



THE EFFECT OF AMBIENT TEMPERATURE AND RELATIVE HUMIDITY IN POSTPARTUM DAIRY COWS ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE AND BIOCHEMICAL BLOOD INDICES IN THE SUBSEQUENT LACTATION

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Abstract

This study evaluated the effects of ambient temperature (T) and relative humidity (RH) during the postpartum transition period on dairy cows' milk performance, fertility, and immunometabolic blood indices in the subsequent lactation. A total of 100 Polish Holstein-Friesian cows originating from five commercial dairy farms were categorized into three groups based on average T (<16°C, 16–20°C and >20°C) and RH (<65%, 65–75%, and >75%) on the calving day (0d), and days 7, 14, 21 after calving. With increasing T and RH postpartum, the average daily milk yield during the first 150 days in milk decreased gradually, and the differences between T <16°C and >20°C and RH <65% and >75% groups were approximately 3.48 and 2.78 kg, respectively. Milk of cows exposed to increased T and RH was lower in protein and lactose and higher in fat, milk urea, and somatic cell count. It was also characterized by altered fat composition. Ambient T during the postpartum period had a negative effect on cows' fertility, which, however, was not affected by RH. The increasing T from <16°C to >20°C resulted in the later manifestation of the first estrus (by 27.7 days), a more extended artificial insemination service period (by 19.4 days), a higher number of insemination services per conception (by 0.92 on average), more days open (by 43.3 days), and a longer calving interval (by 43.3 days). Within ranges used in this study, increasing T and RH during the period from d 0 to d 14 postpartum led to decreased body condition score on day 21 by approximately 0.48 and 0.51 points, respectively. Furthermore, T and RH on a calving day were associated with altered biochemical blood indices on d 21 after calving, indicating a more severe negative energy balance and a state of inflammation. The results of this study suggest that dairy cows that calve when T >16°C should be provided cooling to ensure optimal environmental conditions for high milk production and prevent economic losses associated with reduced milk yield and low fertility.

Key words: fertility, immunometabolic blood indices, heat stress, microclimate, milk production

The climate is changing, and global warming effects, such as higher frequency of hot days and heat waves, significantly impact cattle performance by increasing the chances of animals experiencing heat stress (HS). High-producing dairy cows are particularly susceptible to HS as they generate more heat during digestive and metabolic processes than low-yielders due to their higher feeding requirements associated with increased milk production (Das et al., 2016). Milk yield per cow continuously increases due to genetic selection and improved nutrition and management practices (Gross, 2023). This, coupled with global warming, is thought to make HS an even greater challenge for dairy farmers in the future.

HS arises when hot environmental conditions cause cattle to reach a point where they gain more heat from

metabolism and the environment than they can easily lose. It leads to hyperthermia and triggers several behavioral, physiological, and cellular responses aimed at returning to temperature balance. As reviewed by several papers (Bernabucci et al., 2010; Sammad et al., 2020; Cartwright et al., 2023), typical cows' adaptations to HS include a decrease in dry matter intake (DMI), an increase in sweating and respiration rate, and re-directing blood from the digestive system to the peripheral tissues where heat can dissipate more efficiently. They are accompanied by significant changes in metabolism aimed at decreasing internal heat generation (Lamp et al., 2015; Chen et al., 2024). Although these responses are essential for animals' welfare and survival, they result, among other things, in greater maintenance requirements, de-

*This study was partially supported by a grant financed by the Polish Federation of Cattle Breeders and Dairy Farmers (no. 74/2017/B) and partially by Poznań University of Life Sciences (Poland) as the research program "First grant" (no. 3/2022) and the statutory funds (no. 506.569.04.00).

creased energy availability (Sammad et al., 2020; Kim et al., 2022), altered mammary gland function (Tao et al., 2018; Mendonca et al., 2024), changes in endocrine profile (Bernabucci et al., 2010; Sammad et al., 2020), oxidative stress (Guo et al., 2021), and impaired immune function (Lemal et al., 2022). Consequently, HS in dairy cattle often leads to numerous issues, including reductions in milk production, health problems, and impaired reproduction (Tao et al., 2020; Cartwright et al., 2023).

The extent of adaptive changes in response to hot environment conditions largely depends on animals' nutritional and metabolic status related to the phase of their production cycle. The transition period between late pregnancy and the onset of lactation, ranging between three weeks before and three weeks after calving (Drackley, 1999), is a challenging time for dairy cows. During this stage, cows experience a drop in feed intake combined with a rapid increase in nutrient requirements to support the final stages of foetal growth and initiation of milk synthesis, resulting in a negative energy balance (NEB; de Vries and Veerkamp, 2000). In the state of NEB, body fat reserves are mobilized from adipose tissue to compensate for energy deficit, and non-esterified fatty acids (NEFA) are liberated into the blood circulation to be subsequently oxidized in the liver. Excess of released NEFA is metabolized in hepatocytes to ketone bodies, including β -hydroxybutyrate (BHBA), or re-esterified to triglycerides (TG; Stefanska et al., 2016 a, b; Baumgard et al., 2017). Although NEB is a normal adaptive mechanism in transition cows, severe body fat mobilization can lead to metabolic disorders such as fatty liver and ketosis (Drackley, 1999; McArt et al., 2013). Several studies found that NEB, with concurrent changes in plasma BHBA and NEFA concentrations, is associated with immune suppression (Sordillo and Mavangira, 2014; Bionaz et al., 2007). This, combined with other known immunosuppressants like stress, low calcium levels, and endocrine changes associated with calving, can increase the risk of retained placenta, metritis, and mastitis postpartum (Esposito et al., 2014). Shifts in blood metabolites and hormones related to NEB can also compromise ovarian function and fertility (Butler, 2005; Dubub et al., 2012; Cheong et al., 2016). In addition, multiple reproductive processes are impaired due to postpartum infectious and metabolic disorders (Carvalho et al., 2019; Braga Paiano et al., 2019; Gilbert, 2019).

Heat stress during the transition period threatens dairy cows' already challenged metabolism and immune system, which can further exacerbate their health, fertility, and milk production. However, although many studies analyzing HS effects investigated cows in various phases of their physiological cycle, research focused on the periparturient period, especially in the postpartum transition period, is limited.

