

# Investigating the interaction effects of coat colour and short-term heat-stress on milk traits and metabolic indicators in Holstein Friesian cows

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## ARTICLE INFO

### Keywords:

Insulin  
NEFA  
Prolactin  
Black coat  
THI  
White coat

## ABSTRACT

This research aimed to investigate the interaction effect of coat colour and short-term heat stress (HS) on milk traits and blood parameters in lactating Holstein Friesian cows under a confinement system with controlled outdoor access for 2 h. A total of 660 milk and blood samples were collected between April and September 2021. 55 s-lactation cows (average body weight  $637 \pm 29$  kg) were classified into three groups based on coat colour ratio: 18 white cows (DIM  $98 \pm 15$ ), 18 black cows (DIM  $101 \pm 18$ ), and 19 mixed-colour cows (DIM  $105 \pm 21$ ). The assessment was conducted under two conditions: HS, where the temperature-humidity index (THI) was  $\geq 68$  during the 2-h outdoor exposure, and thermoneutral conditions (NHS), where the THI remained  $< 68$ . No significant interaction between coat colour and HS was observed for milk traits or blood parameters ( $p \geq 0.05$ ). However, cows under NHS exhibited significantly higher milk production (42.4 kg) compared to HS cows (40.7 kg;  $p < 0.05$ ). Milk protein levels were also elevated under NHS (3.29% vs. 3.14%;  $p < 0.05$ ), and lactose content decreased during HS (5.07% to 5.00%;  $p < 0.05$ ). Heat stress resulted in a 194% increase in insulin levels, a 7.03 ng/ml rise in prolactin, and a 67% increase in NEFA. Overall, short-term heat stress significantly affected milk traits and some metabolic indicators, but there was no evidence that black cows were more susceptible to HS than white cows under the conditions of this study.

## 1. Introduction

The changes in climate over the last few years present substantial problems for ecosystems and the survival of various species, along with the long-term viability of animal husbandry systems worldwide (Das et al., 2016). Numerous studies revealed that starting from mid-1970s, there has been a notable increase of  $0.2^\circ\text{C}$  in global temperatures every decade. There is a projected probability greater than 50% that the global temperature increase will reach or exceed  $1.5^\circ\text{C}$  between 2021 and 2040 across all scenarios (Lee et al., 2023). Dairy sector is currently facing an escalating issue of thermal stress, as dairy cattle exhibit high degree of susceptibility to elevated temperatures (Thornton et al., 2022; Xess et al., 2025). This vulnerability is exacerbated by increasing global temperatures due to climate change and further intensified by the cows' high production, which elevates metabolic heat production (Oliveira et al., 2025). This conduct to reduced reproductive performance and milk yield, which is observed not only in hotter temperatures but also in

temperate locations (Baccouri et al., 2025; Hut et al., 2022; Lovarelli et al., 2024). Humidity, temperature, solar radiation and wind speed are crucial environmental elements in that their increase leads to the occurrence of heat stress (Ji et al., 2020). Moreover, there is an indication that heat stress is considered a substantial economic detriment for around 60% of dairy farms worldwide (Habeeb, 2020). This emphasizes the pivotal role that heat stress plays as a significant and financially consequential issue within agricultural sectors (Wankar et al., 2021). Cows respond to heat stress through physiological adjustments such as increased respiration, sweating, and changes in core temperature and endocrine function (Wheelock et al., 2010). Coat colour has been proposed as a factor influencing heat absorption, with lighter coats generally absorbing less solar radiation than darker ones (Silanikove, 2000; Stuart-Fox et al., 2017). However, evidence remains inconsistent: some studies report higher skin temperatures and sweating rates in black-coated Holsteins (Gebremedhin et al., 2007; Hillman et al., 1998), whereas others found no meaningful differences in evaporative heat loss

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<https://doi.org/10.1016/j.jtherbio.2026.104460>

Received 22 August 2025; Received in revised form 28 March 2026; Accepted 29 March 2026

Available online 2 April 2026

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or sweating between black and white cows (Jiang et al., 2005; Lazzari et al., 2024). These contradictory findings highlight the uncertainty regarding the true contribution of coat colour to heat-stress tolerance. Building on this, several studies have examined whether such physiological differences translate into variation in heat-stress sensitivity and milk production between dark- and light-coloured cattle. Brown-Brandl et al. (2006), Gaughan et al. (1998) and Isola et al. (2020) have presented evidence supporting the notion that cows with darker coats are less tolerant to heat stress potentially implying that heat-stressed conditions affect them more adversely. In contrast, Schütz et al. (2009) conducted research that challenges this perspective as they showed the white and black cows behave in the same way under heat stress condition. In the context of milk production, research findings have indicated variations based on coat colour. Several studies have suggested that under heat stress conditions cows with predominantly white coats tend to yield higher milk quantity than black dominant ones (Anzuere-Olvera et al., 2019; Becerril et al., 1993; Hansen, 1990). In contrast, investigations conducted in subtropical regions by Dikmen et al. (2017) and Lee et al. (2016) have shown that in Holstein cows, coat colour had no impact on milk output. The variations in blood characteristics between white and black cows under different environmental conditions, such as heat stress, remain a relatively overlooked topic in scientific research. A limited number of studies have reported no substantial differences in blood parameters between black and white cows when subjected to heat stress conditions (Anzuere-Olvera et al., 2019; Ghassemi Nejad et al., 2017). These contrasting findings and limited research underscore the complexity of the relationship between coat colour, milk production, and blood parameters and the influence of environmental conditions. The debate about whether or not coat colour affects dairy cow's performance under heat stress conditions has always ensued among researchers (Anzuere-Olvera et al., 2019; Gantner et al., 2020; King et al., 1988; Maia et al., 2005). To contribute to this ongoing debate, this study aims to investigate the interaction effect of coat colour and short-term heat stress on milk yield and quality and metabolic indicators in lactating Holstein Friesian cows in order to determine whether black cows are more susceptible to heat stress than their white counterparts.

