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The effects of improving barn cooling on the behaviour, physiological responses, gene expression, and milk yield of dairy cows

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ABSTRACT

Heat stress poses a significant challenge for dairy cows, particularly in warm climates, as it hampers their physiology, behaviour, and milk production. This study evaluated the effectiveness of a modern barn cooling system equipped with temperature-dependent fans in alleviating heat stress and enhancing cow welfare and productivity. The research was conducted on a large-scale Holstein Friesian dairy farm, comparing two high-yielding groups of around 100 cows each. The experimental group was housed in a barn with newly installed louvered, temperature-dependent circulation fans (providing high airspeed), while the control group remained in a barn with box ventilators (low airspeed). Data were collected over four three-day sampling periods during the summer. Environmental conditions were monitored using data loggers that recorded temperature and humidity, enabling calculation of the temperature-humidity index (THI). Cow body temperature was measured intravaginally. Additionally, time spent lying, eating, and ruminating was also tracked. Daily milk yields were obtained from the farm's database. Blood samples were also taken for gene expression measurements. Results indicated that THI values in both barns frequently surpassed the heat stress threshold (THI >68), yet air velocity in the experimental barn was notably higher (1.5–2.5 m/s) than in the control barn (0.2–0.5 m/s). Cows in the cooled barn showed significantly lower body temperatures in three of four periods. While lying time did not vary significantly, eating time increased in the experimental group later in the study. Moreover, milk yield was approximately 3 L/day higher ($p < 0.05$) in the experimental group. Enhanced ventilation led to complex changes in gene expression patterns, suggesting dynamic cellular responses to improved environmental conditions. These outcomes indicate that improved ventilation effectively reduces heat stress and supports better health, feeding behaviour, and milk production in dairy cows.

1. Introduction

Heat stress is a welfare issue for dairy cows during warm periods of the year, and the use of barn cooling is becoming increasingly important, even in continental regions. Ambient temperature, relative humidity, air velocity, and solar radiation are the primary factors contributing to heat stress, which significantly impacts the physiological and health status, as well as the well-being, of animals (Lacetera, 2019). In the case of cows that produce more than 35 kg/d of milk, heat stress begins at a THI of 68 (Collier et al., 2012). However, others (Pinto et al., 2020) observed changes in physiological reactions even at THI levels above 65. Cows adjust their behaviour in response to heat stress to enhance cooling. The

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respiratory rate (RR) of cows experiencing heat stress increases (Strong et al., 2015), while rumination, feed intake, milk yield, and milk composition decrease (Dikmen et al., 2020; Kim et al., 2021). As ambient temperature increases, dairy cows reduce their lying time by 30 % (Schütz et al., 2011). The increased standing time may contribute to lower milk production and higher prevalence of locomotory disorders (Polsky and von Keyserlingk, 2017). Heat stress affects not only physiological indicators and production (Almuhanna et al., 2021) but also rumen metabolism (Zhao et al., 2019). Additionally, heat stress can cause oxidative stress (Yu et al., 2020). In addition to reducing feed intake, cows also adjust their feeding schedules during heat stress to decrease digestive heat production (Min et al., 2019). Heat abatement strategies, including shading, ventilation, and evaporative cooling, mitigate these effects (Gunn et al., 2019). Enhanced ventilation reduces indicators of heat stress and improves milk yield, feed intake, and cow comfort (Ji et al., 2020; Kaufman et al., 2020; Reuscher et al., 2023). Various ventilation solutions are available for dairy farms, with installation costs ranging from 246 to 318 USD per barn; cross-ventilation systems are the most cost-effective option (Mondaca and Cook, 2019). Advanced approaches include perforated air duct systems, which have proven promising for regulating indoor environments in cattle barns. Slot-type outlets, more efficiently distributing air over lying cow surfaces, achieve more uniform skin temperatures (Cao et al., 2022). Modern cooling strategies also incorporate monitoring of individual animal responses using in vivo temperature sensors, providing real-time capabilities and personalised heat-stress mitigation (Levit et al., 2021). The frequency of cooling interventions influences their effectiveness: eight daily cooling sessions achieve superior respiratory rate reduction compared to three, resulting in an average decrease of 14 breaths per minute (Pinto et al., 2019). Selecting an efficient ventilation system is crucial for economic sustainability, as costs and effectiveness vary (Mondaca and Cook, 2019). Studies have shown that tunnel ventilation, combined with irrigation, can reduce heat effects by up to 84 % compared to conventional cooling technologies, such as those using fans and sprinkler systems, or cooling with shaded shelters and fans (Borshch et al., 2021). Wet material (curtain) fans can decrease building temperature while increasing humidity. Still, they generate relatively high wind speeds around animal bodies, which helps prevent heat stress (Chen et al., 2021).

Recent advances in molecular biology have revealed that heat stress triggers complex changes in gene expression patterns related to cellular protection, inflammation, and metabolism (Li et al., 2023). Heat shock proteins (HSPs) such as HSP90, HSP90 alpha family class A member 1 (HSP90AA1), and HSP family D member 1 (HSPD1) are upregulated during thermal stress to protect intracellular proteins from denaturation (Badri et al., 2018; Mendonca et al., 2025). Inflammatory cytokines, such as interleukin-8 (IL-8), increase during heat stress, indicating systemic inflammation (Das et al., 2016). Additionally, heat stress alters the expression of metabolic regulators, including the insulin receptor (IR) and prolactin (PRL), which affects energy metabolism and lactation physiology (Hernández-Cordero et al., 2017; Mendonca et al., 2025). Heat shock factor 1 (HSF1) serves as a master regulator of the heat stress response, activating the transcription of heat shock proteins (Sigdel et al., 2019). These molecular markers are valuable in understanding the cellular mechanisms of heat stress and the effectiveness of mitigation strategies.

Our study aimed to determine whether heat stress can be reduced through more efficient barn cooling, with fans providing higher airspeed. We aimed to assess the effects of increased air velocity on health, production, and behaviour of dairy cows. Furthermore, we investigated the molecular mechanisms underlying these effects by analysing the expression of heat-stress-related genes, including HSPs, inflammatory markers, and metabolic regulators, to provide comprehensive evidence of the cooling system's effectiveness at both physiological and cellular levels.

2. Materials and methods

2.1. Animals, housing, and study set-up

All animal treatments were performed according to the permission of the Government Office in Pest County, Budapest, Hungary (permit no.: PE/EA/1076-5/2020).

The study was conducted on a large-scale Holstein Friesian dairy farm in Hungary, which housed approximately 1100 cows. We formed an experimental (EXP) and a control (CO) group from two high-yielding groups. There were usually no more than 100 animals in both groups. Within each group, we selected 20 animals (group cores) based on the average milk yield of the week preceding the grouping, parity, and the days in milk (DIM), using the cow-pair method. From these animals, we took biological samples and performed individual tests and data collection. We planned four 3-day-long sampling and measurement periods during the summer (28–30 June, 12–14 July, 4–6 August, and 18–20 August). The study required clinically healthy animals, free of metabolic disorders at the beginning of lactation, with high milk production in the middle third of lactation (Table 1).

Three new, louvered circulation temperature-dependent fans (VES ECV Air; VES-Artex, Chippewa Falls, WI, USA; hereinafter 'EXP fans') were equipped in the barn of the EXP group, facing the open side of the barn (Fig. 1). The fans were set to turn on at 20 °C and to operate at full capacity at 25 °C. The barn of the CO group had been equipped with box fans with unknown technical details, installed at least 25 years ago (hereinafter 'CO fans'). There were 3 fans in the entire barn, of which 2 provided airflow to the CO group's resting area (Fig. 2). These CO fans were also set to turn on at 20 °C, but they only had one speed. The fans in both groups operated during the day and night if the ambient temperature reached the set level. There were no other differences in housing and feeding between the two groups. The animals were housed in a straw-yard barn (deep-bedded) that was open on the south side and had a large paddock. Straw was refreshed three times a day during each milking. The littered resting area was 16×35 m $= 560$ m^2 per group, which means at least 5.6 m^2 per cow (depending on group size; assuming a maximum of 100 cows per group). The animals were milked three times daily in a 40-stall Fullwood rotary parlour. A total mixed ration was offered two times daily; water was provided ad libitum. The feed and water troughs were placed outside the barn on the side of the paddock. The cows had access to the outside area and could move in and out freely.

2.2. Measurements

2.2.1. Barn microclimate

To characterise the microclimate in the animals' environment, we used Voltcraft DL-181THP data loggers (Conrad Electronic SE, Hirschau, Germany), which continuously measured ambient air temperature (°C) and relative humidity (%) inside the barn. The measurement frequency was set to 1 h. The data loggers were placed in the middle of the barns, 2 m above the bedding level, in April, and the data were downloaded at the end of August. For the analysis of the production data, values from the entire test period were used. In contrast, temperature values measured during the measurement periods were used for studying individual body temperature and activity data.

From the measured temperature and relative humidity values, we calculated a temperature-humidity index (THI) according to the equa-

Table 1
The data of the animals at the time of group formation.

