervals (CI).

3.4.2. Effects of early-life heat stress on reproductive development and fertility traits

The analysis of AFI showed that prenatal heat stress exposure had period-specific effects. Heat stress during the second trimester of foetal development (T2) was significantly associated with delayed AFI. Specifically, each additional day with $\text{THI} \geq 68$ during foetal T2 increased AFI by 0.20 days (95% CI [0.10, 0.30], $p < 0.001$), corresponding to a delay of 6.01 days per 30 days of exposure (95% CI [3.12, 8.89]). Similarly, heat stress exposure during the third trimester (T3) had a significant but smaller effect, with each additional day with $\text{THI} \geq 68$ increased AFI by 0.12 days (95% CI [0.01, 0.24], $p = 0.032$), equivalent to 3.70 days per 30 days of exposure (95% CI [0.32, 7.08]). In contrast, heat stress during the first trimester of gestation (T1) did not significantly affect AFI (0.04 days/day; 95% CI [-0.08, 0.16], $p = 0.503$), nor did postnatal heat stress (-0.11 days/day; 95% CI [-0.24, 0.02], $p = 0.463$) (Figure 7).

The analysis of AFC revealed clear period-specific effects of prenatal heat stress exposure. Heat stress during the second trimester of foetal development (T2) was significantly associated with a delay in AFC. Each additional day with THI ≥ 68 during foetal T2 increased AFC by 0.19 days (95% CI [0.10, 0.29], $p < 0.001$), corresponding to an increase of 5.82 days per 30 days of exposure (95% CI [2.86, 8.79]). Similarly, heat stress exposure during the third trimester (T3) had a significant, albeit smaller, effect. Each additional day with THI ≥ 68 during T3 was associated with an increase of 0.13 days in AFC (95% CI [0.01, 0.24], $p = 0.030$), equivalent to 3.82 days per 30 days of exposure (95% CI [0.37, 7.27]). In contrast, heat stress exposure during the first trimester of gestation (T1) was not significantly associated with AFC (0.05 days/day; 95% CI [-0.07, 0.18], $p = 0.393$), nor was postnatal heat stress (-0.17 days/day; 95% CI [-0.40, 0.06], $p = 0.259$) (Figure 7).

Consistent with these findings, analyses based on cumulative heat stress load confirmed that prenatal heat stress exposure during the second and third trimesters of gestation was significantly associated with increased AFI and AFC (Table 13).

Heat stress exposure during the foetal period, first, second, and third trimesters, as well as during the postnatal period, showed no significant association with SPC or FSCR (all $p > 0.05$; Incidence Rate Ratios for SPC and Odds Ratios for FSCR were close to 1.00 across all developmental periods). Similarly, analyses based on cumulative heat stress load (THIc) revealed no significant effects on SPC or FSCR. Overall, these findings indicate that prenatal heat stress during mid- to late gestation delays reproductive maturation but does not appear to impair adult conception efficiency.