## 2. Material and methods

### 2.1. Description of research 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  $637 \pm 29$  kg, and varying black and white coat colour ratios, between April 2021 and September 2021. The cows were categorized into three groups according to their coat colour. The first group included 18 cows with  $\geq 75\%$  white coats with days in milk (DIM)  $98 \pm 15$ , the second group comprised 18 cows with  $\geq 75\%$  black coats DIM  $(101 \pm 18)$ , and the third group comprised 19 cows with an equal distribution of 50% black and 50% white coats with DIM  $(105 \pm 21)$ . To assess the percentage of black and white colour on the cows' coats, photos were taken from both the left and right sides of each cow using a Canon EOS 2000D camera with an EF-S 18-55 mm f/3.5-5.6 IS II lens, which has 24.5 megapixels. Subsequently, Specialised custom-developed software, termed 'Teheñ', was created by the computer science department of the University of Szeged to specifically differentiate between the black and white colours was used to determine the percentage of black and white on the cows' coats. The measurements excluded belly areas, tail and legs, and included only the head, neck and trunk.

The cows were housed together under the same environmental conditions during the experiment, in a cooled barn with ventilators and

sprinklers to manage summer heat when the THI was  $\geq 68$ . The barn was fitted with sensors that monitor humidity and temperature, and when the THI reaches  $\geq 68$ , the ventilators and sprinklers were activated automatically. In summary, a collection of ventilators generated air speeds of 2 m/s or above from 06:00 to 24:00 h. Additionally, fans and sprinklers were activated successively, alternating between ventilation (4.5 min) and soaking (0.5 min) cycles. The ventilation speed and sprinkler frequency increased automatically as needed to maintain THI at 68 or below, ensuring a controlled environment that minimised heat stress exposure for all cows while they were housed together indoors in the same area of the barn. This process was repeated for a total of seven 0.5-h intervals, occurring every 1.5 to 2 h between 07:30 and 18:30 h. Water was provided ad libitum the whole year. The cows were fed with a total mixed ration (TMR) as their primary feed, with the same daily ration throughout the experiment, formulated to fulfil their nutritional requirements. The TMR composed of a combination of alfalfa silage, corn silage, alfalfa hay, molasses, and concentrate. The exact proportions of individual feed ingredients are not disclosed because they are considered proprietary information by the commercial farm. However, the nutritional composition of TMR was provided by the farm nutritionist based on the ration formulation system used on the farm. The diet contained 50.35% dry matter, and cows consumed on average 29.37 kg of dry matter per day. On a dry matter basis, the ration supplied 6.52 MJ NEL/kg DM of net energy for lactation, 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 met the nutrient requirements for lactating dairy cows according to the National Research Council (NRC, 2001). Feed was provided ad libitum, meaning it was freely available throughout the day, allowing the cows to consume it as needed. The feed was distributed twice daily, typically at 9:00 a.m. and 4:00 p.m., to ensure fresh availability and encourage consistent intake. Additionally, part of the morning's main meal was distributed in the outdoor yard (uncontrolled environment) feeding area ensuring that the cows had unrestricted access to feed throughout the 2-h exposure period. As part of the experiment, the cows were exposed to a natural outdoor environment (uncontrolled environment under local weather conditions) for 2 h daily, typically between 12:00 p.m. and 2:00 p.m., coinciding with the hottest part of the day in the region. During this time, they had access to unshaded yard that included the feed manger and water troughs. The yard is enclosed by fences and without cooling systems, and conditions such as ambient temperature and humidity were not controlled. In the yard, feed and water were provided ad libitum in designated feeding areas. At the beginning of the experiment, the workers guided the cows outdoors. Eventually, this became routine. The cows grew accustomed to the schedule and voluntarily moved outside when the doors were opened without being forced and the workers simply followed behind without any contact or pressure. Consequently, during the uncontrolled environment period, the cows were managed with minimal human intervention. They could move freely within the yard, with unrestricted access to water and feed, but they could not return to the cooled barn. All cows were exposed simultaneously as a group for a fixed 2-h duration. Although the exact time spent outdoors by each individual cow was not monitored, the management protocol ensured that the entire group was kept in the yard for the full 2-h duration with no access back to the barn, ensuring a consistent heat exposure across all experimental groups.

In 2021, they were 92 days of heat stress (THI  $\geq 68$ ) from June to September 2021. The samples during this period were considered to be under heat stress conditions.

### 2.2. Blood samples

During the experiment from April 2021 to September 2021, the collection of blood samples was done on a 15 days basis, in a 10 ml blood tube containing anticoagulant sodium EDTA (Vacutainer; Becton Dickinson, Franklin Lakes, NJ, USA) from each cow through coccygeal

venipuncture. This consistent bi-monthly schedule ensured that samples were collected across both Non-Heat Stress (NHS; THI <68) and Heat Stress (HS; THI ≥68) conditions throughout the study duration. The samples were taken between 6:00 and 6:30 h in the morning, primarily before the cows get their first meal and to limit the possible impact of feeding on the results. All samples were collected under standardized conditions to reduce variability and enhance comparability. During the summer months, due to the prevalence of continuous heat stress conditions (THI ≥68), all samples naturally followed at least three consecutive days of heat stress. The analysis of these samples primarily focused on comparing the levels of Insulin determined by using Bovine Insulin ELISA (MBS165580, MyBioSource, LLC, San Diego, CA, USA) intra-assay CV < 8%, inter-assay CV < 10%; Prolactin determined by using bovine prolactin ELISA kit (MBS2022462, MyBioSource, LLC, San Diego, CA, USA) intra-assay CV < 10%, inter-assay CV < 12%; and NEFA determined by using Bovine Non-ester Fatty Acid (NEFA) ELISA (MBS748204, MyBioSource, LLC, San Diego, CA, USA) intra-assay CV < 10%, inter-assay CV < 12%; between cows under non-heat stress environments and short-term heat stress.

