

# **DOCTORAL (PhD) DISSERTATION THESES**

**SZILVIA SZALAI**

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**HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES**

**POSSIBILITIES FOR REDUCING HEAT STRESS IN  
DAIRY COWS**

**SZILVIA SZALAI**

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## **Doctoral School**

Name: **Animal Biotechnology and Animal Science**

Discipline: Animal Breeding Science

### **Head of Doctoral School:**

**Prof. Dr. Miklós Mézes**

Professor, full member of MTA

Hungarian University of Agriculture and Life Sciences

Faculty of Agricultural and Environmental Sciences

Institute of Physiology and Nutrition,

Department of Feed Safety

**Supervisor: Dr. Ákos Bodnár**

Associate Professor

Hungarian University of Agriculture and Life Sciences

Faculty of Agricultural and Environmental Sciences

Institute of Animal Science

Department of Animal Husbandry Technology and Animal Welfare

**Co-supervisor: Dr. Viktor Jurkovich**

Senior Research Fellow

University of Veterinary Medicine Budapest

Centre for Animal Welfare

## **1. Background and objectives of the research**

The increasingly frequent occurrence of extremely hot weather conditions impairs the welfare and production parameters of cattle (Kovács et al., 2020), and may also increase their mortality rate (Morignat et al., 2015). Heat stress can have both direct and indirect effects on the animals' normal physiological, metabolic, hormonal functions and immune system. Less attention is paid to the protection of pre-weaning calves against heat stress, since their higher body surface/body weight ratio results in a lower heat load compared to lactating cows (Broucek et al., 2009) however, postnatal heat load can significantly influence the success of rearing (Tao et al., 2013; Dado-Senn et al., 2020). Different strategies can be applied to reduce heat load, such as shifting feeding times to cooler periods of the day (Polsky – Keyserlingk, 2017), or using dietary supplements, but above certain levels of temperature and humidity only evaporative cooling and ventilation systems (fans and water sprinklers) prove to be effective (Armstrong, 1994). Fans that enhance air movement and convection can reduce respiratory rate and rectal temperature, and may also promote dry matter intake (Ji et al., 2020). The protection of dry cows against heat stress is particularly important, as it can have negative effects on several parameters such as birth weight, calving process, onset of lactation, and the risk of transition diseases, all of which significantly influence the animals' lifespan and the profitability of milk production (LeBlanc, 2010). Based on rumination monitoring during the first 10 days of lactation, cows at greater risk of developing health problems in the first month of lactation can be identified early (Calamari et al., 2014). Prepartum rumination information shows promising results in identifying cows at risk of postpartum metritis and subclinical ketosis (Schirmann et al., 2013; Cocco et al., 2021). Poor fertility in summer is the result of cumulative stress effects, which influence the balance of reproductive hormones, impair oocyte quality, and inhibit optimal embryo development through direct and indirect mechanisms (Pereira et al., 2015; Dovolou et al., 2023). Increased body temperature alters the standing and lying behaviour of cows (Allen et al., 2015), and affects the duration and intensity of oestrus (Hansen, 1997).

The aim of our experiments was to explore the effects of heat stress on behavioural and physiological parameters during critical periods such as the pre-weaning calf stage, peak lactation, calving, and oestrus. We examined the impact of shading in calves and cooling in high-yielding cows on various behavioural and physiological parameters, as well as the predictability of periparturient diseases and changes in oestrus behaviour under heat stress conditions.

## Objectives

In my dissertation, I examined the significance of heat stress among Holstein-Friesian dairy cattle during pre-weaning period and in different life stages of adult lactating cows. The increasingly long and hot summers caused by climate change pose a major challenge for the dairy sector. In order to maintain profitable production, rational measures need to be implemented. For this, it is essential to monitor the physiological and behavioural changes of the animals and to provide them with the most optimal environment possible, especially during critical periods.