Group	Average daily milk yield during the week before group forming (L)	Parity	Days in milk
Experimental	54.6 ± 14.2	2.1 ± 0.3	99 ± 10
Control	54.6 ± 9.3	2.2 ± 0.4	98 ± 10

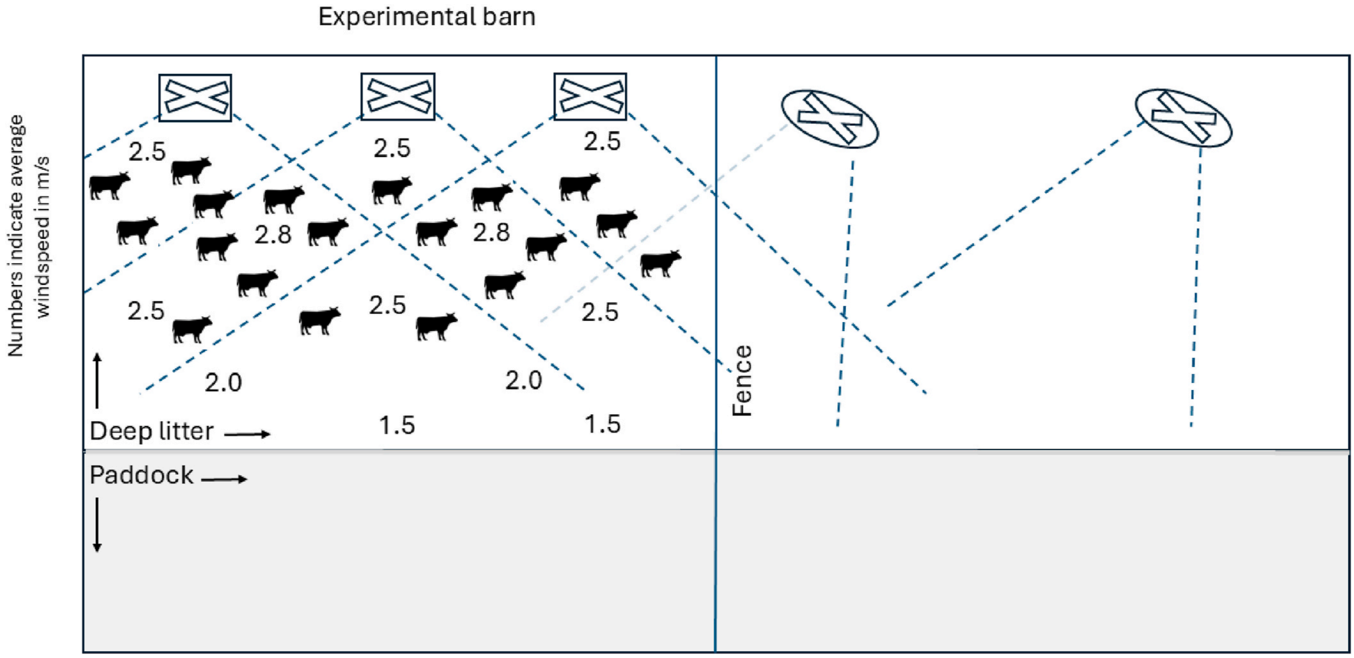

Fig. 1. Schematic layout and map of air velocities in the experimental barn. Image is not to scale.

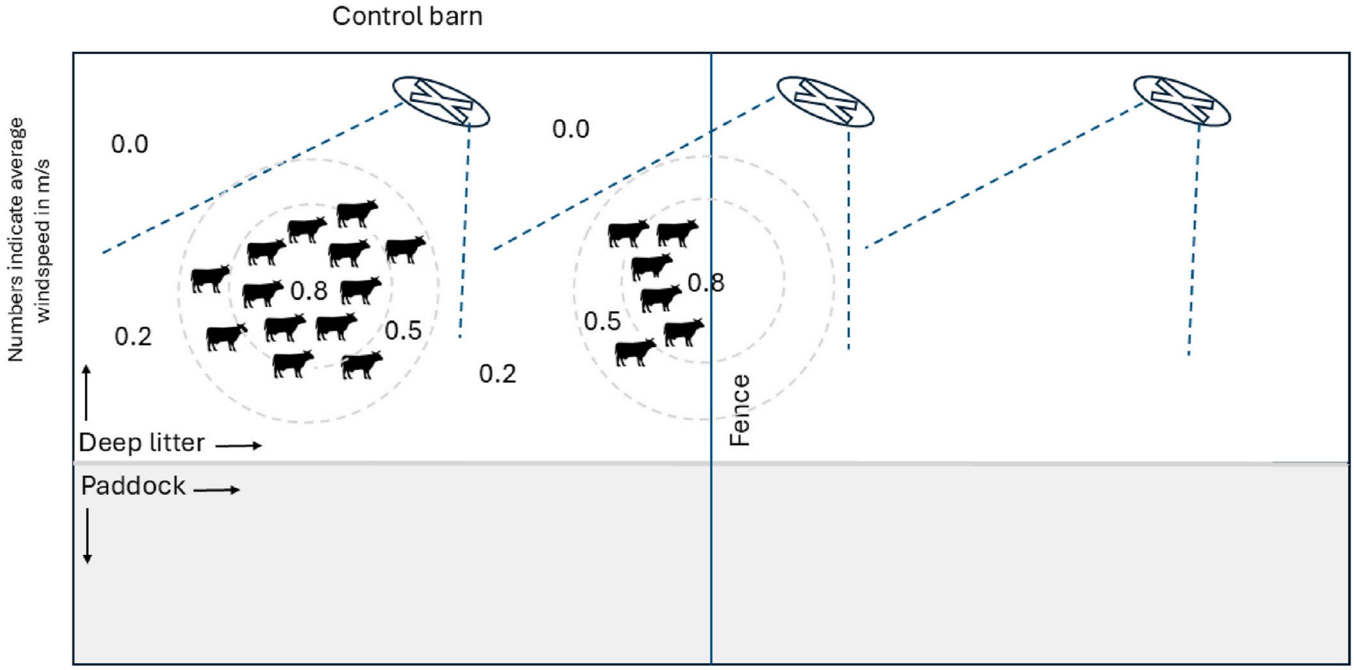

Fig. 2. Schematic layout and map of air velocities in the control barn. Image is not to scale.

tion by Mader et al. (2006):

$$THI = (0.8*T) + \left(\frac{RH}{100}\right)*(T - 14.4) + 46.4$$

where T stands for dry bulb ambient air temperature (°C), and RH stands for relative humidity (%). The threshold value of heat stress was set at 68 (Reiczigel et al., 2009; Collier et al., 2011).

Air velocity in the barns was measured once during the study period using a Testo 480 digital airflow meter (Testo SE & Co. KGaA, Titisee-Neustadt, Germany) on a day with no natural wind, when the fans were operating at full speed. In both the control and experimental barns, we measured the entire resting area by dividing it into 4 × 4 m cells and measuring air velocity in each cell (32 cells per barn).

2.2.2. *Body temperature, behaviour, and production data*

The body temperature of the animals in the core groups was measured with an intravaginally placed iButton (Maxim Integrated Products Inc., San Jose, CA, USA) temperature data logger attached to an empty, drug-free CIDR (Zoetis Co., Parsippany, NJ, USA). The data collectors were inserted at the beginning of the sampling periods and remained in place for 5 days. Continuous measurements were recorded at 30-min intervals.

At the time of iButton insertion, we placed an activity meter (HOBO

Pendant G logger, Onset Computer Corp., Bourne, USA) on the legs of the animals. The device measures acceleration and tilt on three spatial axes (x, y, z), making it suitable for distinguishing between walking, standing, and lying body positions. The device was positioned on the left rear leg of the animal, on the outer side of the midfoot, with cotton wool placed underneath and secured with a self-adhesive bandage. The sampling rate was set at 30-s intervals (Bonk et al., 2013). The device remained in place for 5 days. On the fifth day, the device was removed, the data downloaded, and the time spent lying down was determined from the x-axis acceleration values. Based on the method validated in the literature, the animal was considered to be lying down if the x-axis acceleration was <0.65.

We placed the iButton and HOBO devices in their positions in the morning (around 8 a.m.) before each sampling period. If we consider the sampling period between 28 and 30 June, for instance, the devices were attached in the morning of 27 June. Therefore, the cows were habituated to the devices on the first day of the sampling periods. Measurement of body temperature and lying behaviour began from midnight on the day the instruments were placed, lasted for three days, and concluded on the morning of the fifth day. This way, measurement data was generated for 3 days (0–24 h).

In addition, based on the data extracted from the farm database, we analysed the daily milk yield of the animals (AfiFarm, Afimilk Ltd., Kibbutz Afikim, Israel), as well as the time spent ruminating and eating (AfiCollar, Afimilk Ltd., Kibbutz Afikim, Israel) from May until August, approximately from the 80th DIM until the 180th DIM.

2.2.3. Gene expression analysis

We collected blood samples (3 ml) from the caudal vessels of the animals in the core groups into Tempus Blood RNA Tubes (Applied Biosystems Inc., Waltham, MA, USA) at the time of inserting the iButtons and HOBO loggers at each sampling period (Table 2) and immediately cooled them for transport. All samples were then stored at −20 °C until RNA extraction.

Total RNA was extracted from the samples with the Tempus Spin RNA Isolation Kit (Applied Biosystems, Waltham, MA, USA) according to the instructions. Samples were treated with DNase (catalogue number: 79256; Qiagen NV., Venlo, The Netherlands) to eliminate potential genomic DNA contamination. We measured RNA concentration using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and assessed RNA quality with 1.2 % agarose gel electrophoresis. The samples were then stored at −70 °C in RNase-free tubes until further analysis.