### 2.3. Milk records

The cows were milked three times a day (at 8:00 a.m., 3:00 p.m., and 10:00 p.m.), 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 somatic cell count (SCC) at the laboratory of Livestock Performance Testing LTD; Gödöllő. Milk performance parameters were analysed at the individual cow level and compared among the three coat-colour groups. In summer, due to the continuous nature of heat stress conditions (THI ≥68), all samples were collected following at least three consecutive days of heat stress. Protein, fat, and lactose levels in milk samples were analysed using a mid-infrared spectroscopy method with an FT6000 MilkoScan infrared analyser (Foss A/S, Hillerød, Denmark). The somatic cell count (SCC) was determined using a flow cytometry-based technique with a Fossomatic™ 7DC analyser (Foss A/S, Hillerød, Denmark), following standard protocols for milk quality analysis.

### 2.4. Meteorological data

Meteorological data was collected specifically to characterize the environmental conditions during the daily 2-h heat challenge period. Therefore, relative humidity and ambient temperature were documented from 12:00 p.m. to 2:00 p.m. daily, corresponding to the time the cows were in the unshaded outdoor yard. These data were obtained from a meteorological station operated by the Hungarian Meteorological Service, situated 2 km from the farm. Throughout the research period, ambient temperature varied from 4 °C to 38 °C, while relative humidity fluctuated between 38% and 87%. The THI was computed using the method delineated by Dikmen et al. (2008) and is shown in equation (1):

$$THI = (1.8 \times T + 32) - (0.55 - 0.55 \times RH / 100) \times (1.8 \times T - 26) \quad (1)$$

Where:

T = air temperature (°C)  
RH = relative humidity (%)

Cows were considered in heat stress if THI ≥68. This threshold was selected based on its widespread application in dairy cattle research as the point at which physiological and productive performance begins to decline (Collier et al., 2006). Although heat stress intensity can vary across a wide range of severity levels, the binary classification was considered appropriate for the experimental design of this study, which

aimed to compare coat-colour group responses under clearly contrasting thermal conditions. Thus, heat stress prevalence was ranged and categorized as follows:

THI ≥68 heat stress (HS)  
THI <68 no heat stress (NHS)

### 2.5. Statistical analysis

No formal sample size calculation was performed because all eligible cows that met the inclusion criteria (health status, parity, and coat colour ratio) were enrolled. The sample size was therefore determined by the number of available animals on the commercial farm rather than by an a priori power analysis. The study utilised a repeated measures observational design. The individual cow served as the experimental unit (classified by coat colour), and individual samples (milk yield/composition or blood parameter per sampling occasion) as the observational unit, contributing repeated measurements across the study period. Data were examined using the linear mixed model procedure implemented in R software (version 4.4.1, 2024) (Team, 2020), using Lmer function of the (lme4) package (Bates et al., 2015). The main objective of the study was to determine the interaction effect of coat colour and short-term heat stress on some milk traits and blood biochemical contents. We also determined the single factor effect of all the studied parameters using the following equation (2):

$$Y_{ijk} = \beta_0 + \beta_1 HS_i + \beta_2 CC_j \beta_3 + (HS_i \times CC_j) + u_{1k} + u_{2l} + \epsilon_{ijk} \quad (2)$$

Where:

$Y_{ijk}$  = Response variable for the observation of the heat stress status and colour code (milk yield, protein, fat, lactose, SCC, insulin, prolactin, NEFA).

$\beta_0$  = Intercept

$\beta$  = Regression coefficients for the individual fixed effects ( $\beta_1$ : Coefficient for heat stress ( $HS_i$ ),  $\beta_2$ : Coefficient for colour code ( $CC_j$ ),  $\beta_3$ : Coefficient for the interaction term ( $HS_i \times CC_j$ )

$HS_i$  = Fixed effect of the heat stress status

$CC_j$  = Fixed effect of the colour code.

$(HS_i \times CC_j)$  = Fixed effect of the interaction between the heat stress status and the colour code.

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

$u_{2l}$  = Random effect of the DIM (to account for variation in lactation stage between cows and across repeated measurements within the same cow during the natural progression of lactation from April to September, thereby controlling for individual differences in lactation trajectories and isolating the effects of coat colour and heat stress).

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

Post hoc Tukey test was performed by using the estimate marginal means (emmeans) package of R (Lenth, 2016) and ANOVA was used to check the differences between the groups. Significance was declared at  $p < 0.05$ .

## 3. Results

THI values were consistently below the heat-stress threshold (THI <68) during April and May, corresponding to non-heat-stress (NHS) conditions (Fig. 1). From mid-June through August, THI values exceeded 68 continuously, indicating sustained short-term heat-stress (HS) exposure. In September, THI values gradually decreased and returned to NHS levels. These fluctuations enabled the clear identification of HS and NHS days used in the subsequent analyses.