In the dissertation, I investigate the physiological and behavioural changes arising from heat stress in four different life stages:

1. Examination of the effect of shading on behavioural and physiological changes of dairy calves depending on ambient temperature and humidity. Monitoring of different parameters (body temperature, respiratory rate, heart rate, salivary cortisol) during heat stress and thermoneutral periods.
2. Evaluation of the efficiency of old and new types of fans through the behavioural and physiological parameters of dairy cows, including the study of the animals' microenvironment, body temperature, feed intake and rumination time, lying behaviour, and milk production during heat load periods.
3. Impact of peripartur heat stress on the occurrence of diseases during the transition period (ketosis, mastitis, metritis). Analysis of correlations of data collected by precision tools (neck transponder, rumen bolus) such as rumen temperature, rumination time, activity, as well as the possibilities for predicting the onset of diseases.
4. Behavioural differences underlying reduced fertility results in summer, monitoring of oestrus expression from multiple aspects (video recordings, activity sensors, visual observations). Determination of different hormone levels from blood samples in heat stress and thermoneutral periods, and evaluation of conception success.

## 2. Materials and methods

The effects of heat stress on behavioural and physiological parameters were examined in different age groups and lactation stages, focusing on critical periods affecting the dairy cattle sector, such as the pre-weaning stage, peak lactation, calving, and oestrus. For measuring temperature and relative humidity, VOLTCRAFT DL-181THP data loggers (Conrad Electronic SE, Hirschau, Germany) were placed in the microenvironment of the animals. The temperature-humidity index (THI) was calculated using the formula of Mader et al. (2006):

$$\text{THI} = (0,8 \times T) + (\text{RH}/100) \times (T - 14,4) + 46,4$$

where: T = dry-bulb air temperature (°C), RH = relative humidity (%)

### 2.1. First experiment

The acute physiological responses of pre-weaning dairy calves to heat stress were evaluated under shaded and unshaded environments. Rectal temperature, respiratory rate, and heart rate were measured, seeking correlations between physiological indicators, ambient temperature, relative humidity, and THI. In addition, a non-invasive salivary sampling protocol was developed for cortisol concentration measurement, which allowed the evaluation of the beneficial effect of shading.

#### 2.1.1. Experimental design and animals

Calves were acclimated to the shading structure for 2 days prior to the trials. On the 1st experimental day (control, max. 28.3 °C), all calves (n = 16) (age =  $46.7 \pm 2.4$  days, body weight =  $74.3 \pm 2.6$  kg) were shaded for 24 hours. Thereafter, the shade ( $32.5 \times 3.4$  m green raschel net) was removed from half of the calf hutches, creating shaded (n = 8) and unshaded (n = 8) groups. The 2nd experimental day was considered the heat-stress day (max. 37.7 °C in the shaded calf hutches environment), while days 3–5 represented the post-stress period (day 3 max. 30.3 °C, day 4 max. 26.5 °C, and day 5 max. 24.3 °C in the shaded calf hutches environment).

#### 2.1.2. Behavioural and physiological parameters

Respiratory rate (breaths/min) was observed by counting the movement of the lateral abdominal muscles during breathing and recorded every 4 hours for each experimental animal, between 0:00 on day 1 (first observation) and 24:00 on day 5 (last observation). Immediately following respiratory rate observation, rectal temperature was measured (Digi-Vet SC 12; Jørgen Kruuse A/S, Langeskov, Denmark).

Heart rate was recorded using a Polar V800 watch (electrode belt + sensor) continuously from 0:00 on day 1 until 24:00 on day 5. For saliva sampling, synthetic swabs were used (Salivette cortisol, Sarstedt, Nümbrecht-Rommelsdorf, Germany) at 4-hour intervals. Cortisol concentration was determined using a direct radioimmunoassay (RIA) method without extraction.

## **2.2. Second experiment**

The effectiveness of cow and barn cooling was investigated using fans providing higher air velocity, based on different physiological and behavioural parameters.

### **2.2.1. Experimental design and animals**

From two high-yielding groups, 20 animals each were selected in experimental (barn 1B, new louvered temperature-dependent performance fans; VES ECV Air; VES-Artex, Chippewa Falls, WI, USA) and control (barn 3B, existing old-type fans) barns. Both barns were open on the southern side, deep-bedded, equipped with external paddocks and a covered feeding alley with misting system. Sampling was carried out on four occasions during the summer.