For cDNA synthesis, 300 ng of total RNA was reverse transcribed using the ABI High-Capacity cDNA RT Kit (catalogue number: 4368814; Applied Biosystems Inc., Waltham, MA, USA). The reaction mixture contained 2 μL of 10X RT buffer, 0.8 μL of 25X dNTP mix, 2 μL of 10X RT random primers, 1 μL of MultiScribe reverse transcriptase, 1 μL of RNase inhibitor, and 3.2 μL of nuclease-free water per reaction. The cDNA was diluted 10-fold, and 5 μL was used for each quantitative PCR (qPCR) reaction.

We performed qPCR using PowerUp SYBR Green Master Mix (catalogue number: A25741; Applied Biosystems Inc., Waltham, MA, USA). The thermal cycling conditions were as follows: UDG activation at 50 °C for 2 min, initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. Melting curve analysis was performed from 60 °C to 95 °C at a ramp rate of 0.15 °C/s.

Table 2
Blood sampling time and sample numbers.

Sampling date	Total samples	Control group	Experimental group
June 27	38	20	18
July 11	37	19	18
August 3	31	16	15
August 17	35	18	17

Eight genes of interest were analysed: heat shock proteins (heat shock protein 90 (HSP90), heat shock protein 90 alpha family class A member 1 (HSP90AA1), and heat shock protein family D (Hsp60) member 1 (HSPD1)), heat shock factor 1 (HSF1), interleukin-8 (IL8), insulin receptor (IR), prolactin (PRL) and hypoxanthine phosphoribosyltransferase 1 (HPRT1) as the housekeeping gene. Primer sequences are provided in Table 3. Gene expression was calculated using the Pfaffl method (Pfaffl, 2001).

2.3. Statistical analysis

Descriptive statistics were used to explore the distribution of response variables and patterns of association. Linear mixed models (random intercept models) were fitted to quantify the association between heat stress response variables and predictors, including group (conventional or improved cooling system), production day/week, ambient temperature, sampling time, and hour of the day, and two-way interactions as fixed effects, and cow identification number as a random effect. The model was selected based on the magnitude of the Akaike Information Criterion, primarily by removing non-significant terms. Diagnostic plots were used to check model fit. The Bonferroni-Holm adjustment of p-values was used during post-hoc comparisons between groups. The level of significance was set at 0.05. Data visualisation and hypothesis testing were performed using R 4.4.1 (R Core Team, 2025). The 'Rmisc', 'ggplot2', 'lmer', 'emmeans', and base packages were used. Table 4 summarises the responses and predictors/independent variables.

During statistical analysis of gene expression, independent samples t-tests or Mann-Whitney U-tests were used to compare groups, depending on the data distribution (assessed by the Shapiro-Wilk test) and variance homogeneity (assessed by Levene's test). The SPSS 26.0 (SPSS Inc., Chicago, IL, USA) software was used for this analysis.

3. Results

3.1. Barn microclimate

Figs. 1 and 2 show schematic maps of air velocity in the CO and EXP barns. The airflow ranged from 0.2 to 0.8 m/s in the CO barn and 1.5–2.8 m/s in the EXP barn. Based on the 32 measurement sites evenly distributed in the area of the barns, the average air velocity was 0.32 ± 0.27 m/s in the control barn and 2.3 ± 0.46 m/s in the experimental barn, respectively ($p < 0.001$).

Figs. 3 and 4 display the daily average ambient temperature and THI in the two barns throughout the entire study period, from May to August (ranging from 14 °C in May to 30 °C in June and July). The daily average THI exceeded the heat stress threshold (THI 68) on most summer days from June to August (Fig. 4), with a minimum of 58 in May and a maximum of 78 in June. The two barns showed no significant difference in temperature ($p = 0.804$) or THI ($p = 0.858$).

Figs. 5 and 6 display the average ambient temperature and THI in the barns during the four 3-day-long sampling periods. The nadir in daily temperature and THI occurs around 5 a.m., while the peak occurs around 3 p.m., exceeding the heat-stress threshold. During the second sampling period, a sharp reduction in daily average temperatures was observed, resulting in the heat load falling below the heat-stress threshold. The 1st (28–30 June), the 3rd (4–6 August), and the 4th (18–20 August) sampling periods were the hottest, with the maximum daily THI exceeding 80. The nadir did not fall below the threshold even during the nights of the 1st and 4th periods.

3.2. Body temperature

Fig. 7 shows the average vaginal temperature (VT) of the cows during the four 3-day-long sampling periods. The body temperature of

Table 3
Primer sequences used for qPCR analysis.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
HPRT1	TGCTGAGGATTTGGAGAAGG	CAACAGGTCGGCAAAGAACT
HSF1	CCAGCAACAGAAAGTCGTCA	GGGGGATCTTTCTCTTCACC
HSP90	GGAGGATCACTTGGCTGTC	GGGATTAGCTCCTCGCAGTT
HSP90AA1	GCATTCTCAGTTCATTGGCTATCC	GTCCTTCTTCTCTTCCTCCTCTTC
HSPD1	ACTGGCTCCTCATCTCACTC	TGTTCAATAATCACTGTCCTTCC
IL8	AGAACTTCGATGCCAATGCAT	GGGTTTAGGCAGACCTCGTTT
IR	AGGAGCTGGAGGAGTCCTCGTTCA	CATTCCCCACGTCACCAAGGGCTC
PRL	GAACCTCAGGCCCATCCCT	TCCGGATTCTCCAGCTTCTC

Table 4
The response and predictors/independent variables for statistical analysis.

Response variables	Predictors	Controlling variables
dry bulb temperature	group	sampling period, hour of the day
temperature humidity index	group	sampling period, hour of the day
vaginal temperature	group	sampling period, hour of the day
daily average of time spent lying	group	sampling period
daily average of time spent eating	group	lactation week, average daily THI
daily average of time spent ruminating	group	lactation week, average daily THI
daily milk yield	group	lactation week, temperature-humidity index

the animals followed a pattern similar to the daily temperature and THI curves, with a nadir in the morning and a peak in the afternoon. Hourly comparisons between the groups showed that during the sampling periods with the highest heat loads (the 1st and the 4th periods), the average body temperature of the EXP group was consistently lower than that of the CO group throughout most of the day (Fig. 7, Table 5). The highest body temperatures were observed during the 1st sampling period, when the VT did not fall below the heat-stress threshold of 39.2 °C. The VT values were the lowest during the 2nd sampling period, compared to the other periods.

3.3. *Behaviour of the animals*

Fig. 8 shows the average lying time during the sampling periods. The highest values (CO: 630 ± 22 min/day; EXP: 650 ± 20 min/day) were seen during the second sampling period. There was no difference between the groups in lying times during the sampling periods ($p = 0.301$).

Figs. 9 and 10 display the time spent eating and ruminating. Eating time decreased during the study period in the CO group, from 6.2 ± 0.1 h/day to 5.2 ± 0.4 h/day. Eating time also decreased in the EXP group, but then increased towards the end of the study period from week 18 to 5.9 ± 0.4 h/day, resulting in a significant difference between the groups during the last few weeks ($p < 0.001$). Rumination time decreased as lactation progressed (from 8.1 ± 0.1 h/day in CO and 8.5 ± 0.1 h/day in EXP to 7.6 ± 0.3 h/day in CO and 7.4 ± 0.4 h/day in EXP), without a significant difference between groups ($p = 0.439$).

3.4. *Milk yield*

Fig. 11 shows the average daily milk yield during the study period. Milk yield decreased slightly as lactation progressed (from around 58 L/day to around 42 L/day), and it was higher in the EXP group ($p = 0.039$). The difference between the groups was on average 3.82 ± 1.78 L/day.

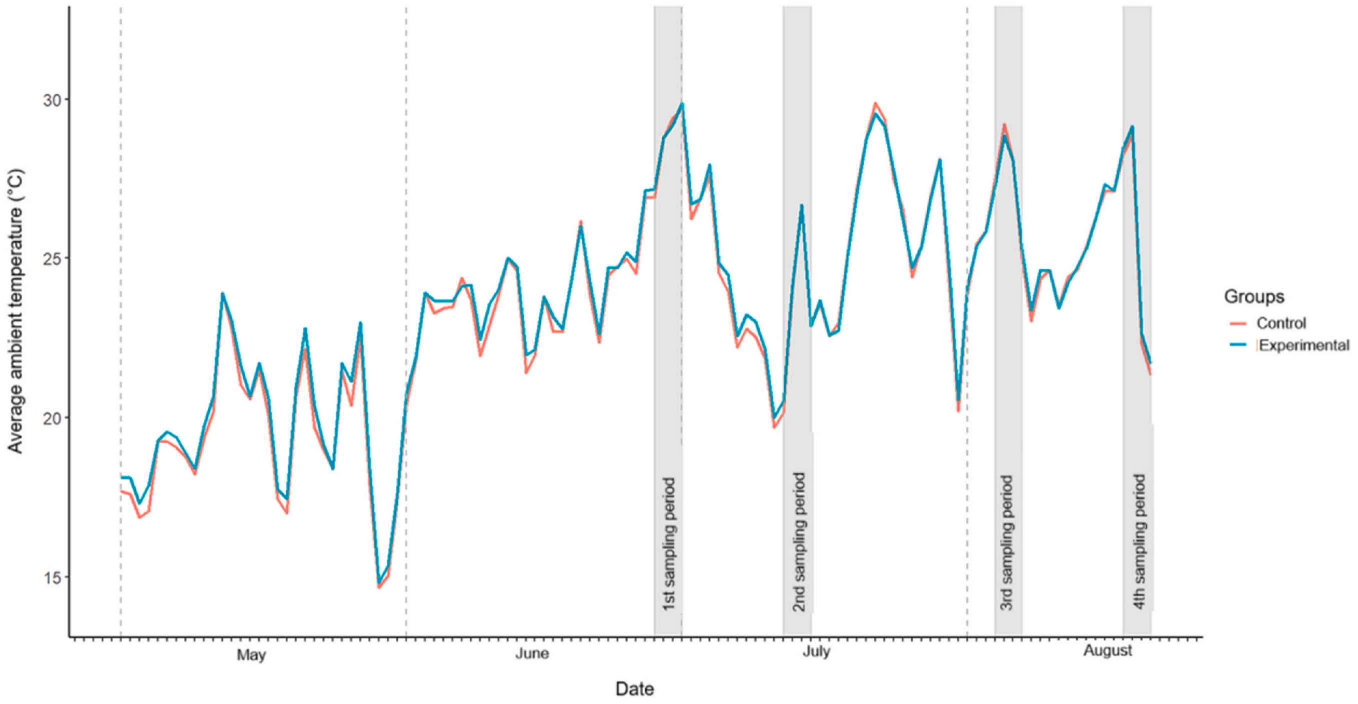

Fig. 3. The daily average ambient temperature in the two barns during the whole study period.