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

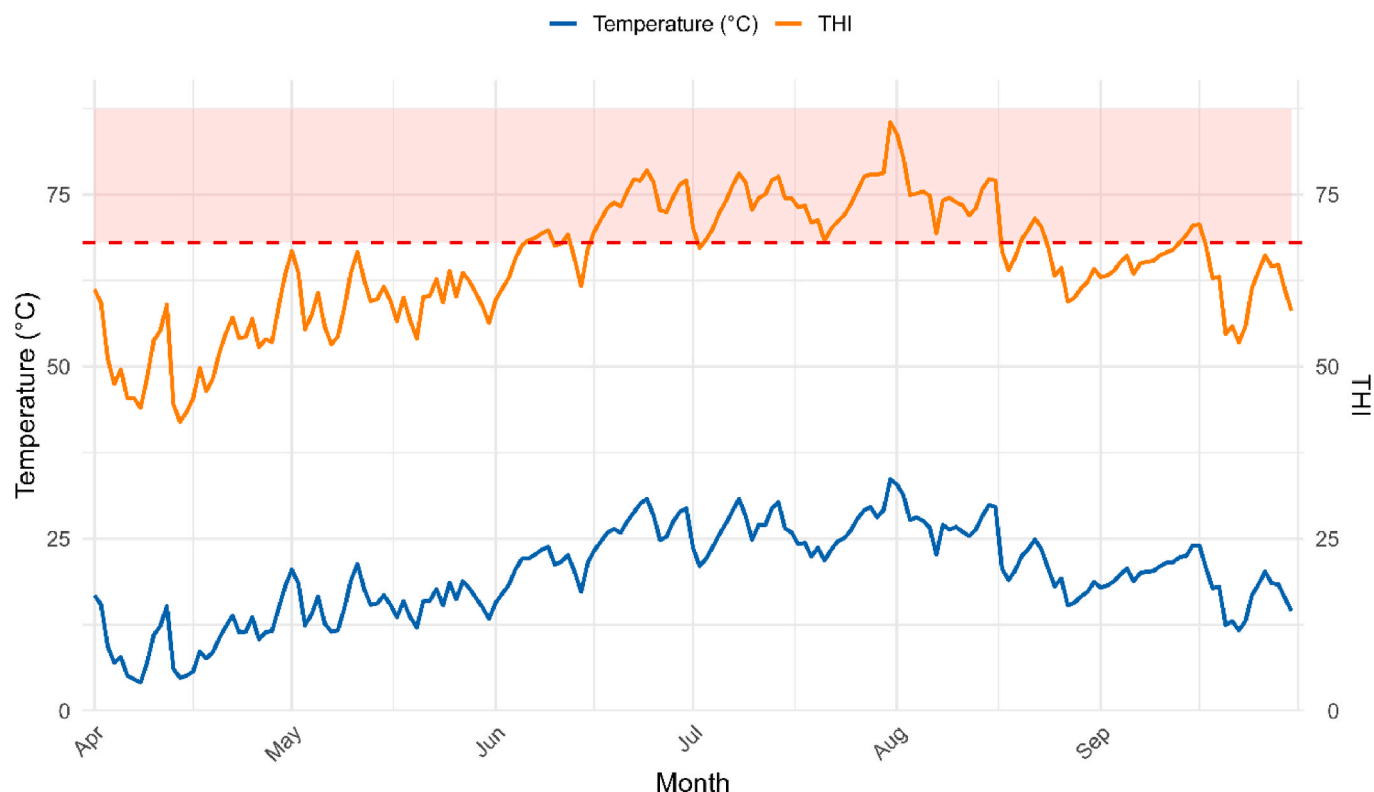


**Fig. 1.** Daily temperature–humidity index (THI) and ambient temperature recorded during the study period. The shaded red area indicates values where THI  $\geq 68$  (heat stress zone), and the dashed line represents the threshold for heat stress classification (THI = 68).

curve and heat stress effects. After adjustment for lactation-stage progression, cows under HS conditions produced a lower MY ( $p < 0.05$ ) than cows in NHS conditions (40.7 kg vs 42.4 kg, respectively), representing a decrease of 1.5 kg. Milk protein levels in cows under HS conditions were also lower ( $p < 0.001$ ) compared with 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 short-term heat stress did not have an effect on milk fat and somatic cells count (Table 1). Additionally, the coat colour did not have any effect on the milk yield and content of the cows under both HS and NHS conditions (Table 2). There was no interaction effect of coat colour and HS on milk traits (Table 3); (see Supplementary material; Supplementary Tables S1–S5 for pairwise interaction effect results).

The effect of the HS on the level of insulin in the blood of cows as under short-term heat stress it increased by 194% compared to its level under thermoneutral conditions (Table 4). Prolactin blood levels were

**Table 1**

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

| Milk Traits         | Stress status     |                   | P value |
|---------------------|-------------------|-------------------|---------|
|                     | HS                | NHS               |         |
|                     | Ls Means $\pm$ SE | Ls Means $\pm$ SE |         |
| Milk Yield (kg)     | 40.70 $\pm$ 3.01  | 42.4 $\pm$ 3.04   | 0.021*  |
| Milk Fat (%)        | 3.89 $\pm$ 0.25   | 4.01 $\pm$ 0.27   | 0.416   |
| Milk Protein (%)    | 3.14 $\pm$ 0.09   | 3.29 $\pm$ 0.09   | 0.001*  |
| Lactose (%)         | 5 $\pm$ 0.06      | 5.07 $\pm$ 0.06   | 0.001*  |
| SCC $10^3$ cells/ml | 235 $\pm$ 53.40   | 171 $\pm$ 67.40   | 0.357   |

HS = heat stress, NHS, = no heat stress, SE = standard error, \* = significant.