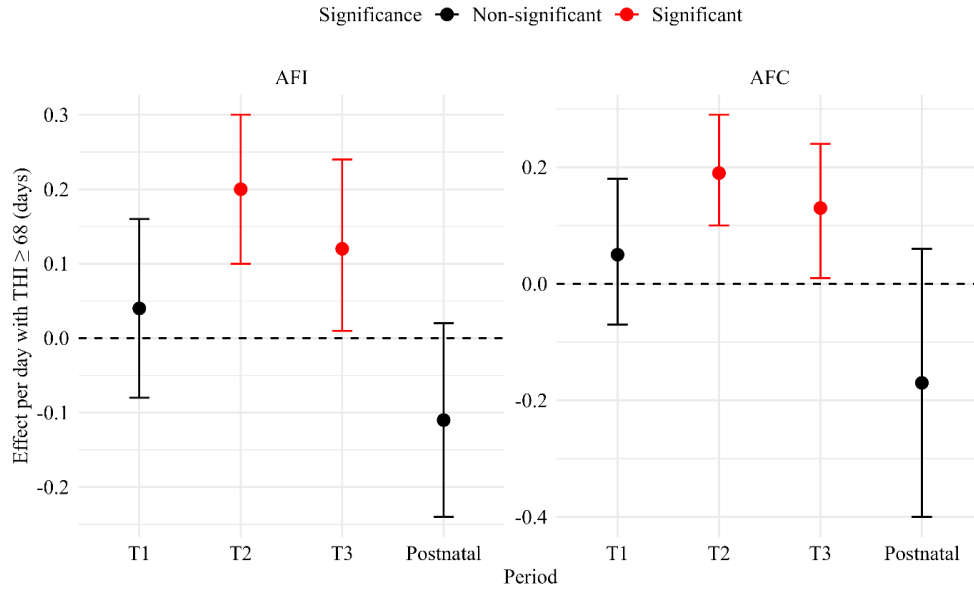


Figure 7. Effects of Early-Life Heat Stress Days on AFI and AFC

Estimated effects of heat stress exposure, expressed as the number of days with a temperature–humidity index (THI) ≥ 68 during distinct early-life periods, first trimester of pregnancy (T1), second trimester (T2), third trimester (T3), and postnatal period (0–3 months), on AFC, AFI in Holstein Friesian cows. Effects are expressed as changes in AFI and AFC (days) per additional day with THI ≥ 68 , together with corresponding 95% confidence intervals (CI).

Table 4. Effects of Early-Life Cumulative Heat Stress (THIc) on AFI and AFC

Parameters	Period	Estimate per unit increase (days)	95% CI (days)	Estimate per 30-unit increase (days)	95% CI (days)	P-value
AFI	Foetal T1	-0.01	[-0.02, 0.02]	-0.09	[-0.84, 0.66]	0.812
	Foetal T2	0.03	[0.01, 0.05]	1.02	[0.38, 1.67]	0.001*
	Foetal T3	0.02	[0.00, 0.04]	0.69	[0.01, 1.37]	0.045*
	Postnatal	-0.04	[-0.09, 0.00]	-1.27	[-2.71, 0.17]	0.085
AFC	Foetal T1	0.00	[-0.02, 0.02]	-0.03	[-0.78, 0.73]	0.945
	Foetal T2	0.03	[0.01, 0.05]	0.95	[0.29, 1.61]	0.005*
	Foetal T3	0.02	[0.00, 0.05]	0.68	[-0.02, 1.37]	0.006*
	Postnatal	-0.04	[-0.01, 0.00]	-1.43	[-2.90, 0.04]	0.564

Estimated effects of cumulative heat stress exposure (THIc), defined as the sum of daily temperature–humidity index values exceeding the threshold (THI ≥ 68), during distinct early-life periods on age at first insemination (AFI) and age at first calving (AFC) in Holstein Friesian cows. Effects are expressed as changes in AFI and AFC (days) per unit increase and per 30-unit increase in cumulative THI above the threshold, together with corresponding 95% confidence intervals (CI).

4. CONCLUSIONS

This dissertation demonstrates the multifaceted effects of heat stress on dairy cows, encompassing metabolism, production, reproduction, and behaviour.

The first study demonstrated that heat stress with short term sun exposure significantly reduced milk yield and milk quality, including fat and protein content, and caused marked alterations in key metabolic hormones such as insulin, prolactin, and NEFA, indicating disruption of energy balance and physiological homeostasis. Importantly, coat colour did not confer any protective effect under confinement housing, suggesting that management practices and environmental conditions are more decisive than phenotypic traits in mitigating heat stress. The second study revealed that in continental climates, spring is already a hot period, with THI frequently reaching or exceeding 68. During these warmer months, insulin and prolactin levels increased, reflecting alterations in glucose metabolism and lactational stress responses, while NEFA levels remained stable, suggesting limited changes in lipid mobilisation. These findings indicate that even early seasonal heat can influence metabolic homeostasis and energy balance, highlighting the need for early-season monitoring and intervention. The third study identified a THI threshold of ≥ 59 on the day of insemination as critical for reducing conception success, with the pre-insemination period from 21 to 6 days before insemination being the most sensitive. Genetic variability in heat stress response was also observed, suggesting potential for selective breeding to improve reproductive resilience. The fourth study showed that severe heat stress disproportionately affected late-lactation cows, with marked reductions in feeding, ruminating, and overall activity, whereas moderate stress had limited impact. These findings indicate that late-lactation cows are more susceptible to behavioural disruption under heat stress, challenging the common perception that this stage is less critical in terms of management priority. The fifth study highlighted long-term, stage-specific effects of prenatal heat stress. Early-gestation exposure led to reductions in first-lactation milk yield and composition, while mid- to late-gestation exposure primarily delayed reproductive development, as reflected in increased age at first insemination and calving, without impairing conception efficiency. These results underscore gestation as a critical window for thermal programming and the importance of cumulative heat exposure in shaping lifetime performance.