### **2.2.2. Behavioural and physiological parameters, data collection**

Body temperature of the animals was recorded every 30 minutes using an iButton (Maxim Integrated Products Inc., San Jose, CA, USA) temperature logger fixed into a non-medicated CIDR applicator (Zoetis Co., Parsippany, NJ, USA) over 5 days per sampling occasion. Lying behaviour was recorded over 3 consecutive days during each sampling period using a sensor-based logger (HOBO Pendant G Logger; Onset Computer Corp., Bourne, USA) attached to the left hind leg with elastic bandage. Feed intake, rumination, and daily milk yield were analysed from herd management system data (AfiCollar; Afimilk, Afikim, Israel) between May and August, corresponding to 80–180 days in milk.

## **2.3. Third experiment**

In this trial, body temperature, activity, and rumination time of cows were monitored during the heat stress period from 5 days before to 14 days after calving, in healthy and transition-disease groups. By analysing data collected through precision devices (neck transponders, rumen boluses), associations were sought for the prediction of diseases, as well as for evaluating the recovery of physiological parameters under heat stress.

### 2.3.1. Experimental design and animals

A total of 40 clinically healthy multiparous cows (average parity:  $3.1 \pm 1.2$ ; body condition score (BCS):  $3.7 \pm 0.3$ ) were included during the heat stress period (June–August). Based on peripartum health status, two groups were formed: healthy (HE,  $n = 26$ ; no disease observed until 14 DIM) and diseased (DI,  $n = 14$ ; diagnosed with transition disorders such as ketosis, mastitis, metritis, or retained placenta).

### 2.3.2. Behavioural and physiological parameters

Rumination time and activity were measured with neck transponders (RuminAct® HR-Tags, SCR Engineers Ltd., Netanya, Israel), recording data at 2-hour intervals. Reticulorumen temperature was recorded every 10 minutes using boluses (SmaXtec Animal Care GmbH, Graz, Austria). Data generated during the experimental period were exported from the Heattime Pro program and the SmaXtec application into Excel spreadsheets for further statistical analysis.

## 2.4. Fourth experiment

The aim of this experiment was to investigate behavioural and physiological changes underlying the marked decrease in conception rates during summer. Under heat stress, oestrous expression is considerably reduced, and hormonal profiles, activity level, and activity distribution are altered.

### 2.4.1. Experimental design and animals

The trial involved 58 clinically healthy cows with synchronised ovulation, studied during thermoneutral (NHS; THI<sub>max</sub> = 48;  $n = 28$ ) and heat-stress (HS; THI<sub>max</sub> = 80;  $n = 30$ ) periods. In both periods, animals were divided into G7G and OvSynch (OVS) groups according to the applied hormonal program, as follows: HSG7G:  $n = 15$ ; DIM:  $68.1 \pm 1.9$ ; parity:  $2.1 \pm 1.2$ ; HSOVS:  $n = 15$ ; DIM:  $160.7 \pm 64.6$ ; parity:  $1.9 \pm 0.6$ ; NHSG7G:  $n = 15$ ; DIM:  $66.1 \pm 1.6$ ; parity:  $2.7 \pm 1.2$ ; NHSOVS:  $n = 13$ ; DIM:  $157.3 \pm 64.1$ ; parity:  $2.5 \pm 1.3$ . Cows at  $48 \pm 3$  DIM were assigned to the G7G protocol, while those failing to conceive after insemination entered the OVS program. The G7G protocol included prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>; cloprostenol, 0.250 mg, 2 ml, Syncoprost; Vetem S.p.A., Porto Empedocle, Italy) injection, followed 2 days later by gonadotropin-releasing hormone (GnRH; gonadorelin, 50 µg, 2 ml, Ovarelin; Ceva Santé Animale, Libourne, France), after which it followed the OVS program described below. Cows with negative pregnancy diagnosis after first insemination entered the OVS protocol. The modified 7-day Ovsynch protocol with double PGF treatment was applied as follows: GnRH, 7 days, PGF<sub>2α</sub>, 24 h, PGF<sub>2α</sub>, 32 h, GnRH, 16 h, followed by timed artificial insemination. Pregnancy diagnosis was performed on day 35 using the membrane slip technique.