Ambient temperature is a significant climate factor determining HS, but humidity is also important because losing body heat is more effective when humidity is low. The temperature-humidity index (THI) combines these

two factors into an indicator of cow thermal comfort often used in research. In our previous study, we investigated the effects of HS based on the THI indicator on cows' performance (Stefanska et al., 2024 a, b). However, using air temperature alone may also be reliable, and temperature is easier to monitor in daily farm management (Hut et al., 2022). Therefore, this study aimed to evaluate the effects of ambient temperatures and relative humidity as HS parameters of cows during early lactation on milk yield and composition, fertility, and immunometabolic blood indices in subsequent lactation.

Material and methods

Ethics approval

This work was carried out in accordance with the EU Directive 2010/63/EU for the protection of animals used for scientific purposes, and all procedures were conventional practices for animal health assessment and monitoring. In particular, blood samples were collected during standard veterinary activities, and the appropriate authorities approved all procedures.

Animals

Data for this study originated from 5 commercial dairy farms and were collected between June and September 2018 in temperate climates. Prospective cohort data were gathered from these farms. The selected farms had more than 80 dairy cows (93 on average; ranging from 85 to 110) and the milk yield of the previous lactation was greater than 9,500 kg (10,173 kg on average; ranging from 9,987 to 10,640 kg). From each farm, 20 Polish Holstein-Friesian cows were selected based on their parity (41 primiparous and 59 multiparous), 305-d milk production (10,055 kg on average; ranging from 9,252 to 10,800 kg), and health status (clinically healthy). Of the 100 selected cows, six were found to have health problems during the first 60 days after calving and were eliminated from the study. Data obtained for these animals were included in the analysis until the time at which the health complications occurred. The herd maintained a similar management and housing system. During the dry period, the cows were housed in shaded free-stall barns with natural ventilation and bedding consisting of chopped straw. After calving, all cows received similar treatment, including access to cooling measures such as shade and fans or air mixers during the lactation phase. The fans or air mixers were automatically activated when the ambient temperature exceeded 21.0°C, and they maintained an air velocity of 1.8 m/s, following the guidelines of Bailey et al. (2016). Representative samples of forages, including corn, grass, and alfalfa silages, were collected monthly. These samples were analyzed using the near-infrared reflectance spectroscopy method as per PN-EN 12099:2017-10, employing a FOSS DS3™ (Foss Electric, Hillerød, Denmark). The analysis covered various nutritional components, including dry matter, crude

protein, neutral detergent fiber, acid detergent fiber, ether extract, and starch. This analysis was conducted consistently throughout the experiment. The nutritional value of the feed components was calculated using the nutrient content obtained through analysis. These calculations were performed using the AMTS.Cattle.Pro version 4.7 (2017, AMTS LLC, Groton, NY). The formulated diets for lactating cows were adjusted monthly to meet the nutritional requirements following the guidelines set forth by the National Research Council (2001). These diets were composed primarily of silages such as corn, grass, and alfalfa, as well as concentrates such as soybean meal, rapeseed meal, barley, and triticale. A mineral–vitamin premix was also included. These diets were delivered to the cows twice daily, at 0600 and 1400 h, and were made available *ad libitum*. After calving, all cows were transitioned to an early FRESH lactation diet up to 21 days in milk (DIM), with an average feed intake of 24 kg dry matter intake (DMI), and 35 kg milk yield. Subsequently, from 22 to 150 DIM, they were fed a TMR I lactation diet, formulated to meet the nutritional requirements of dairy cows consuming 28 kg DMI and producing 40 kg of milk. The early lactation FRESH diet contained a range of 50.8–65.2% forage, 17.5–18.1% CP, 31.1–34.1% NDF, 20.7–24.4% starch, and 255–286 mEq/kg DCAB on a DM basis. The TMR I diet contained a range of 45.5–54.9% forage, 15.9–17.5% CP, 28.1–31.5% NDF, 25.5–28.4% starch, and 268–320 mEq/kg DCAB on a DM basis. The composition and nutritional value of lactation diets are summarized in Stefanska et al. (2024 b). Orts, which refer to leftover feed, were removed daily before the morning feeding. Throughout the experiment, water was supplied to the cows *ad libitum* and was accessible through self-filling troughs.

Environmental data

Temperature (T, °C) and relative humidity (RH, %) in the barns were recorded every 15-minute interval with data loggers (WatchDog A-Series, SpecWare 9 Basic, Spectrim Technologies Inc., Aurora, USA) located 3 m above animals. This positioning aligns with the recommendations put forth by Hut et al. (2022) and Lambertz et al. (2014). These data loggers had a temperature range of –45 to 85°C with an accuracy of $\pm 0.6^\circ\text{C}$ and a humidity range of 0% to 100% with an accuracy of $\pm 3\%$. The cows were classified based on their T and RH independent exposure during the early lactation period, and the average daily T and RH were calculated on the calving day (0 d) and the 7, 14, and 21 days in milk. Temperature and relative humidity values were then divided respectively into three categories: T <16°C, T 16–20°C and T >20°C, and RH <65%, RH 65–75%, and RH >75%. These categories were chosen based on levels of environmental parameters previously indicated as thresholds above which HS effects start to occur in cows raised in temperate climates of Central Europe (Brügemann et al., 2012; Gorniak et al., 2014; Pinto et al., 2020). Also, descriptive statistics of evaluated parameters (explanatory

variables such as T and RH) during early lactation of Polish Holstein-Friesian dairy cows are shown in Table 1.

Table 1. Descriptive statistics of evaluated parameters (explanatory variables such as T and RH) during early lactation of dairy cows

Item ¹	Mean	Minimum	Maximum
T 0 d	23.38	17.40	27.30
T +7 d	22.92	17.70	26.50
T +14 d	22.84	18.29	26.89
T +21 d	22.93	17.77	27.50
RH 0 d	66.36	55.05	82.07
RH +7 d	68.14	55.65	83.61
RH +14 d	67.41	51.04	81.21
RH +21 d	66.82	51.43	80.43

¹T 0 d, +7 d, +14 d, +21 d = temperature calculated on the calving day (0 d) and 7, 14, and 21 days after calving; RH 0 d, +7 d, +14 d, +21 d = relative humidity calculated on the calving day (0 d) and 7, 14, and 21 days after calving.