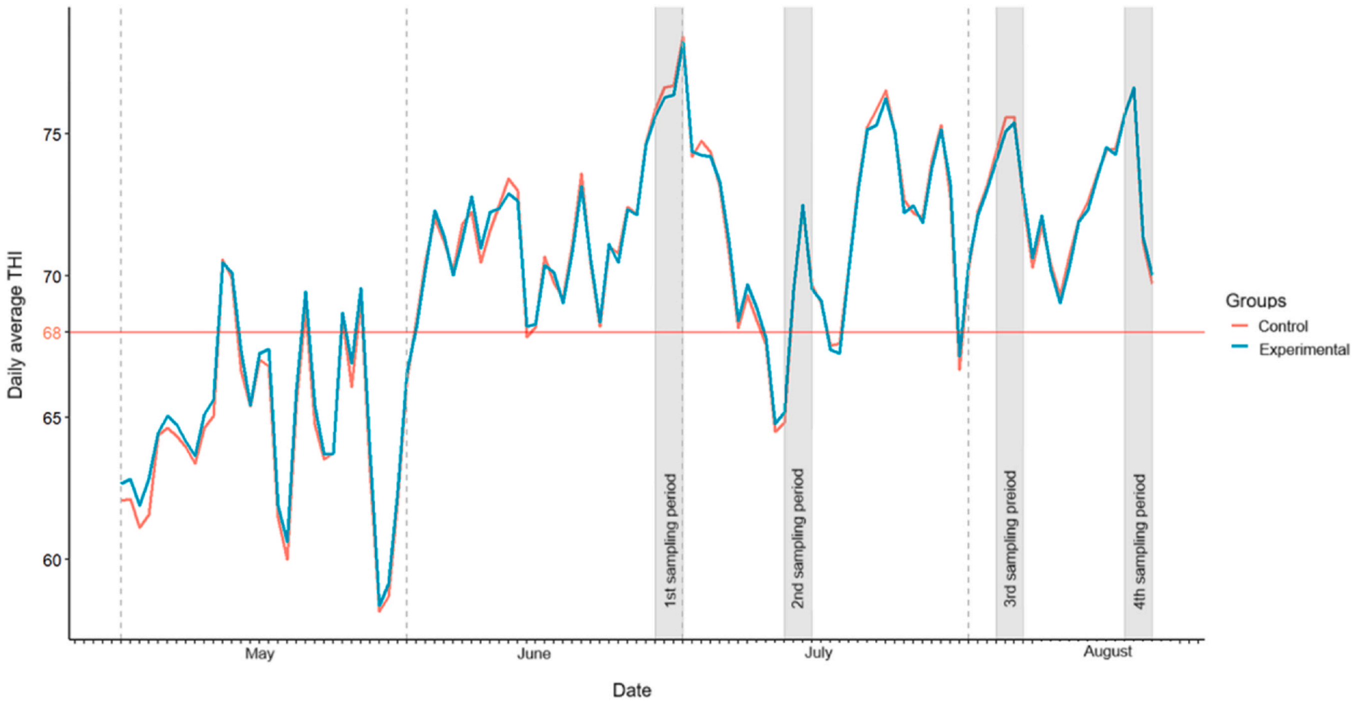

Fig. 4. The daily average temperature-humidity index (THI) in the two barns during the whole study period.

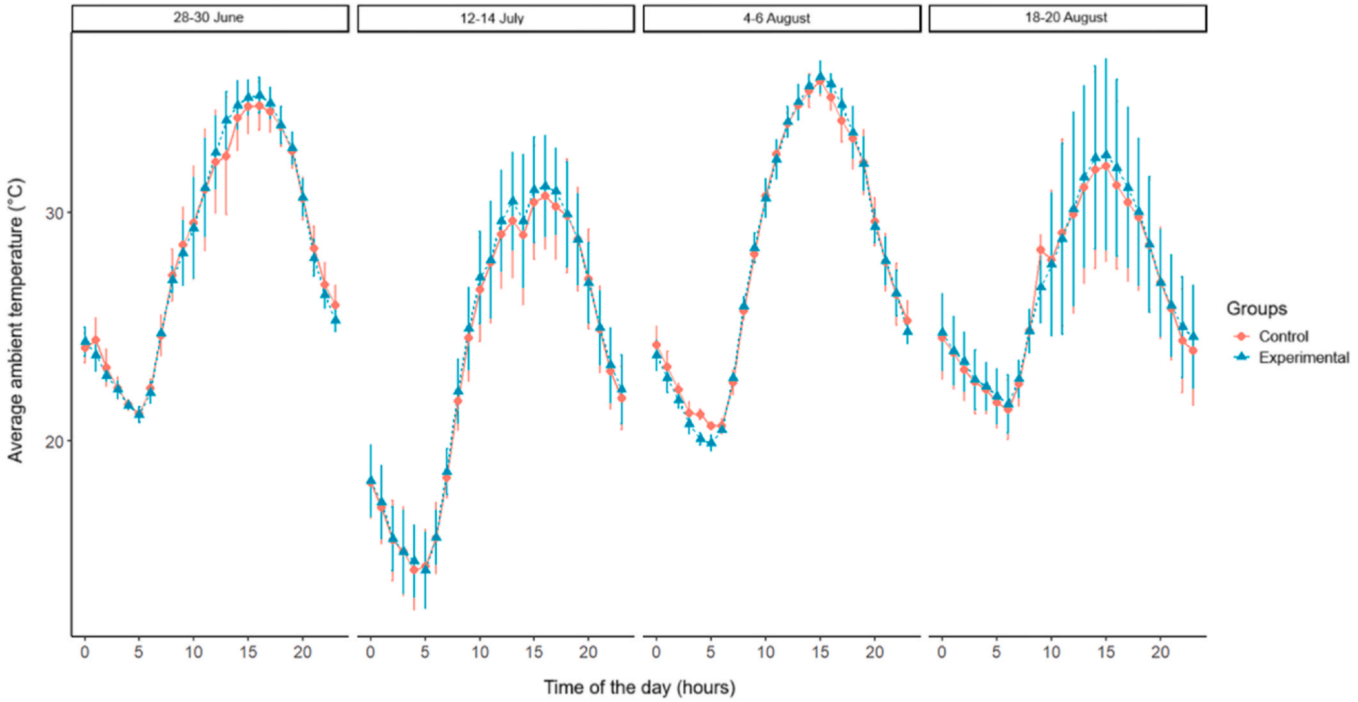

Fig. 5. The average hourly ambient temperature in the barns during the four 3-day-long sampling periods.

3.5. Gene expression analysis

The analysis revealed significant differences between the EXP and CO groups across multiple heat stress-related genes (Fig. 12).

Heat shock proteins: Heat shock protein expression patterns varied across sampling periods. HSF1 expression was lower in the experimental group during three of the four sampling periods (except August 4), with significant differences observed on June 28 and August 4 ($p < 0.05$). HSP90 expression was consistently higher in the EXP group than in CO, except on August 18 when it was significantly lower ($p < 0.05$). HSP90AA1 showed significant differences across all four sampling periods ($p < 0.05$), with the EXP displaying lower expression during three time points (June 28, July 12, August 18) but higher expression on August 4. HSPD1 expression was lower in the EXP group during June and July, but higher during both August sampling periods, with a significant difference on August 18 ($p < 0.05$).