#### 2.4.2. Blood sampling, oestrus detection, camera system, activity monitoring

Prior to insemination, blood samples were collected from the coccygeal vein for hormonal assays. Oestrus signs were scored on a 1–3 scale based on three parameters: amount of oestrous mucus, presence of mounting marks, and uterine tone. Oestrous behaviours were monitored in the 12 h prior to insemination using a Smart PSS night-vision camera system (DH Vision Inc., Hannover, MD, USA) covering the entire barn, the drive lane, and the holding pen. Activity data were recorded with neck transponders (BouMatic HeatSeeker; BouMatic, Madison, WI, USA).

### 3. Statistical methods

Data were tested for constant variance (Levene's test) and the Shapiro-Wilk test was used for testing the equality of error variances both for shaded and unshaded groups. Correlations between animal-based heat stress indicators and thermal parameters were assessed using Pearson correlation. Differences between correlation coefficients according to environment (shaded vs. unshaded) were tested at a significance level of 0,05 using a z-test based on Fisher's z-transformation. The effect of shading on the thermal microenvironment of the animals was compared using a t-test based on meteorological data at  $p < 0,05$ . Salivary cortisol concentrations in shaded and unshaded calves were compared using linear mixed models.

For evaluating the relationships between responses to heat stress and explanatory variables under more efficient cooling technology, linear mixed models were fitted. Fixed effects included in the models were: group (experimental vs. control), day/week of lactation, ambient temperature, sampling time, time of day, and their two-way interactions. The animal ID (ear tag) was included as a random effect to account for individual variation. Model selection was based on the Akaike Information Criterion (AIC), primarily by gradually removing non-significant factors. Post hoc comparisons between groups were performed using Bonferroni-Holm adjusted p-values. The significance level was set at  $p < 0.05$ .

The relationship between average and maximum daily rumen temperature and average and maximum daily THI was characterized using correlation coefficients. Daily average rumination time and activity were compared between groups using linear mixed models.

For the analysis of oestrus synchronization protocols and seasonal effects, categorical variables were compared within groups by season using Fisher's exact test. For continuous variables, parameter means were evaluated using two-way analysis of variance (ANOVA) with group, season, and their interaction as factors. Tukey's method was applied for post hoc multiple comparison corrections. Statistical significance was set at  $p < 0,05$  in all cases.

## 4. Results and evaluation

### 4.1. First experiment

#### 4.1.1. Relationship between physiological parameters and THI

On day 1 (control), no statistically significant differences were observed in the maximum and mean environmental temperatures ( $p = 0,875$ ,  $p = 0,920$ ) or in the THI ( $p = 0,930$ ,  $p = 0,945$ ). Values recorded between day 2 (00:00) and day 4 (24:00) demonstrated the positive effect of shading on the thermal environment. On day 2, the maximum temperature and THI measured at 16:00 in the unshaded individual pen environment were higher than in the shaded experimental group ( $43,7 \pm 0,1$  vs.  $37,7 \pm 0,1$  °C,  $p = 0,005$  and  $86,4 \pm 0,1$  vs.  $78,2 \pm 0,1$ ,  $p < 0,001$ ). Heat load was reduced in the shaded individual pens compared to the unshaded group, which was also reflected in the mean temperature on heat stress days ( $28,5 \pm 0,1$  vs.  $24,2 \pm 0,1$  °C;  $p < 0,001$ ) and in THI ( $78,1 \pm 0,1$  vs.  $71,3 \pm 0,1$ ;  $p = 0,011$ ).

The maximum and mean daily environmental temperatures in the shaded group were lower than those in the unshaded individual pen environment on the 3rd experimental day ( $p < 0,001$  and  $p = 0,005$ ) and 4th day ( $p = 0,008$  and  $p = 0,012$ ). Maximum and mean THI showed similar differences between shaded and unshaded groups during the first two post-stress days (day 3:  $p = 0,003$ ,  $p = 0,014$ ; day 4:  $p = 0,015$ ,  $p = 0,026$ ). On day 5, no differences were observed in maximum or mean daily environmental temperature ( $p = 0,395$ ,  $p = 0,420$ ) or THI ( $p = 0,526$ ,  $p = 0,545$ ). Rectal temperature showed moderate correlation, whereas physiological parameters were strongly correlated with meteorological values.