Milk production performance

The milk performance data used in this study included the early lactation period from the calving day until 150 DIM. Farms housing animals used in this study milked cows twice a day at approximately 0500 and 1700 h. Milk yield was recorded daily using automated electronic milk meters (DeLaval MM27BC, Wrocław, Poland). Milk samples for the determination of the chemical composition of milk were collected from each cow during the morning and afternoon milking of every month's routine test days conducted by the Polish Federation of Cattle Breeders and Dairy Farmers. Concentrations of fat (%), protein (%), lactose (%), casein (%), milk urea (MU, mg/mL), and fatty acids (FA, g/100 g of fat) in milk were predicted by Fourier transform infrared spectroscopy using a CombiFoss FT 6000 apparatus (Foss Electric, Hillerød, Denmark), and somatic cell count (SCC, 10³ cells/mL) was determined using Foss-o-Matic equipment (FOSS Electric, Hillerød, Denmark). FA included four individual FA (C14:0, C16:0, C18:0, and C18:1) and eight groups, specifically saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), trans-unsaturated FA (trans-UFA), short-chain FA (SCFA), medium-chain FA (MCFA), and long-chain FA (LCFA). More details about milk fatty acids analysis are presented by Komisarek et al. (2022). The milk yield and composition data for this study were collected until 150 DIM. They were subsequently used to calculate 3.5% fat-corrected (FCM), 3.5% fat-and-protein-corrected (FPCM), and energy-corrected (ECM) milk yields according to formulas described in Stefanska et al. (2024 a).

Reproductive performance and body condition score

Reproductive data included the length of the anestrus period (number of days from calving to the first artificial insemination (AI) service), the length of the AI service

period (number of days from the first to successful AI service), the number of services per conception, days open (number of days between parturition and the following conception), and calving interval (number of days from one to the next calving). The body condition score (BCS) of the cows, rated on a scale of 1 (thin) to 5 (fat) according to Edmonson et al. (1989), was recorded at three weeks after calving (on d 21±3).

Blood sampling and analyses

Blood samples were collected from the tail vein 4±0.5 h after morning feeding on d 21±3 after calving. The vacutainers with samples were transported to the laboratory and centrifuged at 3,000 × g for 15 min at 4°C. The separated serum was stored at -20°C until analyzed. Concentrations of the immunometabolic blood indices were then determined in the samples using a microplate spectrophotometer (Synergy 2, BioTek Biokom). The metabolites determined in the blood samples were glucose (g/dL), TG (mg/dL), NEFA (mmol/L), BHBA (mmol/L), aspartate aminotransferase (AST; IU/L), alanine aminotransferase (ALT; IU/L), cholesterol (mg/dL), low-density lipoproteins (LDL; mg/dL), high-density lipoproteins (HDL; mg/dL), blood urea nitrogen (BUN; mg/dL), total serum protein (TSP; g/dL), albumin (g/L), tumor necrosis factor α (TNF-α; ng/L), interleukin 1 (IL-1; ng/L), interleukin 6 (IL-6; ng/L), serum amyloid A (SAA; μg/mL), haptoglobin (Hpt; μg/mL), heat shock 70 kDa protein 1A (HSP70; ng/mL), calcium (Ca; mg/dL) and hormones such as insulin (ng/mL), insulin-like growth factor I (IGF-I; ng/mL), follicle-stimulating hormone (FSH; ng/mL), luteinizing hormone (LH; ng/mL), adiponectin (ADP; μg/mL), leptin (LEP; ng/mL). Details on blood indices determination methods are given in Stefanska et al. (2024 a).

Statistical analysis

Before analysis the PROC MEANS was used and then all data underwent normality screening using PROC UNIVARIATE in SAS version 9.4, SAS Institute. Fertility parameters, such as service per conception and AI period service were subjected to Box-Cox transformation, and milk SCC to a logistic transformation function before statistical analysis. The results for the above fertility parameters and milk SCC were presented in Tables as means before a transformation, but the significance between the explanatory variables for these indices after transformation. The explanatory variables, T and RH during early lactation (at days 0, 7, 14, and 21), were divided into three categories, and this classification was consistent across the analysis of productive performance, fertility, and biochemical blood indices. The effect of barn environmental microclimate such as T and RH indices during early lactation on productive performance, fertility, and immunometabolic blood indices was determined using the GLM procedure of SAS separately for each explanatory variable under the models described in the equations. Milk production

performance also including FA composition was analyzed using the following model:

$$Y_{ijklmno} = \mu + L_i + H_j + B_k + R_l + \beta_1 afc_m + \beta_2 dl_n + e_{ijklmno}$$

where: $Y_{ijklmno}$ = the value of the dependent variable; μ = the overall mean; L_i – fixed effect of cow's parity ($i = 1, 2$); H_j – fixed effect of the farm ($j = 1, 2, 3, 4, 5$); B_k – is the explanatory variable – fixed effect of the category of T on the 0, or 7, or 14, or 21 DIM during early lactation ($k = 1, 2, 3$); R_l – is the explanatory variable – fixed effect of the category of RH on the 0, or 7, or 14, or 21 DIM during early lactation ($l = 1, 2, 3$); β_1 and β_2 – partial linear regression coefficients; afc_m – the age at first calving; dl_n – DIM; and $e_{ijklmno}$ – is the random error. The fertility indices were analyzed using the same statistical model but without the second partial regression coefficient ($\beta_2 dl_n$). The immunometabolic blood indices were analyzed using the following model:

$$Y_{ijklmno} = \mu + L_i + H_j + B_k + R_l + \beta_1 afc_m + \beta_2 ds_n + e_{ijklmno}$$

where: $Y_{ijklmno}$ = the value of the dependent variable; μ = the overall mean; L_i – fixed effect of cow's parity ($i = 1, 2$); H_j – fixed effect of the farm ($j = 1, 2, 3, 4, 5$); B_k – is the explanatory variable – fixed effect of the category of T on the 0, or 7, or 14, or 21 DIM during early lactation ($k = 1, 2, 3$); R_l – is the explanatory variable – fixed effect of the category of RH on the 0, or 7, or 14, or 21 DIM during early lactation ($l = 1, 2, 3$); β_1 and β_2 – partial linear regression coefficients; afc_m – the age at first calving; ds_n – day of blood sampling; and $e_{ijklmno}$ – is the random error. In cases of the significance of the explanatory variables, individual comparisons were made using Duncan's adjustment. Statistical significance was declared when $P \leq 0.05$.