**Table 2**

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

| Milk Traits         | Coat colour       |                   |                   | P value |
|---------------------|-------------------|-------------------|-------------------|---------|
|                     | White             | Black             | Mixed             |         |
|                     | Ls means $\pm$ SE | Ls means $\pm$ SE | Ls means $\pm$ SE |         |
| Milk Yield (kg)     | 41.1 $\pm$ 3.19   | 42.7 $\pm$ 3.18   | 40.8 $\pm$ 3.18   | 0.545   |
| Milk Fat (%)        | 3.73 $\pm$ 0.29   | 4.08 $\pm$ 0.28   | 4.04 $\pm$ 0.28   | 0.289   |
| Milk Protein (%)    | 3.25 $\pm$ 0.09   | 3.17 $\pm$ 0.09   | 3.22 $\pm$ 0.09   | 0.365   |
| Lactose (%)         | 5.05 $\pm$ 0.06   | 5.01 $\pm$ 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   |

SE = standard error.

affected by the short-term heat stress ( $p < 0.05$ ) as it increased from 3.21 (ng/ml) in non-heat stress conditions to 10.24 (ng/ml) which increased by 319%. NEFA was affected by HS as it increased from 395 ( $\mu$ M) in NHS conditions to 583 ( $\mu$ M) in HS conditions, which means an increase by 67%. However, the coat colour did not have any significant effect on the blood parameters of the cows under heat stress and non-heat stress conditions (Table 5). In addition, Table 6 shows that there was no significant interaction effect of coat colour and HS on blood biochemical content; (see Supplementary material; Supplementary Tables S6–S8 for pairwise interaction effect results).

#### 4. Discussion

In this study, we aimed to explore the effects of the association between short-term heat stress and coat colour on milk yield, quality, and blood biochemical composition. Given the projected increases in global temperature, understanding factors that may influence heat tolerance in dairy cattle is important for informing breeding strategies. This study may provide evidence on whether coat colour could play a role in such

**Table 3**Least square means ( $\pm$  standard error) for the interaction of coat colour and heat stress effect on milk yield and composition.

| Milk Traits                  | Coat colour   |                |               |               |               |               | P value |
|------------------------------|---------------|----------------|---------------|---------------|---------------|---------------|---------|
|                              | White         |                | Black         |               | Mixed         |               |         |
|                              | HS            | NHS            | HS            | NHS           | HS            | NHS           |         |
|                              | Ls means ± SE | Ls means ± SE  | Ls means ± SE | Ls means ± SE | Ls means ± SE | Ls means ± SE |         |
| Milk Yield (kg)              | 40.1 ± 1.28   | 39.9 ± 1.61    | 41.7 ± 1.32   | 42.1 ± 1.44   | 39.9 ± 1.23   | 39.1 ± 1.62   | 0.996   |
| Milk Fat (%)                 | 3.77 ± 0.29   | 3.70 ± 0.34    | 3.96 ± 0.30   | 4.20 ± 0.31   | 3.95 ± 0.29   | 4.13 ± 0.34   | 0.658   |
| Milk Protein (%)             | 3.19 ± 0.10   | 3.32 ± 0.10    | 3.11 ± 0.10   | 3.23 ± 0.10   | 3.12 ± 0.10   | 3.33 ± 0.10   | 0.316   |
| Lactose (%)                  | 5.01 ± 0.06   | 5.08 ± 0.06    | 4.99 ± 0.06   | 5.03 ± 0.06   | 5.01 ± 0.55   | 5.08 ± 0.06   | 0.762   |
| SCC 10 <sup>3</sup> cells/ml | 215.5 ± 78.40 | 223.1 ± 110.80 | 235.4 ± 82.20 | 190.1 ± 91.90 | 253.6 ± 75.80 | 98.3 ± 112    | 0.629   |

HS = heat stress, NHS, = no heat stress, SE = standard error.

**Table 4**Least square means ( $\pm$  standard error) for effect of heat stress on blood parameters.

| Blood parameters  | Stress status     |                   | P value |
|-------------------|-------------------|-------------------|---------|
|                   | HS                | NHS               |         |
|                   | Ls Means $\pm$ SE | Ls Means $\pm$ SE |         |
| Insulin (mIU/L)   | 11.72 $\pm$ 1.45  | 6.03 $\pm$ 2.25   | 0.004*  |
| Prolactin (ng/ml) | 10.24 $\pm$ 1.40  | 3.21 $\pm$ 3.11   | 0.028*  |
| NEFA ( $\mu$ M)   | 583 $\pm$ 63.20   | 395 $\pm$ 86.40   | 0.010*  |

HS = heat stress, NHS, = no heat stress, SE = standard error, \* = significant.

**Table 5**Least square means ( $\pm$  standard error) for effect of coat colour on blood parameters.

| Blood parameters  | Coat colour       |                   |                   | P value |
|-------------------|-------------------|-------------------|-------------------|---------|
|                   | White             | Black             | Mixed             |         |
|                   | Ls means $\pm$ SE | Ls means $\pm$ SE | Ls means $\pm$ SE |         |
| Insulin (mIU/L)   | 10.21 $\pm$ 2.83  | 11.12 $\pm$ 2.55  | 5.31 $\pm$ 3.09   | 0.325   |
| Prolactin (ng/ml) | 6.72 $\pm$ 3.16   | 5.70 $\pm$ 2.41   | 7.76 $\pm$ 3.80   | 0.890   |
| NEFA ( $\mu$ M)   | 397 $\pm$ 113     | 643 $\pm$ 111     | 427 $\pm$ 110     | 0.242   |