These results indicate that respiratory rate and heart rate are more appropriate than rectal temperature for assessing acute stress in dairy calves.

#### 4.1.2. Rectal temperature, respiratory rate, heart rate, cortisol

Although mean rectal temperature did not reflect the positive effect of shading on day 2 ( $p = 0,865$ ), significant but minor group differences were found in maximum rectal temperatures ( $0,5$  °C,  $p = 0,046$ ). These results suggest that the experimental animals' thermoregulatory capacity functioned properly. Larger differences were observed between days 2 and 3, as the maximum respiratory rate of calves in the unshaded environment was on average 25,9 and 17,8 breaths/min higher. Mean daily respiratory rate was also higher in unshaded calves compared to shaded peers on days 2 ( $p = 0,008$ ) and 3 ( $p = 0,010$ ). Unshaded calves continued to show significantly higher maximum respiratory rates on day 4 ( $p = 0,025$ ), indicating a longer acclimatization period to the heat-stressed environment.

Maximum and mean heart rates of unshaded calves were higher on day 2 ( $p<0,001$  and  $p=0,010$ ) and day 3 ( $p=0,005$  and  $p=0,012$ ), likely due to increased physical activity associated with more frequent breaths. Heart rate gradually increased during the day, peaking between 12:00 and 14:00, with minimum values observed at 02:00 for both shaded and unshaded calves throughout the experiment, except on day 5 when minimal occurred at 04:00.

Mean salivary cortisol in shaded calves ranged from 6,5 to 10,2 ng/g on day 1 (control), and in unshaded calves from 6,7 to 9,3 ng/g. On day 2, cortisol remained stable until 08:00 in both groups, followed by a sudden increase of 51% in shaded and 342% in unshaded calves, peaking around 12:00 ( $p=0,586$  and  $p<0,001$ ). Higher cortisol concentrations were observed in the unshaded group at 12:00, 16:00, and 24:00 ( $p<0,001$ ;  $p<0,001$ ;  $p=0,046$ ). In shaded calves, the decrease between 20:00 and 24:00 ( $p=0,009$ ) occurred similarly to the daytime rise (12:00–16:00,  $p=0,025$ ), but with a four-hour delay compared to unshaded calves. On day 3, both groups peaked at 12:00, then in unshaded calves cortisol decreased to half the peak value by 16:00 (48.3%,  $p=0,039$ ), whereas in shaded calves the decrease was milder (26.1%,  $p=0,675$ ). Day 4 values were similar to day 3 in both groups. On day 5, shaded calves ranged between 7,2 and 9,4 ng/g, and unshaded calves between 8,0 and 11,9 ng/g, returning to control-day levels in both groups.

## **4.2. Second experiment**

### **4.2.1. Barn microclimate, air velocity**

During the experiment, the daily average temperature exceeded 20 °C and occasionally reached daily means around 30 °C. The daily average THI between June and August, with a few exceptions, exceeded the heat stress threshold of 68. Temperature and THI were particularly high during the sampling periods. In both experimental and control barns, on a typical summer heat-stressed day, as expected, the lowest temperatures and THI values were recorded at dawn, between 03:00 and 05:00, after which heat load gradually increased, reaching maximum levels around 15:00.

Air velocity in the control barn ranged from 0,2–0,8 m/s, whereas in the experimental barn it ranged from 1,5–2,8 m/s, which is also illustrated schematically (Appendices 1–2). Based on 12 evenly distributed measurement points across the barns, the calculated average air velocity was  $0,32 \pm 0,27$  m/s in the control barn and  $2,3 \pm 0,46$  m/s in the experimental barn ( $p<0,001$ ).

### **4.2.2. Vaginal temperature, lying behavior, feed intake, rumination, milk production**

Vaginal temperature of the animals showed a characteristic daily rhythm during the three-day sampling periods across all four occasions and correlated well with barn temperature and THI. During heat stress periods, mean body temperature exceeded the 39,2 °C threshold, reaching maximum values up to 41,5 °C. The extra cooling effect of the new fans, providing higher air

velocity, was clearly reflected in the experimental group, where average body temperature values were lower.