Inflammatory marker: The expression of IL8 was consistently higher in the EXP group across all sampling periods, with a significant

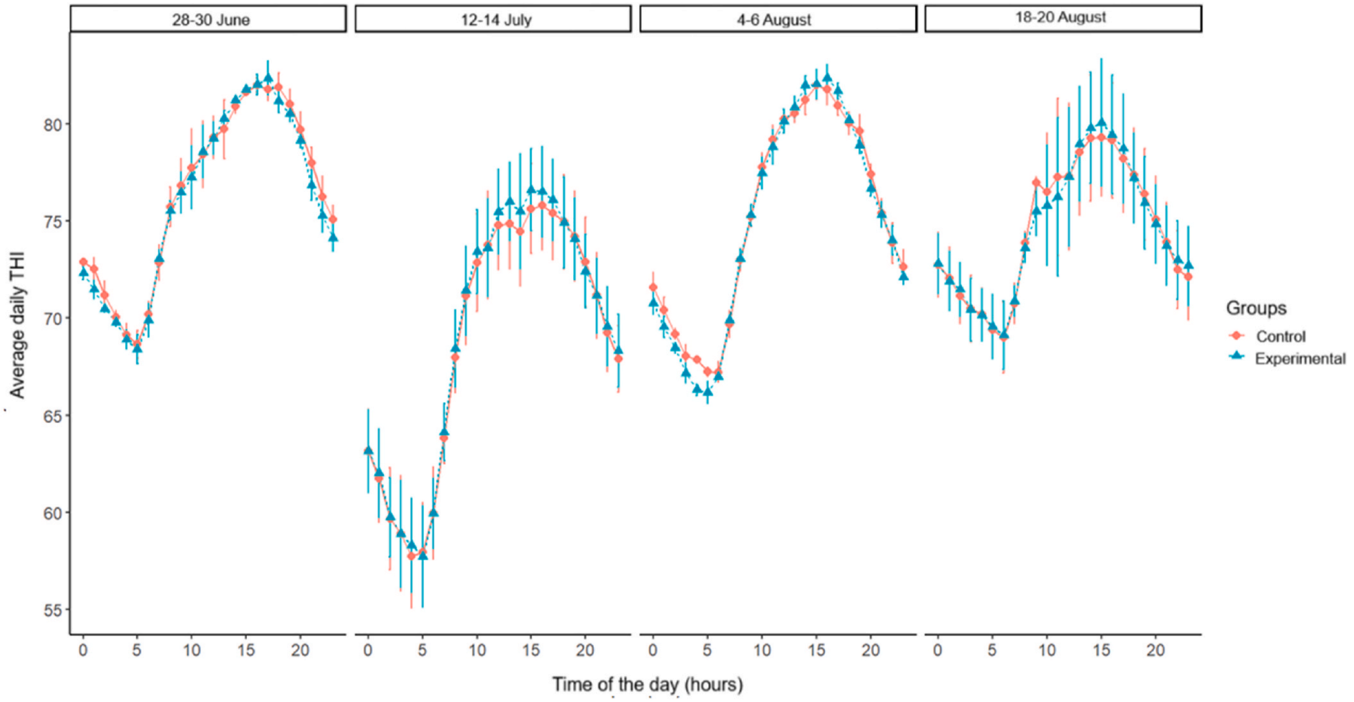

Fig. 6. The average temperature-humidity index (THI) in the barns during the four 3-day-long sampling periods.

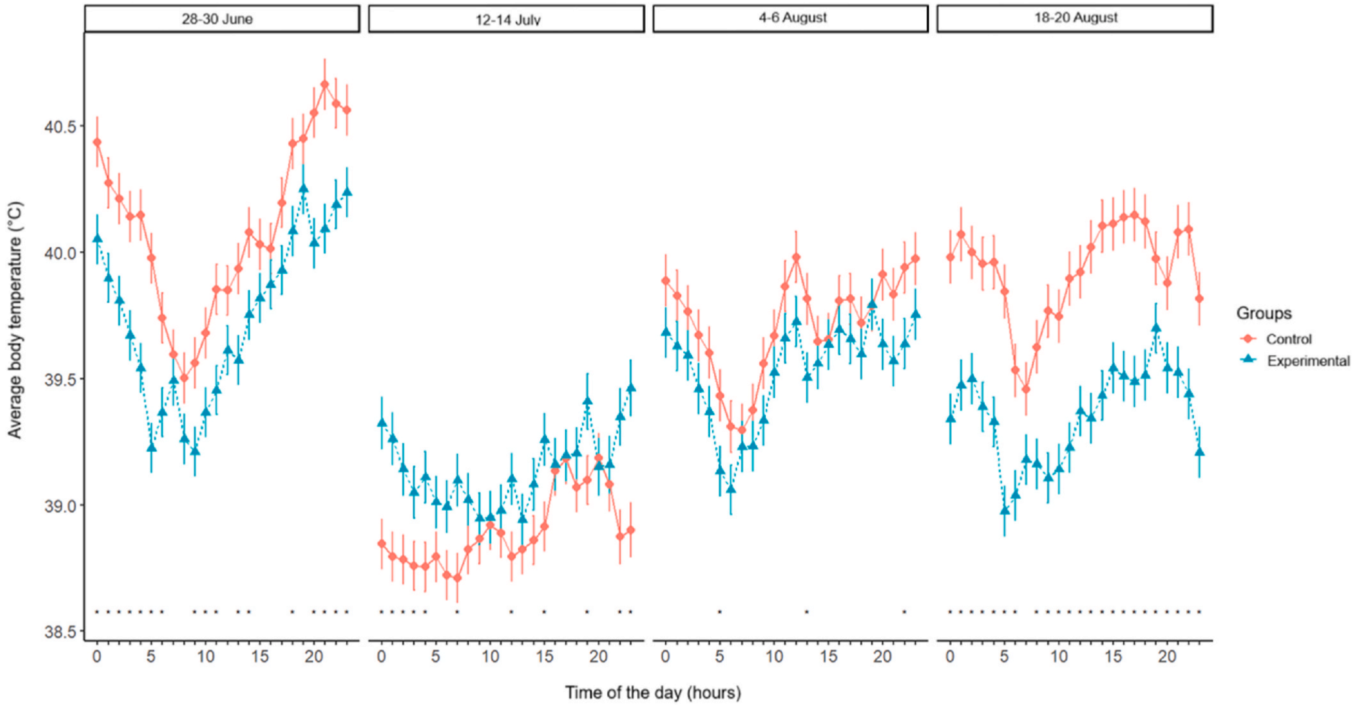

Fig. 7. The average vaginal temperature of the cows during the four 3-day-long sampling periods. Asterisks indicate significant differences between the two groups in the hourly average body temperatures ($p < 0.05$).

difference observed on July 12 ($p < 0.05$).

Stress response regulation: HSF1, the primary regulator of the heat stress response, showed significantly reduced expression ($p < 0.05$) in the EXP group, indicating a less activated stress response pathway.

Metabolic regulators: IR expression showed no significant differences between groups, although the EXP group exhibited higher expression only on August 3, with lower expression during the other periods. PRL showed significant differences during three sampling periods: June 28, July 12, and August 18 ($p < 0.05$). The experimental group exhibited higher PRL expression on June 28 and August 4, but lower expression on July 12 and August 18, compared to the controls.

These molecular findings demonstrate that enhanced ventilation creates complex changes in gene expression patterns, with effects varying across different cellular pathways and time points, suggesting dynamic cellular responses to improved environmental conditions.

Table 5
Average body temperature of cows in the control and treatment groups in the sampling periods.

Sampling period	Control	Treatment	SEM	Difference	Diff SEM	p-value
June 28–30	40.1	39.7	0.08	0.4	0.11	0.0020
July 12–14	38.9	39.1	0.08	−0.2	0.11	0.0376
Aug 4–6	39.7	39.5	0.08	0.2	0.11	0.0908
Aug 18–20	39.9	39.4	0.08	0.5	0.11	>0.0001

4. Discussion

We measured the effects of increased airspeed in barns, achieved by installing higher-speed fans, on thermal comfort, physiological responses, and the yield of lactating dairy cows. The thermoneutral zone of dairy cows is between 5 and 25 °C, where the animal expends the least energy on thermoregulation, thereby maximising the energy available for milk production (Becker and Collier, 2020). During the examined period, there was no significant difference in either temperature or THI values between the two barns. Among the measured parameters, only air velocity differed, with an average of 0.2–0.5 m/s in the control barn and 1.5–2.5 m/s in the experimental barn. On most summer days, the daily average THI exceeded the heat stress threshold (THI 68). As shown in Fig. 4, heat exposure, when the daily average THI exceeded the threshold, lasted at least a week before the sampling periods 1 and 4 (more precisely, almost a month). A cold front occurred before the 2nd sampling period. The heat wave was again continuous before the 3rd sampling period, with a day-long cooler period 5 days earlier. This mild cooling lasted only a day, so we still considered the cows to be under heat stress in the weeks leading up to the 3rd period. The average ambient temperature and THI were above the thermoneutral zone of the cows in the barns during the four 3-day sampling periods. The nadir in daily temperature and THI occurred around 5 a.m., while the peak was reached around 3 p.m., elevating above the heat stress threshold.

The options for measuring body temperature in cows include rectal (RT), vaginal (VT), rumen temperature, and ear canal (Rejeb et al., 2016) methods. The VT of the studied animals followed the daily rhythms of temperature and THI in the barns, indicating that heat stress affected the animals. A body temperature above 39.2 °C indicates heat stress, when the animals are unable to maintain thermoregulation under high-temperature conditions (Bakony et al., 2019). The average body temperature exceeded this threshold on most sampling days, indicating that the animals were unable to cool down properly, even during the night, except during the 2nd sampling period. During the warm sampling periods (1st, 3rd, and 4th), the average body temperature consistently exceeded the heat-stress threshold, reaching a maximum of 41.5 °C. The second period was slightly cooler, so the body temperature of the animals was also lower during this period. During the hot periods (1st, 3rd, and 4th), the extra cooling effect of the EXP fans, due to the higher airspeed, was evident, with average body temperatures in the experimental group lower. The elevated air velocity in the experimental barn enhanced convective heat loss, enabling cows to dissipate excess body heat more effectively. The reduction in body temperature suggests that the higher-speed fans effectively improved the heat dissipation, thus alleviating heat stress. Similar studies have shown that increasing air movement can enhance evaporative cooling, particularly when cows cannot rely solely on sweating due to high humidity (Smith et al., 2016). This finding is supported by previous research emphasising the importance of air velocity in barn cooling strategies as a cost-effective and sustainable intervention for dairy farmers facing heat stress challenges (Izhboldina et al., 2020).