Overall, these findings emphasise that both the timing and severity of heat stress critically influence dairy cow performance and resilience.

5. NEW SCIENTIFIC RESULTS

1. Coat colour and heat stress with short-term sun exposure: No interaction was detected between coat colour and heat stress with short-term sun exposure on milk yield, milk composition, or metabolic biomarkers (insulin, prolactin, and NEFA). Black- and white-predominant Holstein-Friesian cows responded similarly under confined housing conditions with active cooling. This clarifies conflicting reports in the literature, showing coat colour is not a thermophysiological advantage under modern management.
2. Spring heat stress: In continental climates, spring now may exceed the heat-stress threshold (average THI 69.5), which was associated with elevated insulin (9.86 mIU/L) and prolactin (8.58 ng/mL) compared to seasons without heat stress (insulin 5.92–6.30; prolactin 3.78–6.49; $P < 0.05$). These findings suggest that heat stress conditions may occur during spring under certain environmental circumstances.
3. Heat stress and reproduction: Heat stress during the long pre-insemination period (21–6 days before insemination, P1) most strongly reduces conception probability (OR = 0.697–0.823, $p < 0.001$), indicating this is a critical window for fertility.
4. Genetic resilience: Heritability of insemination success increases under severe heat stress (e.g., P1: 0.048 $\rightarrow$ 0.129), highlighting a genetic component for heat resilience and providing a target for selection of heat-tolerant cows.
5. Behavioural impacts: Severe heat stress (THI ≥ 77) markedly reduces eating (–46 to –60 min/day) and ruminating time (–37 min/day in late-lactation cows), while inactivity increases (+61 to +95 min/day). Late-lactation cows are most sensitive behaviourally and physiologically.
6. Prenatal heat stress: First-trimester exposure reduces 305-day milk yield (–48.7 kg/30 days of THI ≥ 68), milk protein (–1.66 kg), and milk fat (–1.29 kg), while second- and third-trimester stress delays reproductive milestones (age at first insemination +6 days, age at first calving +5.8 days). Postnatal heat stress has no significant long-term effects. This identifies gestation, especially the first trimester, as a critical window for thermal programming of productive traits.

6. PRACTICAL RESULTS

1. Coat colour: Not a useful selection or management criterion for heat tolerance under cooled housing systems.
2. Seasonal heat stress management: Spring heat stress (THI 69.5) necessitates implementing heat mitigation earlier in the year, not just in summer.
3. Reproductive management: Cooling from three weeks before insemination through one month after is critical. Genetic selection for heat-resilient cows could improve fertility under thermal stress.
4. Behavioural management: Prioritise late-lactation cows during severe heat stress ($\text{THI} \geq 77$) with cooling, feeding adjustments, or nutritional support to mitigate reductions in eating/rumination and increased inactivity.
5. Gestational management: Minimise heat stress during early pregnancy, particularly during the first trimester, by implementing effective cooling strategies and by strategically planning insemination or breeding schedules to avoid peak heat stress periods, when possible, as this period is highly sensitive and may influence both foetal development and long-term productive and reproductive performance.

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List of publications related to the dissertation

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Total IF of journals (all publications): 20,2

Total IF of journals (publications related to the dissertation): 13,3

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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