With rising temperatures, animals spent less time lying down. During the second sampling period, which was slightly cooler than the others, lying time approached the desired level (10-12 hours). In other periods, the effect of heat stress was apparent, as animals spent less time lying (7-8 hours). The lowest lying time values were observed during the first period, when temperatures were highest.

Time spent on feed intake ranged from 4,5 to 6,5 hours/day during the experiment. A significant difference ( $p < 0,001$ ) between the two groups appeared from approximately the 21st week of lactation, primarily related to milk production.

Rumination time of the studied cows fell within the acceptable normal range under intensive management, with an average of 8 hours per day, which is excellent, especially under heat stress, likely due to high-quality, heat stress-optimized feeding. Rumination time slightly decreased as lactation progressed, but no significant differences were observed between the two groups.

Milk production analysis and graphs were prepared from the 80th day of lactation, when the animals were definitively allocated to experimental and control groups. The two groups differed by approximately 3 liters/day ( $3,82 \pm 1,78$ ), which was statistically significant at all time points ( $p = 0,0390$ ). This difference is partly explained by the experimental group cows spending more time feeding, presumably resulting in higher dry matter intake, and partly by lower body temperature due to better cooling, reducing the biological cost of heat stress (energy needed for thermoregulation), leaving more energy available for milk production.

### **4.3. Third experiment**

#### **4.3.1. Temperature-humidity index, rumen temperature**

During the experimental period, animals were exposed to substantial heat stress, as supported by rumen and ambient temperature measurements. Calvings occurred during this heat-stressed interval, potentially contributing to peripartum diseases due to the well-known immunosuppressive effect of heat stress. Rumen temperature ranged between 39 °C and 41 °C, indicating high heat load. A significant but moderate correlation was observed between rumen temperature and the temperature-humidity index (THI) ( $r = 0,27$ ;  $p < 0,0001$ ).

Average rumen temperature followed a similar pattern during the peripartum period for both healthy and diseased cows, with a pronounced physiological decrease on the day of calving. Daily mean rumen temperature was significantly associated with days to calving and days postpartum ( $p < 0,0001$ ), as well as THI ( $p < 0,0001$ ). However, the presence of postpartum diseases during the experimental period (-5 to +14 days relative to calving) did not significantly affect rumen

temperature. Rumen temperature fluctuations appeared to be influenced more by calving than by THI changes during this period. The physiological drop in body temperature around calving was observable even under heat stress. Maximum body temperature occasionally reached 41 °C, representing a substantial physiological load.

#### 4.3.2. Rumination, activity

Healthy cows had an average daily rumination time of  $360 \pm 45$  minutes prepartum and  $400 \pm 37$  minutes postpartum, whereas diseased cows showed lower values ( $330 \pm 37$  and  $291 \pm 31$  minutes, respectively). Daily average rumination time (-5 to +14 days relative to calving) was significantly influenced by days relative to calving ( $p < 0,0001$ ), presence of postpartum disorders ( $p < 0,0001$ ), and THI ( $p = 0,0293$ ).

Rumination gradually decreased in the last days of gestation, with the lowest value on the day before calving in the healthy group and on the second day postpartum (2 DIM) in the diseased group. Significant differences between groups were observed on day -1 and day 2. Rumination in healthy cows decreased but normalized within a few days, whereas in diseased cows recovery took considerably longer. Activity increased before calving and returned to normal by the fourth day postpartum in healthy cows, and by days 5–7 in the diseased group.

### 4.4. Fourth experiment

#### 4.4.1. Barn climate, hormonal analysis

Maximum THI values during the summer experiment frequently exceeded the threshold of 68, representing heat-stressed periods for cows, which did not provide favorable conditions for conception, embryo implantation and development. Among estrogen hormones, estradiol, responsible for estrous behavior, showed similar average concentrations in the G7G program regardless of season, whereas in the OVS program, concentrations were significantly higher in winter compared to summer ( $p < 0,001$ ). No significant seasonal differences were observed for other hormones examined (LH, insulin, prolactin, IGF-1).