Results

The effects of environmental conditions during the early postpartum period on cows' lactation performance are shown in Tables 2–5. With increasing ambient T and RH from the calving day to day 21, the average daily milk yield during the first 150 days of lactation decreased gradually ($P \leq 0.05$). The differences between T <16°C and >20°C and RH <65% and >75% groups were approximately 3.48 and 2.78 kg, respectively. Changes in 3.5% FCM, 3.5% FPCM, and ECM due to rising T and RH were similar to those for actual milk yield. Also, the chemical composition of milk was associated with ambient T and RH during the early postpartum period ($P \leq 0.05$). Cows exposed to T >20°C and RH >75% produced milk that was lower in protein (by approximately 0.2 and 0.1%, respectively) and higher in fat (by approximately 0.4 and 0.3%, respectively) than those housed in T <16°C and RH <65%. The milk lactose concentration was associated with barn microcli-

mate indices during early lactation. With an increasing T on the +14 d and RH on the calving day (0d) and on the +14 d during early lactation, we recorded decreased milk lactose content ($P \leq 0.05$). Also, increasing T from the calving day up to +21 d during early lactation was associated with higher milk casein, but increasing RH was associated with decreasing that milk compound ($P \leq 0.05$). Also, increased T and RH were associated with increasing the MU and SCC contents in milk ($P \leq 0.05$). We observed an association of T and RH values from day 0 to day 21 after calving with milk fatty acids composition during the first 150 days of lactation. Among individual FA analyzed in this study, only C14:0 was associated with T and RH categories, and lower C14:0 values ($P \leq 0.05$) were found in the milk of $T \geq 16^\circ\text{C}$ and $\text{RH} \geq 65\%$ compared to $T < 16^\circ\text{C}$ and $\text{RH} < 65\%$ cows, respectively. With rising T and RH early after calving, SFA gradually increased, and UFA decreased ($P \leq 0.05$). We also observed that LCFA increased ($P \leq 0.05$) at the expense of SCFA and MCFA ($P \leq 0.05$) with rising T and RH. Additionally, both environmental parameters were associated with trans-UFA content in milk fat. The BCS on day 21 after calving was associated with both environmental parameters we used in this study (Table 2–5; $P \leq 0.05$). With rising T (from $T < 16^\circ\text{C}$ to $T > 20^\circ\text{C}$) and RH (from $\text{RH} < 65\%$ to $\text{RH} > 75\%$) up to d 14 postpartum, the BCS decreased by approximately 0.48 and 0.51 points, respectively.

We found the detrimental effect of ambient T from the calving day (d 0) to day 21 after calving on the subsequent fertility of cows ($P \leq 0.05$), which, however, was not affected by RH (Table 6). The increasing air temperature postpartum from $T < 16^\circ\text{C}$ to $T > 20^\circ\text{C}$ was related to the later manifestation of the first estrus (by 27.7 days on average), a more extended AI service period (by 19.4 days on average), and higher number of AI services per conception (by 0.92 on average). Consequently, the days open and calving interval in $T > 20^\circ\text{C}$ cows were elongated by 47.3 days on average compared to the $T < 16^\circ\text{C}$ group.

The associations between ambient T and RH on a calving day and biochemical blood indices on d 21 after calving are presented in Table 7. With increasing T and RH, we observed increased concentrations of TG, NEFA, BHBA, LEP, TSP, TNF- α , IL-1, IL-6, and HSP70 (the latest was affected only by temperature) and lower levels of glucose, IGF-I, ADP, FSH, and LH ($P \leq 0.05$). The remaining blood indices included in this study, such as insulin, AST, ALT, cholesterol and its fractions, BUN, albumin, SAA, Hpt, and Ca, were not influenced by barn microclimate conditions at calving.

Discussion

The results of this study indicate that exposure of dairy cows to increased ambient T and RH during

the postpartum transition period is associated with changes in their metabolic status and has a prolonged effect on productive and reproductive performance. The reduction in milk yield is a frequent observation in lactating cows suffering deterioration of barn microclimate indices and HS occurrence. An important contributor to this reduction is a lower feed intake, leading to insufficient energy and nutrient supply to an animal to support high milk production (Rhoads et al., 2009; Baumgard et al., 2011; Lamp et al., 2015). The other factors include increased energy expenditure for heat loss mechanisms such as panting and sweating and changes in postabsorptive metabolism, independent of feed intake and whole-animal energy balance, which may alter the availability of nutrients to the mammary gland (Baumgard and Rhoads, 2012). Although many papers reported lower milk production during HS conditions, limited data exists about the longer-term effects of early-lactation cows' exposure to hot environments. Safa et al. (2019) observed that cows cooled during the first seven weeks after calving yielded 1442 kg more milk over the whole lactation period than noncooled cows. Menta et al. (2022) recently showed that cows suffering from HS during PRE (28 days before calving), POST (28 days after calving), or both PRE and POST periods displayed similar reductions in daily milk yield (1.7 kg and 2.4 kg in nulliparous and parous cows, respectively) until 90 DIM. These cows were also at a greater risk of morbidity in early lactation, which could contribute to their lower milk production. In this study, ambient $T \geq 16^\circ\text{C}$ and $\text{RH} > 75\%$ conditions from calving day (d 0) to d 21 postpartum were associated with reduced daily milk yield (by approximately 3.5 kg) until 150 DIM. Even though individual feed intake data were not included in our data set, it seems likely that this reduction partly resulted from decreased DMI and changed metabolism during the first weeks after calving. Also, similarly to Menta et al. (2022), HS cows in our study were characterized by a higher SCC in milk, indicating poorer udder health, and mastitis, even in the subclinical form, negatively affects cows' productivity. Increased plasma levels of pro-inflammatory cytokines IL-1 α , IL-6, and TNF- α that we observed in cows exposed to higher ambient T and RH might also be important. There is evidence that postpartum inflammation negatively affects milk production, although mechanisms underlying long-term inflammatory effects on milk yield remain unclear. Bionaz et al. (2007) showed that cows with the greatest plasma paraoxonase activity associated with increased markers of inflammation during the first 30 DIM produced 1,971 kg more milk over 305 d lactation than animals with the lowest enzyme activity. In line with these results, Carpenter et al. (2016) reported that treating cows for three days early after calving with anti-inflammatory drugs increased whole-lactation milk yield by 7 to 9%.