SE = standard error.

strategies under short-term heat stress conditions. Under the specific conditions of this study, however, the results revealed no differences in milk yield, milk fat content, milk protein content, lactose content, or somatic cell count between black-predominant and white-predominant Holstein-Friesian cows under both heat stress and non-heat stress conditions, similar results have been reported by previous studies (Gantner et al., 2020; Godfrey and Hansen, 1996; King et al., 1988; Valle, 1985). In contrast, other studies have reported some effects of coat colour on dairy cows under HS Anzuere-Olvera et al. (2019) in Mexico; Becerril et al. (1993) in the United States (Florida); Hansen (1990) in the United States (Florida); Maia et al. (2005) in Brazil; Mehany et al. (2025) in Egypt. The variations in these findings may be explained by several factors, such as different housing systems. For example, Hansen (1990) housed cows in an open area, subjecting them to chronic heat stress,

which differs from our study where cows experienced 2 h of heat stress as the rest of the day they were housed in a controlled, cooled barn where THI remained below 68. Variations may also arise from differences in the method of detecting the percentage of black colour. As an example, visual detection has been used by Becerril et al. (1993) and Maia et al. (2005), while our study employed image software. Additionally, climatic conditions across regions may play a role; Maia et al. (2005) conducted their study in an equatorial climate in Brazil characterised by a summer average temperature of 31 °C and humidity 85%, Becerril et al. (1993) in a subtropical climate with average temperature 32 °C and humidity 70%, whereas our research was conducted under a continental climate where the with lower summer average temperature 28% and humidity 59%. Moreover, differences in our results compared to others might also be attributed to variations in solar radiation and wind speed, as both elements affect cattle–environment thermal exchange (Ji et al., 2020; Oliveira et al., 2025; Wang et al., 2018). Higher wind speeds might alleviate heat stress through convective cooling, particularly when core body temperatures exceed ambient air temperature (Becker and Stone, 2020; Ji et al., 2020). Conversely, increased solar radiation can intensify heat load, especially for black cattle in tropical regions, open lots, or poorly insulated barns (Herbut and Angrecka, 2013; Ji et al., 2020; Mader et al., 2006; Silva et al., 2007; Wang et al., 2018). Other factors including dairy facility types, cow handling practices, feed, and the use of different sires could also contribute to these variations (Bernabucci et al., 2014). Regarding blood parameters, coat colour did not show any effect under both heat stress and non-heat stress conditions. Although black cows exhibited higher values of insulin and NEFA compared to white cows, the difference was not significant. These results align with Anzuere-Olvera et al. (2019), as they determined in their study that there is no effect of black coat and white coat on blood parameters, such as the complete blood count under heat stress conditions. In general, white and black cows reacted in the same way under short-term HS, but it is important to consider the specific conditions of our study. The relatively short duration of heat exposure (2 h daily), combined with controlled cooling for the remaining 22 h of the day, reflects practical management conditions on modern commercial farms but may have attenuated the cumulative thermal load experienced by the cows compared to continuous, unmitigated heat stress. Consequently, the absence of detectable coat-colour

**Table 6**Least square means ( $\pm$  standard error) for the interaction of coat colour and heat stress effect on blood parameters.

| Blood Parameters  | Coat colour       |                   |                   |                   |                   |                   | P value |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------|
|                   | White             |                   | Black             |                   | Mixed             |                   |         |
|                   | HS                | NHS               | HS                | NHS               | HS                | NHS               |         |
|                   | Ls means $\pm$ SE | Ls means $\pm$ SE | Ls means $\pm$ SE | Ls means $\pm$ SE | Ls means $\pm$ SE | Ls means $\pm$ SE |         |
| Insulin (mIU/L)   | 12.32 $\pm$ 2.50  | 8.11 $\pm$ 3.84   | 15.10 $\pm$ 2.63  | 7.14 $\pm$ 2.94   | 7.76 $\pm$ 2.41   | 2.85 $\pm$ 4.73   | 0.584   |
| Prolactin (ng/ml) | 15.15 $\pm$ 2.31  | −1.70 $\pm$ 5.33  | 6.57 $\pm$ 2.69   | 4.82 $\pm$ 3.33   | 8.99 $\pm$ 2.24   | 6.53 $\pm$ 6.89   | 0.054   |
| NEFA ( $\mu$ M)   | 491 $\pm$ 109     | 303 $\pm$ 127     | 737 $\pm$ 112     | 550 $\pm$ 120     | 521 $\pm$ 105     | 333 $\pm$ 124     | 0.772   |

HS = heat stress, NHS, = no heat stress, SE = standard error.

effects should be interpreted within this specific context. It remains possible that under more prolonged heat exposure, such as in open lots without cooling or in tropical regions with sustained high THI, darker coats might confer a greater thermal disadvantage, as suggested by previous studies (Anzuers-Olvera et al., 2019; Becerril et al., 1993; Hansen, 1990). However, it is noteworthy that despite the limited daily exposure, cows in our study exhibited significant physiological and productive responses consistent with heat stress (reduced milk yield, decreased protein and lactose, elevated insulin, prolactin, and NEFA), indicating that the thermal challenge was sufficient to elicit measurable stress.