#### 4.4.2. Estrous behavior, conception

During heat stress, most cows did not show estrus during the day. Some individuals moved actively, followed and sniffed, but typical standing and mounting behavior was not observed in the summer study. Standing and mounting behaviors were mainly seen in the morning when cows were waiting for insemination after milking. In contrast, during winter, most cows began displaying estrous behavior on the day before TAI, after the afternoon GnRH injection. Frequency of standing and mounting behavior also differed by season: five times more mounts in winter (total

125 winter vs. 25 summer, average  $10,4 \pm 12,4$ /cow in winter vs.  $2,1 \pm 4,0$ /cow in summer) and more than seven times as many standing estrus events (total 124 winter vs. 16 summer, average  $10,3 \pm 11,6$ /cow winter vs.  $1,3 \pm 11,6$ /cow summer) were observed based on camera recordings. Estrous behavior was more intense during winter in the G7G program.

When cows with low and high-intensity estrus were grouped together, the proportion of estrous cows was 53% in summer and 87% in winter. Almost half of the cows showed no estrous signs in summer. For the OVS program, no statistically significant difference was observed in the number of estrous cows between seasons, although the expression and timing of estrous behavior differed significantly.

Conception rates by season demonstrated that both hormone programs were more successful under thermoneutral conditions. In the G7G program, seasonal differences were not significant, whereas in the OVS program, significantly more cows conceived in the thermoneutral period ( $p=0,035$ ). In winter, 39% of cows conceived, compared to only 10% in summer, all receiving the G7G hormone program.

## **5. Conclusions and recommendations**

In our experiments, the effects of heat stress were investigated across different life stages, highlighting the importance of interventions to ensure both animal welfare and economic efficiency during periods of heat load.

Shading effectively mitigated the negative impacts of heat stress on physiological and endocrine parameters in pre-weaning dairy calves. Our findings clearly support the establishment of an optimal microclimate—primarily through shading—during summer months, as this improves calf comfort, reduces acute stress, and contributes to a more favorable housing environment in terms of welfare and animal health. Heart rate and respiratory rate, which can be measured in real time using non-invasive methods, represent useful tools for rapid detection of heat stress and targeted implementation of preventive measures.

Increased air velocity improved thermal comfort, facilitated body temperature regulation, and supported higher feed intake and milk production. Considering global temperature increases and their negative effects on livestock, optimizing cow and barn cooling - including increased airflow - offers a cost-effective and sustainable strategy for managing heat stress.

Heat stress during the peripartum period significantly affects the health of lactating cows, influencing rumination time, reticulorumen temperature, and activity. Our study confirmed that rumination time decreases in the final stages of gestation, even before the onset of postpartum diseases, suggesting its potential as an early disease indicator. Reticulorumen temperature

decreased around calving, primarily influenced by the calving event itself, with only moderate correlation observed with the temperature-humidity index (THI). Activity increased before calving, but in diseased animals, this heightened activity persisted longer, likely reflecting discomfort and stress. These results underscore the value of precision monitoring systems for early detection and intervention, which can improve animal health and support the transition period.

Accurate estrus detection is fundamental for reproductive performance and herd productivity in dairy cattle. During heat stress, secondary estrus indicators—such as increased activity, mucus discharge, vulvar swelling, restlessness, vocalization, mounting, following, sniffing, and licking behaviors—become particularly important, as mounting may be absent or present as silent estrus. In our study, the G7G protocol maintained more balanced hormone status and stable estradiol levels even in summer, improving conception rates. Estradiol levels in G7G cows were similar in both summer and winter, whereas OVS cows showed significantly lower levels in summer.

The combined use of activity monitoring systems and ovulation synchronization, when applied appropriately, may represent an effective strategy for improving reproductive efficiency in dairy herds. Implementation of cooling systems prevents excessive body temperature elevation, supporting hormonal and metabolic balance. Ensuring consistent conception outcomes avoids fluctuations in calving intervals, contributing to sustained high milk production and overall farm profitability.