The ethological requirement of cows is 10–12 h of rest per day (Kilgour, 2012). The lying time approached the reference value in our second sampling period, which was slightly cooler. As temperatures increase, animals spend less time resting (Hut et al., 2022; Reuscher et al., 2023), as observed during the warmer sampling periods in our study. The lowest values were observed during the first period, which was the warmest. Interestingly, the average lying times did not differ between the groups during the sampling periods, which is in contrast with others (Reuscher et al., 2023).

Cows cannot adequately dissipate accumulated heat in hot environments, leading to hyperthermia (West, 2003). Consequently, heat-stressed dairy cows reduce their energy metabolism (Joo et al., 2021). Heat stress affects rumen fermentation, increasing lactate and

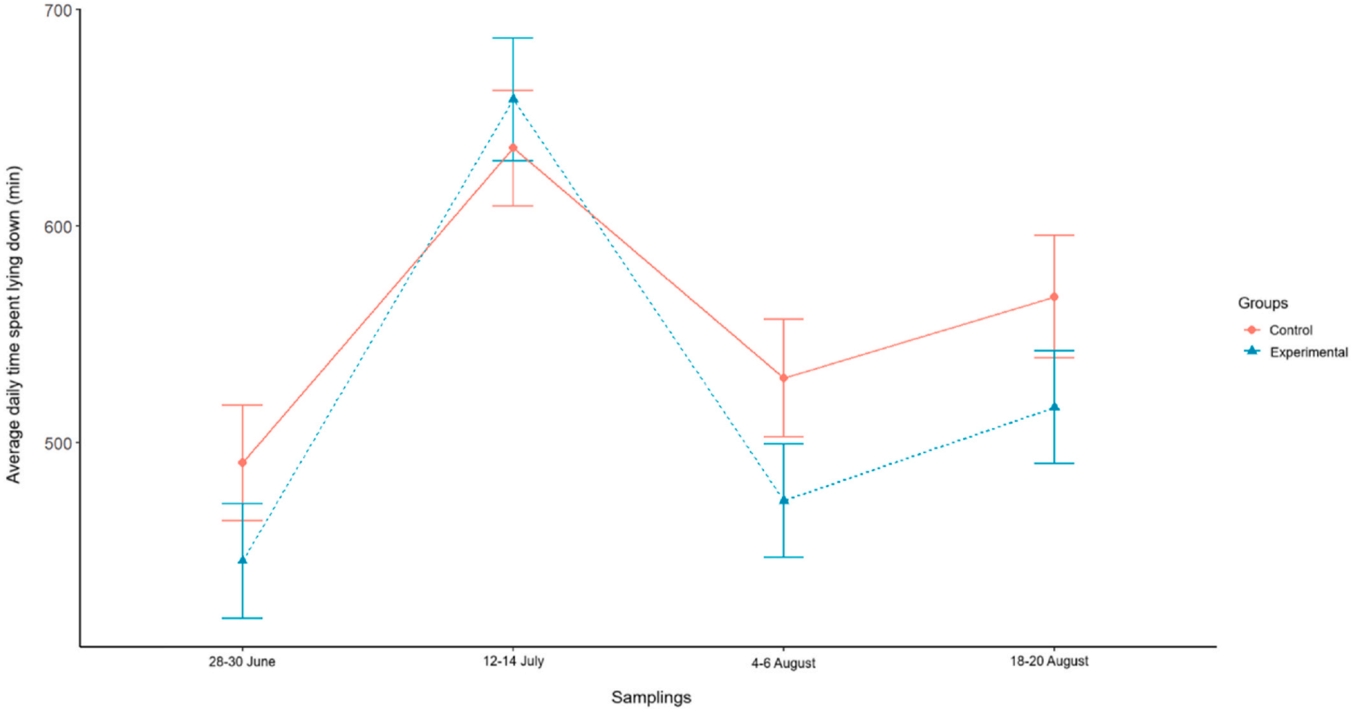

Fig. 8. The average time spent lying down during the sampling periods.

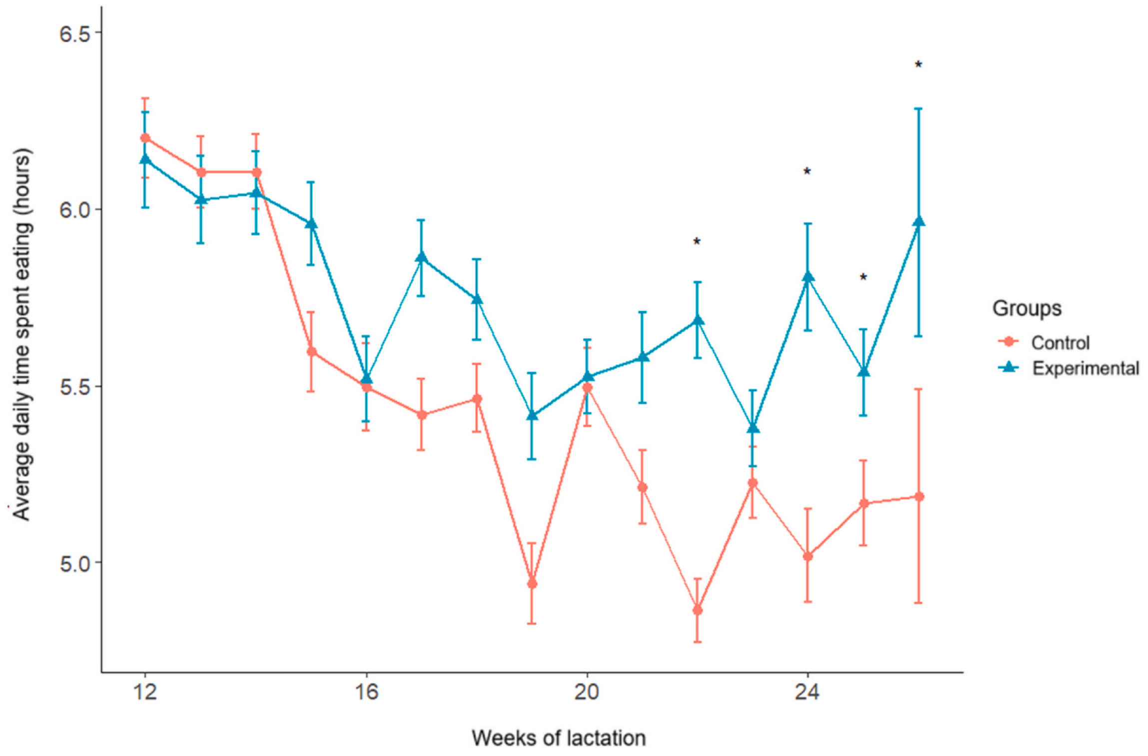

Fig. 9. The daily average time spent eating during the sampling periods. Asterisks indicate significant differences between the two groups ($p < 0.05$).

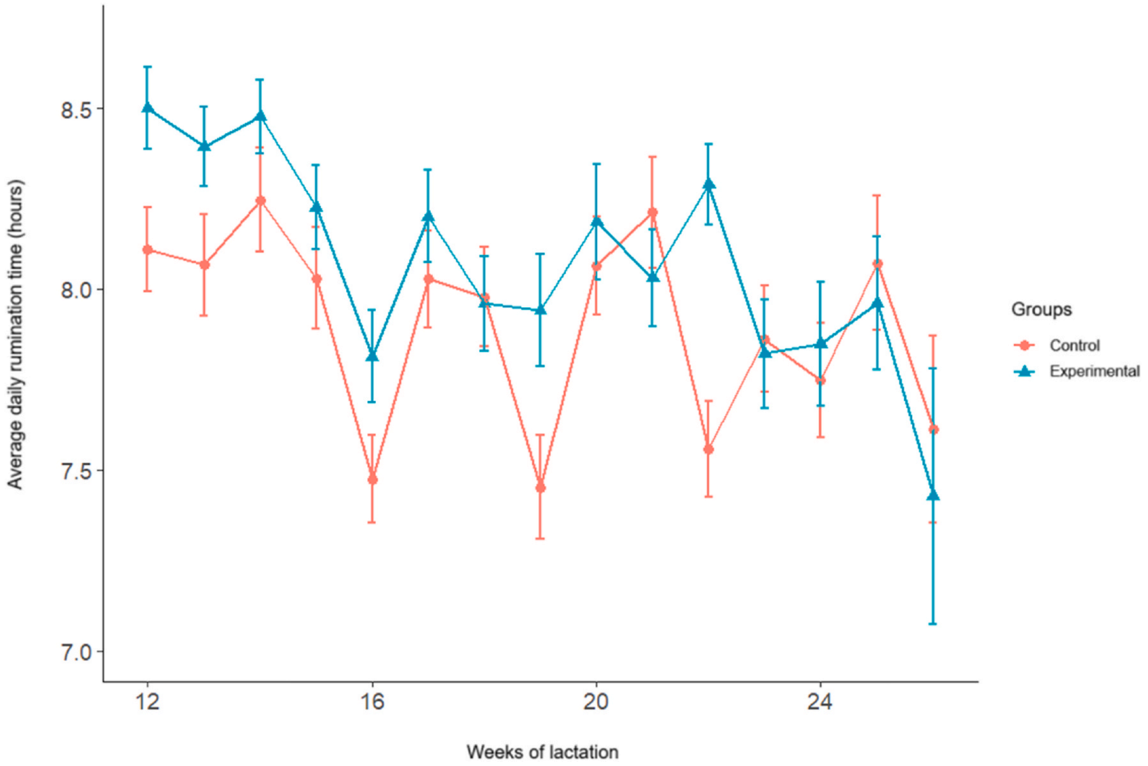

Fig. 10. The daily average time spent ruminating during the sampling periods.