Table 2. The effect of the temperature (T) or relative humidity (RH) on the calving day (0 d) on dairy cows' lactation performance and body condition score

Item	T			RH			SEM	P-value					
	<16 °C	16–20 °C	>20 °C	<65%	65–75%	>75%		farm (H_1)	dam parity (L_1)	T (B_1)	RH (B_1)	AFC ¹ (β_1 atc ₁)	DIM ² (β_2 dl ₁)
Milk yield (kg)	32.4 a	28.7 b	29.0 b	30.2 a	29.4 b	29.4 b	0.26	0.0001	0.0001	0.0362	0.0201	0.8911	0.0001
3.5% FCM (kg)	30.4 a	28.9 b	28.8 b	29.1 a	29.8 a	27.9 b	0.24	0.0001	0.0001	0.0223	0.0141	0.5070	0.0001
3.5% FPCM (kg)	30.8 a	29.3 b	29.2 b	30.2 a	29.4 ab	28.5 b	0.23	0.0001	0.0001	0.0379	0.0328	0.6588	0.0001
ECM (kg)	33.8 a	32.1 b	32.0 b	33.1 a	32.3 ab	31.3 b	0.26	0.0001	0.0001	0.0417	0.0224	0.6699	0.0001
Milk fat (%)	3.66 b	4.08 a	4.04 a	3.69 b	4.01 a	4.03 a	0.03	0.0001	0.4519	0.0249	0.0128	0.4186	0.0001
Milk protein (%)	3.51a	3.55 a	3.32 b	3.53 a	3.49 ab	3.43 b	0.01	0.0001	0.0162	0.0197	0.0276	0.6846	0.0001
Milk lactose (%)	4.81	4.83	4.79	4.85 a	4.80 b	4.80 b	0.01	0.0001	0.0001	0.3228	0.0228	0.1392	0.0001
Milk casein (%)	2.60 b	2.78 a	2.75 a	2.77 a	2.73 ab	2.69 b	0.01	0.0001	0.1184	0.0305	0.0228	0.6039	0.0001
SCC (10^3 cells/mL)	238 b	362 b	478 a	279 b	439 a	436 a	36.0	0.0001	0.0001	0.0186	0.0289	0.6080	0.0004
MU (mg/mL)	219 b	208 b	239 a	203 b	227 a	232 a	2.42	0.0001	0.0828	0.0145	0.0168	0.7685	0.0001
Milk fatty acids (FA), g/100 g fat ³													
C14:0	11.8 a	11.5 b	11.4 b	12.0 a	11.4 b	11.4 b	0.05	0.0001	0.7457	0.0425	0.0141	0.9026	0.0001
C16:0	29.7	29.8	29.9	30.1	29.9	30.1	0.10	0.0001	0.1073	0.2781	0.3209	0.9275	0.0001
C18:0	10.2	9.60	9.60	10.0	9.50	9.10	0.06	0.0001	0.6532	0.9939	0.2325	0.7712	0.0001
C18:1	19.0	18.8	18.8	18.5	19.1	18.8	0.13	0.0001	0.0001	0.6736	0.2131	0.8230	0.0001
SFA	65.0 b	65.7 ab	66.0 a	65.2 b	65.5 ab	66.4 a	0.14	0.0244	0.0001	0.0430	0.0312	0.8740	0.0001
UFA	27.6 a	27.6 a	28.5 b	27.9 a	27.8 a	26.5 b	0.14	0.0001	0.0003	0.0324	0.0471	0.5267	0.0001
MUFA	22.9	22.6	22.7	22.8	22.9	22.6	0.13	0.0001	0.0001	0.4309	0.1411	0.9842	0.0001
PUFA	2.91	2.86	2.93	2.92	2.98	2.82	0.02	0.0001	0.0615	0.1509	0.4049	0.4684	0.8748
TRANS-UFA	1.45 b	1.67 ab	1.73 a	1.47 b	1.70 b	2.10 a	0.05	0.0001	0.5111	0.0332	0.0460	0.3086	0.1484
SCFA	10.6 a	10.0 ab	9.89 b	10.3 a	10.1 ab	9.94 b	0.04	0.0001	0.0091	0.0122	0.0199	0.7153	0.9812
MCFA	44.0 a	43.4 b	43.3 b	43.1	43.9	43.6	0.19	0.0001	0.0039	0.0410	0.3312	0.9891	0.0001
LCFA	31.6 b	31.9 b	32.5 a	30.7 b	31.1 b	32.3 a	0.19	0.0001	0.0001	0.0289	0.0242	0.3718	0.0001
BCS 21 d after calving	3.43 a	3.37 ab	3.03 b	3.30 a	3.24 b	2.72 b	0.07	0.0523	0.4634	0.0348	0.0226	0.8221	0.6531

¹AFC = age at first calving.²DIM = days in milk.³Milk fatty acids (g/100 g fat): SFA – saturated fatty acids (FA); UFA – unsaturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; trans-UFA – trans-unsaturated FA; SCFA – short-chain FA; MCFA – medium-chain FA; LCFA – long-chain FA.a, b – means of the explanatory variable within the category of T or RH on the calving day (0 d) within the row with different letters differed significantly ($P \leq 0.05$).

Table 3. The effect of the temperature (T) or relative humidity (RH) on the +7 day of lactation on dairy cows' lactation performance and body condition score