The second aim of the study was to understand how a short, 2-h period of heat stress could affect cows' performance, given that they spent the remaining 22 h of the day in a cooled barn where the THI was consistently below 68. Our findings elucidate the direct impact of heat stress on bovine performance. Milk production of cows under short-term heat stress conditions was 1.5 kg significantly lower than that under NHS conditions. Consistent with the current research findings, previous studies have also documented a decrease in milk production among dairy cows subjected to heat stress (Chen et al., 2024; Gorniak et al., 2014; Mendonca et al., 2025). This decrease might be explained, on the one hand, by changes in the metabolism of the cows. It may be attributed to separate alterations in glucose and lipid regulation during the post-absorptive state (Wheelock et al., 2010). On the other hand, the decline in dry matter intake (DMI) under HS (Jo and Lee, 2025; Nascimento et al., 2025; West, 2003) is a significant factor contributing to the drop in milk production, with the decrease in DMI responsible for 50% of the noted drop in milk yield (Wheelock et al., 2010). In addition, heat stress has been associated with impaired intestinal integrity, including damage to intestinal villi, which may reduce nutrient absorption and further contribute to decreased productive performance (Baumgard and Rhoads Jr, 2013).

In line with the present results, the decrease in protein content of milk under heat stress conditions was reported by previous studies (Besteiro et al., 2025; Gao et al., 2017; Habimana et al., 2024). According to earlier research (Cragle et al., 1986; Guo et al., 2021), the protein content in the milk is more affected by the feed than other factors. As the DMI decreases under HS conditions (Chang-Fung-Martel et al., 2021), a consequent decrease in nutrient consumption during lactation is commonly accompanied by a decline in protein content (Emery, 1978; Shi et al., 2023). Rhoads et al. (2009) explained this decrease, suggesting that rough alterations in the somatotrophic axis could account for a minor fraction of the decline in milk protein yield during heat stress.

The lactose level was lower when the cows were under HS conditions compared to the levels under NHS conditions which is consistent with prior investigations (Dobranic et al., 2008; Fabris et al., 2019; Mehaba et al., 2019; Yasmin et al., 2012). This decrease might be explained by the alteration in the level of energy and nutrient intake caused by heat stress (Baumgard and Rhoads Jr, 2013). According to Costa et al. (2019) lactose content mainly affected by energy and metabolism imbalance in cows.

The present findings show no effect of short-term HS on milk fat. These results are supported by previous findings (Chen et al., 2024; Knapp and Grummer, 1991; Wheelock et al., 2010) which found that heat stress does not lead to a significant drop in the fat content of dairy cows. Our results did not reveal significant change in SCC due to HS same as Lengi et al. (2022) and Mendonca et al. (2025), which differs from previous research findings indicating an increase in hot periods of the year with high temperature-humidity indices compared to cold periods (Bouraoui et al., 2002; Lambertz et al., 2014; M'Hamdi et al., 2021). As reported by several researchers (Cartwright et al., 2023; Dahl et al., 2020; Vermunt and Tranter, 2011), chronic heat stress affects immunity of an animal increasing their susceptibility to mastitis hence increasing their SCC. Nevertheless, our findings align with those of previous studies that observed no rise in SCC levels under short-term

heat stress when cows were kept in controlled chambers, in contrast to outdoor circumstances (Lengi et al., 2022; Wegner et al., 1976). This suggests that the observed rise in SCC under heat stress conditions in certain studies may not be solely attributed to temperature. Instead, the favourable conditions during warmer months likely facilitate the growth of pathogens, leading to increased contamination exposure for cows (Smith et al., 2013).

Despite the blood samples being collected early in the morning on the day following short-term heat stress, 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). The increase in insulin levels under short-term HS conditions compared to non-heat stress conditions in the present findings are in line with several investigations (Ellett et al., 2024; Stefanska et al., 2024; Wheelock et al., 2010). The rise in insulin might be explained as a compensatory mechanism designed to decrease thermal output. According to Winn et al. (2023), insulin, the primary factor that triggers the absorption and processing of glucose, plays a crucial role in thermogenic activities. Rhoads et al. (2013) emphasise that optimal insulin functionality is essential for effective heat stress adaptation and for preventing heat-induced cellular damage. In further detail, during short-term heat stress, dairy cows undergo a physiological response characterized by elevated concentrations of insulin. In response to elevated ambient temperatures, stress hormones such as cortisol and catecholamines are released (Jo et al., 2021; Lamp et al., 2015; Idris et al., 2021). Cortisol, in particular, stimulates gluconeogenesis, the metabolic pathway that converts non-carbohydrate precursors, including amino acids, into glucose, thereby supplying an immediate energy source and resulting in elevated blood glucose levels (Ma et al., 2023; Ndibualonji et al., 1995; Thau et al., 2019). The pancreas responds by increasing insulin secretion to facilitate glucose uptake by peripheral tissues (Baumgard and Rhoads Jr, 2013).

In the present findings the blood levels of NEFA in cows under short-term HS conditions were higher compared to those under NHS conditions. Guo et al. (2018) and Zachut et al. (2020), observed also an increase in NEFA levels in dairy cows. Moreover, the drop in DMI in cows exposed to HS prolongs a state where energy intake is lower than energy expenditure, resulting in elevated levels of NEFA (De Rensis and Scaramuzzi, 2003; Kim et al., 2021; Woo et al., 2024). Additionally, heat stress-induced response is linked to disruption in lipid metabolism, including elevated NEFA levels and enhanced lipolysis (Rius et al., 2022), as the body attempts to meet the higher energy demands required to maintain a stable body temperature (Amaral-Phillips et al., 1993). This process it might be explained as the catecholamines released by the sympathetic nervous system trigger lipolysis, the degradation of accumulated triglycerides in adipose tissue (Mullins et al., 2014). This process releases NEFAs into the bloodstream, which can be used as an alternative energy source, reducing the reliance on glucose (Sammad et al., 2020). The increased NEFA levels reflect the body's attempt to adapt to stress and conserve glucose for essential functions (Sundrum, 2015).