## **6. New scientific results**

1. Supplemental shading effectively mitigated the physiological and endocrine effects of heat stress in bull calves. On heat stress days, rectal temperature showed a moderate but significant correlation with meteorological parameters ( $r=0,44$ ;  $p=0,046$ ), whereas respiratory rate and heart rate exhibited strong significant correlations ( $r=0,74$ ;  $p=0,008$  and  $r=0,63$ ;  $p<0,001$ , respectively). Shaded calves displayed significantly lower salivary cortisol levels at 12:00, 16:00, and 24:00 ( $p<0001$ ;  $p<0,001$ ;  $p=0,046$ ).
2. Higher air velocity (1,5–2,8 vs. 0,2–0,8 m/s) effectively reduced body temperature in heat-stressed cows, with vaginal temperature averaging 0,5 °C lower in the experimental group. Significant differences between the experimental and control groups were observed in three out of four sampling periods ( $p=0,002$ ,  $p=0,037$ ,  $p=0,900$ ,  $p<0,0001$ ). Based on twelve evenly distributed measurement points in the barns, the average air velocity was  $0,32 \pm 0,27$  m/s in the control barn and  $2,3 \pm 0,46$  m/s in the experimental barn ( $p<0,001$ ).

3. During the heat stress period, increased prepartum activity was observed in both healthy and diseased cows. Activity returned to normal by the fourth day postpartum in healthy cows, whereas in diseased cows it remained significantly elevated ( $p < 0,05$ ) and normalized only between days 5 and 7. Rumination time, compared to the typical 7–8 hours for lactating cows, was reduced to 5–6 hours in healthy cows and only 4–5 hours in diseased cows during the days preceding calving, serving as an early indicator of potential postpartum health risks.
4. Reticulorumen temperature decreased physiologically around calving despite heat stress in both groups, showing a significant association ( $p < 0,0001$ ) with days remaining until calving, days postpartum, and THI. Rumination time was significantly influenced ( $p < 0,0001$ ) by the number of days around calving, the presence of postpartum disorders, and the temperature-humidity index ( $p = 0,029$ ).
5. The G7G protocol proved more effective than the shorter OvSynch program during the summer heat stress period, maintaining a more stable estradiol level (G7G mean 35,2 pg/ml [min. 27,6; max. 42,9]; OVS 20,7 pg/ml [min. 13,0; max. 28,3]) and achieving a higher conception rate (G7G 39%; OVS 10%).
6. Heat stress markedly reduced the expression and intensity of estrus behaviors, particularly the frequency of mounting and standing estrus events. During thermoneutral periods, cows exhibited an average of  $10,4 \pm 12,4$  mounts per cow, while during heat stress, only  $2,1 \pm 4,0$  mounts per cow occurred within the 12 hours preceding insemination. Temporally, cows exhibited mounting behavior only in the early morning around milking during summer, whereas in winter, active estrus signs appeared after the afternoon GnRH injection, providing a considerably longer window for estrus detection.

## **Publications Related to the Dissertation**

### **Publication in foreign scientific journal**

**Szalai, S., Bodnár, Á., Fébel, H., Bakony, M., & Jurkovich, V. (2025).** Rumination Time, Reticulorumen Temperature, and Activity in Relation to Postpartum Health Status in Dairy Cows During Heat Stress. *Animals*, 15(11), 1616. <https://doi.org/10.3390/ani15111616>



**Szalai, S.,** Bodnár, Á., Fébel, H., Bakony, M., & Jurkovich, V. (2025). Effects of Heat Stress on Estrus Expression and Pregnancy in Dairy Cows. *Animals*, 15(12), 1688. <https://doi.org/10.3390/ani15121688>

Jurkovich V., **Szalai Sz.**, Várhidi Zs., Dávid B., Kovács-Wéber M., Bakony M., Hejel P. (2025): The effect of improving barn cooling on the behavior and milk yield of dairy cows. *Journal of Thermal Biology*. Under review.

### **Publications in Hungarian scientific journal**

**Szalai Szilvia;** Bodnár, Ákos; Jurkovich, Viktor (2025): A hőstressz hatása az élettani és viselkedési paraméterek alakulására választás előtti borjaknál és tejelő teheneknél. *Scientia et Securitas* (5): Under review.

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