Item	T			RH			SEM	P-value					
	<16 °C	16–20 °C	>20 °C	<65%	65–75%	>75%		farm (<i>H</i> _i)	dam parity (<i>L</i> _i)	T (<i>B</i> _i)	RH (<i>B</i> _i)	AFC ¹ (<i>β</i> _{afc})	DIM ² (<i>β</i> _d)
Milk yield (kg)	32.3 a	29.0 b	28.6 b	30.8 a	30.6 a	28.2 b	0.26	0.0001	0.0001	0.0270	0.0104	0.3650	0.0001
3.3–3.5% FCM (kg)	30.6 a	28.1 b	28.9 b	29.9 a	29.3 ab	28.3 b	0.24	0.0001	0.0001	0.0225	0.0297	0.2393	0.0001
3.3–3.5% FPCM (kg)	31.0 a	28.5 b	29.3 b	29.9 a	30.3 a	28.6 b	0.23	0.0001	0.0001	0.0208	0.0265	0.3076	0.0001
ECM (kg)	34.0 a	31.2 b	32.1 b	32.9 a	33.2 a	31.4 b	0.26	0.0001	0.0001	0.0118	0.0368	0.3028	0.0001
Milk fat (%)	3.73 b	3.83 b	4.16 a	3.81 b	3.89 b	4.08 a	0.03	0.0001	0.1433	0.0218	0.0368	0.6917	0.0001
Milk protein (%)	3.56 a	3.44 b	3.35 c	3.51 a	3.53 a	3.40 b	0.01	0.0001	0.1223	0.0211	0.0161	0.1954	0.0001
Milk lactose (%)	4.82	4.83	4.80	4.83	4.80	4.80	0.01	0.0001	0.0001	0.2346	0.9274	0.3116	0.0001
Milk casein (%)	2.63 c	2.70 b	2.79 a	2.75 a	2.77 a	2.66 b	0.01	0.0001	0.4252	0.0223	0.0309	0.1652	0.0001
SCC (10 ³ cells/mL)	308 b	404 b	607 a	212 b	476 a	438 a	36.0	0.0055	0.0001	0.0218	0.0346	0.0338	0.0003
MU (mg/mL)	214 b	219 b	272 a	207 b	227 a	230 a	2.42	0.0001	0.0954	0.0307	0.0232	0.2024	0.0001
Milk fatty acids (FA), g/100 g fat ³													
C14:0	11.9 a	11.5 b	11.6 b	11.7 a	11.5 b	11.2 b	0.05	0.0001	0.7714	0.0229	0.0143	0.9006	0.0001
C16:0	29.8	29.8	29.9	29.8	30.0	29.8	0.10	0.0001	0.1296	0.4472	0.1702	0.7787	0.0001
C18:0	9.99	9.55	9.54	10.2	9.48	9.69	0.06	0.0001	0.4688	0.1172	0.1116	0.5688	0.0001
C18:1	18.7	19.3	18.7	19.3	18.6	18.9	0.13	0.0003	0.0006	0.9840	0.1500	0.2382	0.0001
SFA	65.0 b	65.5 ab	66.1 a	65.7 b	66.3 ab	65.6 a	0.14	0.0420	0.0001	0.0121	0.0258	0.1695	0.0001
UFA	28.3 a	28.0 a	27.4 b	28.3 a	28.1 a	27.2 b	0.14	0.0001	0.0010	0.0321	0.0138	0.0472	0.0001
MUFA	22.5	23.4	22.5	23.5	22.4	22.7	0.13	0.0001	0.0001	0.2803	0.1790	0.1317	0.0001
PUFA	2.86	3.00	2.88	3.11	2.92	2.81	0.02	0.0001	0.1691	0.7621	0.1156	0.2890	0.9989
TRANS-UFA	1.50 b	1.65 ab	1.79 a	1.48 b	1.65 b	2.05 a	0.05	0.0001	0.0037	0.0121	0.0258	0.0395	0.5872
SCFA	10.5 a	10.0 ab	9.87 b	10.4 a	10.1 ab	9.89 b	0.04	0.0001	0.0037	0.0321	0.0138	0.0395	0.5872
MCFA	44.5 a	43.8 b	42.4 b	43.6	43.1	43.8	0.19	0.0001	0.0314	0.0128	0.6700	0.3436	0.0001
LCFA	31.5 b	31.3 b	32.0 a	31.9 b	31.3 b	32.6 a	0.19	0.0001	0.0002	0.0379	0.0128	0.2263	0.0001
BCS 21 d after calving	3.48 a	3.27 ab	3.05 b	3.32 a	3.24 b	2.76 b	0.07	0.5283	0.4346	0.0124	0.0221	0.3421	0.8221

¹AFC = age at first calving.²DIM = days in milk.³Milk fatty acids (g/100 g fat): SFA – saturated fatty acids (FA); UFA – unsaturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; trans-UFA – trans-unsaturated FA; SCFA – short-chain FA; MCFA – medium-chain FA; LCFA – long-chain FA.

a, b, c – means of the explanatory variable within the category of T or RH on the +7 d of lactation within the row with different letters differed significantly (P≤0.05).

Table 4. The effect of the temperature (T) or relative humidity (RH) on the +14 day of lactation on dairy cows' lactation performance and body condition score