This simultaneous increase in insulin and NEFA might be explained as heat stress has been shown to impair insulin sensitivity, meaning that the cells of the body become increasingly insensitive to insulin, leading to higher blood glucose levels even with elevated insulin secretion. This physiological phenomenon leads to the development of insulin resistance (Geiger and Gupte, 2011; Xie et al., 2016; Zachut et al., 2020). Moreover, insulin resistance is often induced by decreased nutrient consumption and negative energy balance due to heat stress. This condition manifests as a reduction in insulin sensitivity in peripheral tissues, leading to an elevated insulin-to-glucose ratio, increased lipolysis, and higher levels of NEFA in the plasma (De Koster and Opsomer, 2013). Furthermore, insulin plays a crucial role in inhibiting lipolysis in adipose tissue, thereby regulating the release of NEFA into the bloodstream. Under conditions of insulin resistance, this inhibitory effect is

compromised, resulting in enhanced basal lipolysis and elevated serum NEFA concentrations (Morigny et al., 2016; Pal and Méndez-Sánchez, 2023). In the context of selected dairy cows for highly milk yield, the metabolic adaptation to lipolysis facilitates the increased utilization of lipids for routine energy and metabolic activities. Concurrently, glucose is conserved for milk production, which further contributes to lower insulin synthesis and the onset of insulin resistance (Majkić et al., 2017).

In our findings, blood prolactin levels in cows increased due to short-term heat stress, a result consistent with previous studies (Cartwright et al., 2023; Djelailia et al., 2021; Do Amaral et al., 2010). This rise may be due to the important role of prolactin in thermoregulatory mechanisms when the animal is exposed to heat (Clarke et al., 2025), as suppression of its production in the bloodstream diminishes the body's capacity to regulate temperature (Al-Saiadi et al., 1995). Furthermore, prolactin has been demonstrated to regulate the movement of electrolytes and water in different fluid compartments of organisms, including the kidney, mammary gland and the gut (Clarke et al., 2025; Collier et al., 1982). Additionally, the genetic activity related to prolactin receptors, it may have a positive effect on the activation of sweat glands (Langan, 2024; Soos et al., 1993).

The possible limitations of this study include the low number of animals, as it was conducted on a commercial farm. This relatively small sample size may have reduced the statistical power of the study, potentially limiting the ability to detect significant coat-colour-dependent differences if they exist. The setting was chosen to closely mirror practical farming conditions, which adds ecological validity but also presents logistical challenges, as the primary focus is production rather than experimentation, limiting both the availability and selection of cows. Additionally, finding purely black or white cows was difficult, as mixed-coloured cows are more common. Furthermore, the meteorological station from which temperature and humidity data were obtained was located 2 km from the farm, which may not fully capture microclimatic variations and could introduce minor inaccuracies in THI calculations, although previous studies have successfully used stations located further away (Moore et al., 2023). Another limitation is that dry matter intake was not measured, as the cows were group-fed under commercial farm conditions; this may have obscured energetic responses to heat stress that could have further explained the metabolic changes observed. A further limitation of the present study relates to the temporal overlap between lactation progression and seasonal heat stress exposure. Because the study was conducted from April to September under commercial farm conditions, cows naturally advanced in DIM while environmental temperatures increased. Although DIM was included as a random effect in the mixed model to account for lactation-stage variation, it is not possible to completely disentangle the potential effects of lactation progression from seasonal heat stress in this study design. Therefore, the results should be interpreted with this consideration in mind. Finally, the absence of additional physiological indicators, such as rectal temperature and respiration rate, limits the comprehensiveness of the heat stress assessment, although the observed reductions in milk yield and alterations in blood metabolic parameters indicate that the animals experienced physiological responses consistent with heat stress. Despite these limitations, we believe the data collected provide valuable insights into the interaction between coat colour and heat stress. We acknowledge these constraints and suggest that increasing the sample size in future studies, and using on-site temperature and humidity data will help strengthen and expand upon these findings, as noted in previous research (Anzuere-Olvera et al., 2019; Dikmen et al., 2017; Flores et al., 2002; Gantner et al., 2020; Godfrey and Hansen, 1996; King et al., 1988; Lee et al., 2016; Valle, 1985).

## 5. Conclusion

Short-term heat stress, experienced during a 2-h outdoor exposure period, was associated with reduced milk yield, protein content, and lactose levels in lactating Holstein-Friesian cows. Additionally, increases

in insulin, prolactin, and NEFA were observed, indicating shifts in the metabolic parameters measured. These findings suggest that even limited heat exposure, when combined with cooling for the remainder of the day, can elicit some measurable physiological and productive responses. No interaction between coat colour and heat stress was detected, suggesting that under the specific management conditions, short-term exposure with active cooling, black and white cows did not differ in their responses. In conclusion, under the conditions of this study, coat colour did not appear to influence heat stress susceptibility. These results reinforce the importance of implementing heat abatement strategies, such as cooling systems and limiting outdoor access during peak heat, to mitigate the effects of heat stress on dairy cattle. Further research incorporating a broader range of metabolic indicators, direct physiological measurements, and longer or more intense heat exposure would help clarify the generalisability of these findings.

## Funding

The research was funded by the National Research, Development and Innovation Office (VKE-1.3.1-2018-00033, "A novel research of using lecithin and substituting palm fat with domestic raw material in the diet of high yielding dairy cattle, with special regard to improve the reproduction parameters and the possibilities of producing functional milk").

## CRediT authorship contribution statement

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

## Declaration of competing interest

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

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2026.104460>.

## Data availability

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

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