decreasing acetate-producing bacteria, and also affects blood parameters and metabolites (Jo et al., 2021). Cows in thermoneutral conditions consume their feed in 12–15 bouts per day, but decrease their eating frequency to 3 to 5 meals per day during heat stress (Kadzere et al., 2002; Sammad et al., 2020). Lower feed intake and reduced rumination decrease saliva production and, consequently, buffering capacity, resulting in a lower rumen fluid pH (Sammad et al., 2020). An interesting aspect of our findings is the increase in time spent eating towards the end of lactation in cows housed in the experimental barn. The increased airspeed likely improved the barn's climate, allowing cows to

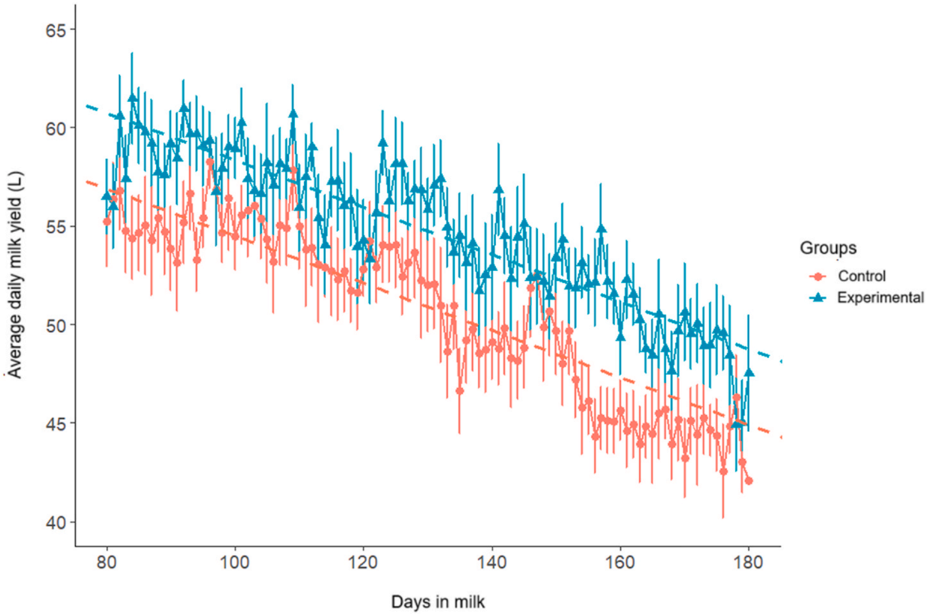

Fig. 11. The average daily milk yield of the cows between days in milk (DIM) 80 and 180.

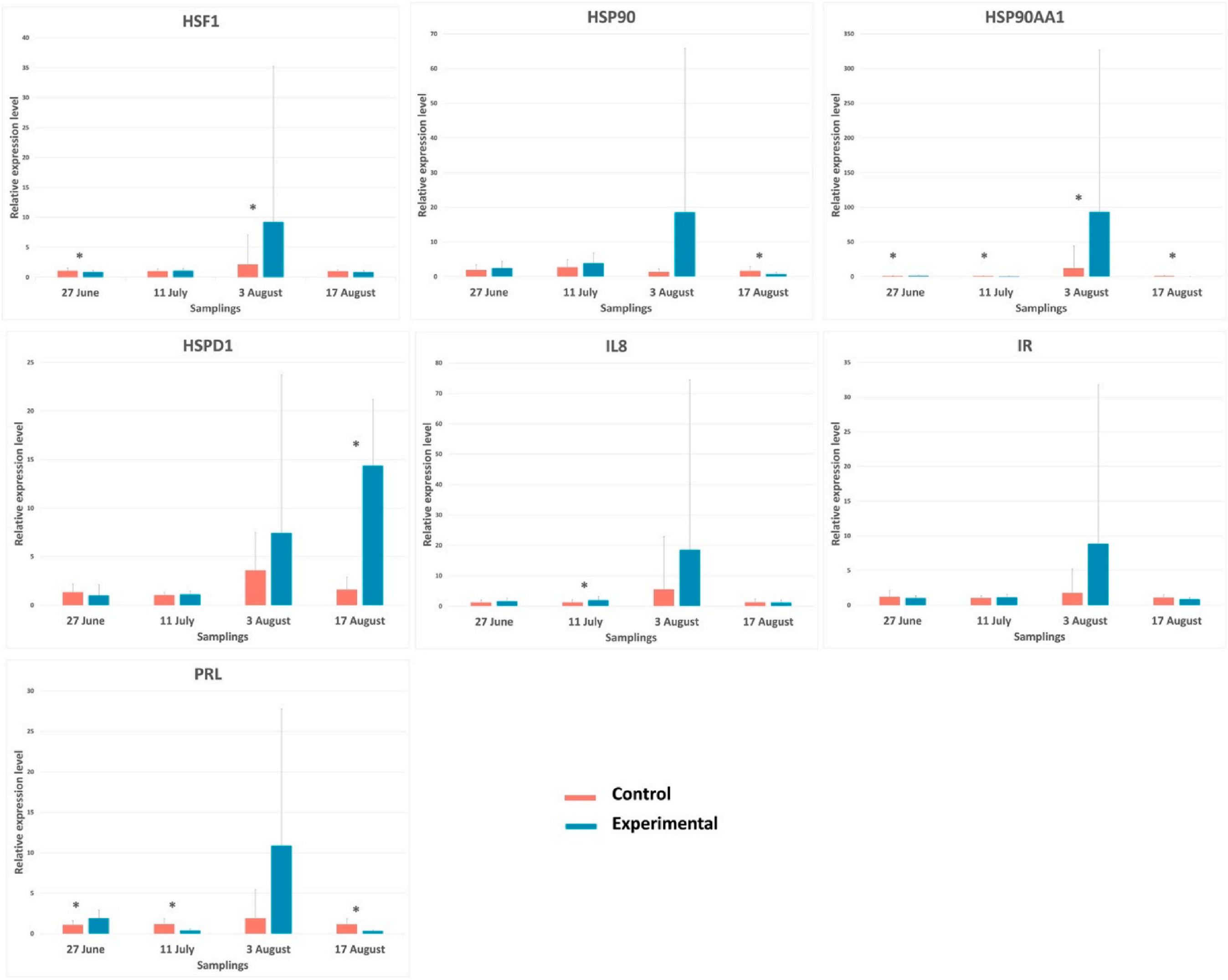

Fig. 12. Relative gene expression of studied genes in control and experimental groups across sampling periods. * indicates $p \leq 0.05$.

consume more feed comfortably, which is crucial for supporting milk production during late lactation. Increased feed intake during this period may help sustain body reserves that would otherwise be depleted by prolonged exposure to heat stress, allowing cows to maintain their lactation curve effectively. This outcome is consistent with reduced heat-stress markers, such as body temperature, indicating that cows were under less thermal strain, enabling them to consistently meet their nutritional needs (Reuscher et al., 2023). A limitation of our study is that we were unable to follow the animals and do not know how much time they spent in the barn or in the outside area.

In intensive farming, where the animals receive feed in several portions per day as a complete mixture, the cows' eating time averages 4.5 h (ranging from 2.5 to 8.5 h; Beauchemin, 2018). The eating time was physiological, ranging from 4.5 to 6.5 h per day during our study. We observed a significant difference between the two groups from approximately the 21st week of lactation. Interestingly, the time spent ruminating did not differ between the experimental and control groups, indicating that increased airspeed did not directly influence rumination behaviour. The feeding technology of intensive farming affects the rumination time, primarily through the fibre ratio of the feed. However, a healthy rumination time is from 2.5 to 10.5 h daily (7 h, on average; Beauchemin, 2018). The rumination time of the cows in our study was within the normal range acceptable for intensive farming, averaging 8 h daily. Rumination time decreased as lactation progressed, without a significant difference between groups in our study. The lack of a substantial difference in rumination time suggests that while airspeed influenced the thermal comfort and feeding behaviour of cows, it did not disrupt or alter their normal rumination patterns. This finding could imply that the improved barn climate was sufficient to alleviate heat stress without affecting rumination. Another aspect may be that adaptation to daily temperature, and THI can be noticeable at relatively low mean temperatures of 12 °C or mean THI of 56 or above, when dairy cows started spending less time lying and eating and more time standing (Hut et al., 2022).