Item	T			RH			SEM	P-value					DIM ² (β_{2m})
	<16 °C	16–20 °C	>20 °C	<65%	65–75%	>75%		farm (H)	dam parity (L)	T (B _t)	RH (B _r)	AFC ¹ (β_{afc})	
Milk yield (kg)	31.8 a	29.5 b	28.5 b	30.8 a	30.6 a	27.1 b	0.26	0.0001	0.0001	0.0141	0.0376	0.6303	0.0001
3.5% FCM (kg)	30.0 a	29.0 ab	28.6 b	29.8 a	29.6 a	27.5 b	0.24	0.0001	0.0001	0.0118	0.0460	0.4119	0.0001
3.5% FPCM (kg)	30.4 a	29.3 b	29.0 b	30.4 a	30.0 a	27.7 b	0.23	0.0001	0.0001	0.0142	0.0107	0.4720	0.0001
ECM (kg)	33.4 a	32.1 b	31.8 b	33.3 a	32.9 a	30.4 b	0.26	0.0001	0.0001	0.0249	0.0186	0.5050	0.0001
Milk fat (%)	3.68 b	3.96 a	4.10 a	3.83 b	3.86 b	4.18 a	0.03	0.0001	0.8752	0.0113	0.0122	0.0892	0.0001
Milk protein (%)	3.57 a	3.44 b	3.35 c	3.50 a	3.47 b	3.45 b	0.01	0.0001	0.2916	0.0127	0.0391	0.1789	0.0001
Milk lactose (%)	4.85 a	4.80 b	4.80 b	4.81 a	4.77 b	4.73 c	0.01	0.0001	0.0001	0.0230	0.0366	0.5523	0.0001
Milk casein (%)	2.62 c	2.69 b	2.80 a	2.74	2.72	2.70	0.01	0.0001	0.8878	0.0159	0.2442	0.1852	0.0001
SCC (10 ³ cells/mL)	318 b	371 b	590 a	319 b	432 a	464 a	36.0	0.0001	0.0001	0.0174	0.0393	0.8959	0.0001
MU (mg/mL)	215 b	223 b	244 a	208 b	241 a	248 a	2.42	0.0001	0.6880	0.0243	0.0287	0.1427	0.0001
Milk fatty acids (FA), g/100 g fat ³													
C14:0	11.7 a	11.4 b	11.5 b	11.6 a	11.3 b	11.3 b	0.05	0.0001	0.9231	0.0169	0.0383	0.2927	0.0001
C16:0	30.0	29.7	30.1	30.6	30.4	30.6	0.10	0.0001	0.0463	0.1134	0.2403	0.0167	0.0001
C18:0	9.94	9.67	9.63	9.93	9.12	9.06	0.06	0.0001	0.7686	0.8819	0.5618	0.9112	0.0001
C18:1	18.5	19.4	18.4	18.9	19.2	18.1	0.13	0.0013	0.0002	0.1107	0.1362	0.1586	0.0005
SFA	65.2 b	65.6 ab	66.2 a	65.3 b	65.7 ab	66.2 a	0.14	0.0008	0.0001	0.0151	0.0161	0.2917	0.0001
UFA	27.9 a	28.2 a	27.2 b	28.8 a	28.4 a	27.2 b	0.14	0.0001	0.0001	0.0262	0.0200	0.1092	0.0001
MUFA	22.5	23.3	22.2	22.9	23.1	22.0	0.13	0.0001	0.0001	0.2183	0.2194	0.2923	0.0057
PUFA	2.89	2.93	2.89	2.99	3.00	2.77	0.02	0.0001	0.0129	0.6300	0.1223	0.1106	0.5356
TRANS-UFA	1.42 b	1.65 ab	1.72 a	1.59 b	1.61 b	2.28 a	0.05	0.0001	0.1758	0.0183	0.0194	0.2085	0.5839
SCFA	10.4 a	10.0 ab	9.89 b	10.4 a	10.2 ab	10.1 b	0.04	0.0001	0.0038	0.0419	0.0228	0.0840	0.2014
MCFA	44.7 a	43.2 b	42.9 b	43.7	43.2	44.1	0.19	0.0001	0.0026	0.0136	0.3236	0.1110	0.0001
LCFA	30.9 b	32.5 b	32.0 a	30.8 b	31.3 b	33.2 a	0.19	0.0001	0.0003	0.0410	0.0135	0.0725	0.0001
BCS 21 d after calving	3.24 a	2.82 ab	2.62 b	3.24 a	3.25 b	2.86 b	0.07	0.0523	0.4634	0.0148	0.0221	0.6531	0.8221

¹AFC = age at first calving.²DIM = days in milk.³Milk fatty acids (g/100 g fat): SFA – saturated fatty acids (FA); UFA – unsaturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; trans-UFA – trans-unsaturated FA; SCFA – short-chain FA; MCFA – medium-chain FA; LCFA – long-chain FA.a, b, c – means of the explanatory variable within the category of T or RH on the +14 d of lactation within the row with different letters differed significantly ($P \leq 0.05$).

Table 5. The effect of the temperature (T) or relative humidity (RH) on the +21 day of lactation on dairy cows' lactation performance and body condition score

Item	T			RH			SEM	P-value					
	<16 °C	16–20 °C	>20 °C	<65%	65–75%	>75%		farm (H _f)	dam parity (L _d)	T (B _t)	RH (B _r)	AFC ¹ (β ₁ atc ₀)	DIM ² (β ₂ dI ₀)
Milk yield (kg)	31.8 a	29.4 b	28.3 b	31.3 a	30.9 a	27.3 b	0.26	0.0001	0.0001	0.0173	0.0173	0.6987	0.0001
3.5% FCM (kg)	30.0 a	28.9 b	28.4 b	30.5 a	29.9 a	27.1 b	0.24	0.0001	0.0001	0.0148	0.0314	0.3668	0.0001
3.5% FPCM (kg)	30.4 a	29.2 b	28.8 b	30.3 a	30.9 a	27.6 b	0.23	0.0001	0.0001	0.0196	0.0390	0.4913	0.0001
ECM (kg)	33.4 a	32.0 b	31.6 b	33.9 a	33.3 a	30.2 b	0.26	0.0001	0.0001	0.0185	0.0408	0.5169	0.0001
Milk fat (%)	3.68 b	3.96 a	4.09 a	3.87 b	3.89 ab	4.04 a	0.03	0.0001	0.3012	0.0289	0.0189	0.1098	0.0001
Milk protein (%)	3.56 a	3.45 b	3.36 c	3.56 a	3.42 b	3.42 b	0.01	0.0001	0.0007	0.0116	0.0292	0.9390	0.0001
Milk lactose (%)	4.84	4.81	4.81	4.77	4.78	4.78	0.01	0.0001	0.0001	0.4322	0.3922	0.3890	0.0001
Milk casein (%)	2.63 c	2.69 b	2.81 a	2.71 b	2.79 a	2.68 b	0.01	0.0001	0.0187	0.0413	0.0433	0.9865	0.0001
SCC (10 ³ cells/mL)	352 b	396 b	421 a	334 b	447 a	442 a	36.0	0.0004	0.0001	0.0311	0.0411	0.4755	0.0001
MU (mg/mL)	217 b	212 b	225 a	209 b	238 a	243 a	2.42	0.0001	0.8752	0.0320	0.0230	0.6960	0.0001
Milk fatty acids (FA), g/100 g fat ³													
C14:0	11.8 a	11.6 b	11.5 b	11.9 a	11.3 b	11.4 b	0.05	0.0001	0.3470	0.0165	0.0260	0.7593	0.0001
C16:0	30.0	30.2	30.0	30.3	30.2	30.3	0.10	0.0001	0.2106	0.6523	0.2235	0.2890	0.0035
C18:0	10.1	9.52	9.25	9.95	9.34	9.21	0.06	0.0001	0.6732	0.3221	0.9231	0.7564	0.0001
C18:1	18.4	18.6	18.5	18.9	18.5	18.3	0.13	0.0001	0.0015	0.4575	0.2815	0.4649	0.1433
SFA	65.7 b	65.9 ab	66.1 a	65.2 b	65.9 ab	66.2 a	0.14	0.0002	0.0001	0.0163	0.0349	0.9601	0.0001
UFA	27.8 a	27.5 a	27.2 b	28.6 a	28.2 a	27.5 b	0.14	0.0001	0.0006	0.0135	0.0403	0.3915	0.0265
MUFA	22.3	22.5	22.4	22.7	22.4	22.2	0.13	0.0001	0.0001	0.7292	0.1966	0.8684	0.4647
PUFA	2.85	2.90	2.89	3.09	2.90	2.78	0.02	0.0001	0.0696	0.3159	0.1813	0.6884	0.1750
TRANS-UFA	1.51 b	1.53 ab	1.69 a	1.48 b	1.57 b	2.13 a	0.05	0.0001	0.3000	0.0282	0.0410	0.2462	0.7732
SCFA	10.7 a	10.1 ab	9.91 b	10.5 a	10.3 ab	10.1 b	0.04	0.0001	0.0091	0.0255	0.0305	0.1495	0.0200
MCFA	44.3 a	44.5 b	42.4 b	43.7	44.3	44.0	0.19	0.0001	0.0152	0.0199	0.3231	0.6271	0.0001
LCFA	31.0 b	31.6 b	31.7 a	30.5 b	30.1 b	32.6 a	0.19	0.0001	0.0019	0.0333	0.0368	0.1752	0.0242