Heat stress hampers milk production and its composition (Bakony et al., 2019), as milk fat and protein content decrease, and somatic cell count increases during summer (Bouraoui et al., 2002). Milk production begins to decline at 27 °C, and the adverse effects typically become apparent within two days after heat stress (Spiers et al., 2004). Collier et al. (2011) found that milk fat and protein content, as well as milk yield, were negatively affected by THI values. They determined a heat stress threshold THI of 68 (Collier et al., 2011). Reduction in milk production occurs due to the direct and indirect effects of heat stress. As a direct effect, cows adjust their energy and protein metabolism to thermoregulation (Tao et al., 2020). As an indirect effect, oxidative stress increases during heat stress, altering cellular efficiency in synthesising milk ingredients (Gao et al., 2019). Meanwhile, nutrient availability is limited due to reduced feed intake (Min et al., 2017). The improved milk production in our experimental group further highlights the effect of enhanced airspeed in mitigating heat stress. The increase in milk yield among cows housed in the experimental barn suggests that reducing thermal strain may allow cows to sustain a higher metabolic rate conducive to lactation. Our results demonstrate that improving the barn climate with higher-speed fans can mitigate these adverse effects, leading to sustained milk production even during high heat stress, as shown earlier by others (Reuscher et al., 2023). Gunn et al. (2019) predict the need for stronger heat abatement methods to mitigate economic losses for dairy farmers. Several studies have demonstrated the effectiveness of sprinklers in reducing body temperature and respiratory rate while increasing milk yield (Kaufman et al., 2020). Ventilation systems can reduce respiratory rate and rectal temperature, and increase dry matter intake (Ji et al., 2020). Izhboldina et al. (2020) showed that fans in a naturally ventilated barn can reduce THI and also cows' body surface temperature during the warmest periods. Reuscher et al. (2023) studied 128 lactating Holstein cows housed in three barns. In the barn of their control group, where fans were not used, the airspeed was 0.4 m/s; one of the experimental groups' barn fans worked at 60 % capacity, and the airspeed was 1.7 m/s; in the other experimental group, with fans working at 100 % capacity, the airspeed was 2.4 m/s (Reuscher et al., 2023). The cows in the experimental groups spent significantly longer time lying daily (14.2 and 13.9 h/day) than the control group (13.2 h/day). The fans reduced vaginal temperature, respiratory rate, and skin temperature, while milk production and daily DMI increased significantly in the ventilated groups compared to the control group (Reuscher et al., 2023).

The gene expression analysis provides molecular evidence supporting the physiological benefits observed in the experimental group, revealing complex temporal patterns that illustrate the dynamic cellular responses to enhanced ventilation conditions. The expression patterns varied significantly across sampling periods, suggesting that enhanced ventilation affects different molecular pathways in a time-dependent manner rather than producing uniform changes. Heat shock protein responses showed complex temporal dynamics in our study. HSF1 expression was generally lower in the EXP group during three of four sampling periods (except August 4), with significant differences on June 28 and August 4. This pattern suggests that enhanced ventilation reduced activation of the master heat stress response regulator during most periods, consistent with previous findings that HSF1 activation is among the earliest responses to thermal stress, triggering the transcription of various heat shock proteins (Sigdel et al., 2019; Fu et al., 2021). HSP90 expression showed an interesting pattern: it was consistently higher in the experimental group, except on August 18, when it was significantly lower. While enhanced ventilation may initially maintain higher HSP90 levels (possibly as a protective mechanism), it effectively reduces stress-induced upregulation during the hottest period. HSP90AA1 displayed significant differences across all sampling periods, with lower expression in the experimental group during three time points but higher expression on August 4. HSPs are usually upregulated during thermal stress to protect cell proteins from denaturation and maintain cellular homeostasis (Badri et al., 2018; Li et al., 2023; Mendonca et al., 2025), with HSP90AA1 being the key gene associated with HS responses in dairy cows (Fang et al., 2021; Hu et al., 2024). The increased expression of HSP90AA1 under HS is a self-protective mechanism that maintains cell survival by inhibiting apoptosis and promoting autophagy (Hu et al., 2024). The variable expression patterns observed suggest that enhanced ventilation may allow for more flexible cellular stress responses rather than chronic activation. HSPD1 expression was lower in the experimental group during June and July but higher during both August periods, with significant differences on August 18 ($p < 0.05$). This temporal shift may reflect seasonal adaptation or varying environmental stressors throughout the study period.

Inflammatory responses showed predominantly elevated patterns in the enhanced ventilation group. Interleukin-8 expression was consistently higher in the experimental group across the study periods, with a significant difference on July 12 ($p < 0.05$). This unexpected finding contrasts with the traditional view that reduced stress should decrease inflammatory markers (Bagath et al., 2019). Interleukin-8 is a key pro-inflammatory cytokine involved in the immune response (e.g., to mastitis) in dairy cows (Puppel et al., 2024). Heat stress induced upregulation of inflammatory cytokines (including IL8) of bovine endometrial epithelial cells in response to bacterial components in an earlier study (Molinari and Bromfield, 2023). The IL8 elevation in our study might reflect enhanced immune capacity rather than pathological inflammation, as heat stress typically suppresses immune function (Das et al., 2016). However, this requires further examination, as variable stress responses among cows suggest that some respond differently to heat stress (Komadan et al., 2025). The improved barn environment may have enabled more robust immune responses, which could benefit overall health.

Metabolic regulators demonstrated complex adaptation patterns. Prolactin showed significant differences during three sampling periods (June 28, July 12, and August 18), with the EXP group exhibiting higher expression on June 28 and August 18, but lower expression on July 12. This variable pattern suggests that enhanced ventilation may optimise prolactin regulation in response to environmental conditions, potentially improving lactation physiology during different stress periods, as PRL performs multiple functions in the body, affecting a range of physiological processes, especially in heat stress (Hernández-Cordero et al., 2017; Clarke et al., 2025; Mendonca et al., 2025). Insulin receptor expression showed no significant differences between our groups, though trends suggested improved metabolic stability in the experimental group. Other studies have shown that heat stress increases IR expression (Mendonca et al., 2025) or blood insulin concentration (Blond et al., 2024). The molecular findings reveal that enhanced ventilation creates dynamic cellular responses rather than uniform stress reduction. The temporal variability in gene expression patterns suggests that improved environmental conditions allow for more flexible and adaptive molecular responses to changing stressors. Rather than simply reducing all stress-related gene expression, enhanced ventilation appears to optimise cellular homeostasis, enabling appropriate responses when needed while preventing chronic stress activation. This interpretation aligns with the improved physiological parameters and production performance observed in the experimental group, suggesting that the molecular changes support enhanced adaptive capacity under varying environmental conditions.

Dairy farms have a variety of ventilation solutions to choose from. Choosing the right technical solution can significantly impact the return on investment, ultimately affecting the farm's profitability. In their study, Mondaca and Cook (2019) compared seven ventilation solutions using a cost model that accounted for daily maximum, minimum, and average temperatures, as well as seasonal system settings. The installation cost ranged from $246 to $318 per barn, with the cross-ventilation system being the most cost-effective. Operating costs also varied significantly. The costs of mechanical systems are higher than those of natural ventilating systems, highlighting the importance of selecting a suitable ventilation system (Mondaca and Cook, 2019).

Overall, the findings of this study underscore the importance of higher airspeeds in reducing the adverse effects of heat stress on dairy cows at both physiological and molecular levels. By enhancing convective heat loss, the high-speed fans in the EXP barn provided a more thermally comfortable environment, resulting in reduced body temperatures, increased feed intake, and higher milk yields. These physiological improvements are complemented by molecular evidence of complex temporal changes in gene expression patterns, demonstrating that enhanced ventilation enables more dynamic and adaptive cellular responses to environmental stressors rather than uniform stress suppression. The molecular findings provide mechanistic insights into the improved performance observed in the experimental group, confirming that the benefits extend beyond surface-level comfort to fundamental cellular adaptation processes. These outcomes highlight the potential of barn climate modifications, such as increasing airspeed, as cost-effective and sustainable strategies for managing heat stress in dairy operations. Given projected increases in global temperatures and anticipated impacts on livestock welfare and productivity, implementing climate control measures, such as high-speed fans, can effectively improve dairy farms' resilience to heat stress by providing immediate physiological benefits and long-term cellular protection mechanisms.

5. Conclusions

Optimising barn climate through increased air velocity can significantly reduce heat stress and improve the welfare and productivity of dairy cows. The enhanced ventilation system improved physiological parameters and behavioural responses, also induced complex temporal changes in gene expression patterns, providing molecular evidence of enhanced cellular adaptability. Future research should consider the combined effects of airspeed, humidity control, and additional cooling methods, such as misting or sprinkling, to determine the most effective barn climate management strategies for different environmental conditions.

CRediT authorship contribution statement

Viktor Jurkovich: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Szilvia Szalai:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition. **Zsóka Várhidi:** Writing – review & editing, Writing – original draft, Investigation. **Szilvia Kusza:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Zoltán Bagi:** Writing – review & editing, Writing – original draft, Investigation. **Lilla Bodrogi:** Writing – review & editing, Writing – original draft, Investigation. **Blanka Dávid:** Writing – review & editing, Writing – original draft, Investigation. **Mária Kovács-Weber:** Writing – review & editing, Writing – original draft, Validation, Funding acquisition, Conceptualization. **Róbert Kővágó:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Mikolt Bakony:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Péter Hejel:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Data accessibility statement

The raw/processed data required to reproduce the above findings cannot be shared at this time due to technical limitations.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Viktor Jurkovich reports financial support was provided by National Office for Research, Developmend and Innovation. Szilvia Szalai reports financial support was provided by National Office for Research, Developmend and Innovation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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