¹AFC = age at first calving.²DIM = days in milk.³Milk fatty acids (g/100 g fat): SFA – saturated fatty acids (FA); UFA – unsaturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; trans-UFA – trans-unsaturated FA; SCFA – short-chain FA; MCFA – medium-chain FA; LCFA – long-chain FA.

a, b, c – means of the explanatory variable within the category of T or RH on the +21 d of lactation within the row with different letters differed significantly (P≤0.05).

Table 6. The effect of the temperature (T) or relative humidity (RH) during early lactation on dairy cows' fertility indices

Item	T			RH			SEM	P-value				
	<16 °C	16–20 °C	>20 °C	<65%	65–75%	>75%		farm (H_f)	dam parity (L_d)	T (B_k)	RH (B_k)	AFC ¹ (β_{afc})
Anestrus period (d)	0 d	74.8 b	85.1 b	104 a	87.4	92.6	98.4	0.0251	0.7219	0.0181	0.3573	0.8118
	7 d	71.8 b	82.2 b	98.3 a	86.8	91.7	96.3	0.0287	0.5880	0.0123	0.2895	0.8047
	14 d	70.3 b	81.6 b	99.9 a	89.7	91.5	93.2	0.0791	0.6791	0.0190	0.2948	0.9586
	21 d	75.5 b	88.7 b	101 a	85.7	86.9	91.1	0.0177	0.7716	0.0283	0.4631	0.9265
AI service period (d)	0 d	39.3 b	39.9 b	58.5 a	40.8	41.3	46.0	0.0245	0.6214	0.0348	0.5159	0.5049
	7 d	34.4 b	42.0 b	54.4 a	38.0	45.5	46.5	0.0151	0.3356	0.0409	0.5633	0.4680
	14 d	36.5 b	42.8 b	57.4 a	34.3	42.6	42.3	0.0268	0.8181	0.0156	0.8758	0.5004
	21 d	38.0 b	40.8 b	55.5 a	40.8	42.0	44.3	0.0090	0.8538	0.0185	0.1497	0.7053
Services per conception	0 d	1.87 b	1.90 b	2.79 a	1.94	1.97	2.19	0.0245	0.6214	0.0348	0.5159	0.5049
	7 d	1.64 b	2.00 ab	2.59 a	1.81	2.17	2.21	0.0151	0.3356	0.0409	0.5633	0.4680
	14 d	1.74 b	2.04 ab	2.73 a	1.63	2.03	2.01	0.0268	0.8181	0.0156	0.8758	0.5004
	21 d	1.81 b	1.94 ab	2.64 a	1.94	2.00	2.11	0.0090	0.8538	0.0185	0.1497	0.7053
Days open (d)	0 d	114 b	125 ab	163 a	128	134	144	0.7686	0.5084	0.0116	0.2246	0.5751
	7 d	106 b	124 ab	153 a	125	137	143	0.7275	0.2677	0.0479	0.6091	0.5152
	14 d	107 b	124 ab	157 a	124	134	136	0.6969	0.5331	0.0197	0.3703	0.6763
	21 d	114 b	130 ab	157 a	127	129	135	0.6213	0.6943	0.0143	0.1070	0.9222
Calving interval (d)	0 d	390 b	401 ab	439 a	404	410	420	0.1148	0.4215	0.0137	0.1404	0.6035
	7 d	382 b	400 ab	429 a	401	413	419	0.4342	0.5350	0.0253	0.8851	0.7115
	14 d	383 b	400 ab	433 a	400	410	412	0.3242	0.2181	0.0180	0.2964	0.5788
	21 d	390 b	406 ab	433 a	403	405	411	0.2483	0.72138	0.0234	0.2260	0.5210

¹AFC=age at first calving.a, b – means of the explanatory variable within the category of T or RH within the row with different letters differed significantly ($P \leq 0.05$).

Table 7. The effect of the temperature (T) or relative humidity (RH) on the calving day on dairy cows' immunometabolic blood indices

Item	T			RH			SEM	P-value					
	<16 °C	16–20 °C	>20 °C	<65%	65–75%	>75%		farm (H _i)	dam parity (L _j)	T (B _k)	RH (B _k)	AFC ¹ (β ₁ afci _j)	BSD ² (β ₂ dl _{im})
Glucose (g/dL)	50.1 a	43.4 ab	41.6 b	47.3 a	43.5 ab	38.4 b	1.36	0.1201	0.0624	0.0488	0.0450	0.4698	0.8259
Insulin (ng/mL)	5.37	5.53	5.64	5.41	5.60	5.72	0.09	0.0267	0.4190	0.4272	0.2567	0.1840	0.3688
IGF-I (ng/mL)	82.8 a	68.3 ab	54.1 b	89.5 a	67.9 ab	54.8 b	2.27	0.0019	0.8856	0.0225	0.0121	0.0022	0.5582
TG (mg/dL)	31.9 b	38.7 ab	48.7 a	35.7 b	35.4 b	48.8 a	1.57	0.0453	0.7021	0.0467	0.0296	0.0546	0.2509
NEFA (mmol/L)	0.55 b	0.57 ab	0.75 a	0.55 b	0.57 b	0.73 a	1.60	0.1196	0.7257	0.0398	0.0198	0.7676	0.1858
BHBA (mmol/L)	0.41 b	0.72 ab	0.98 a	0.59 b	0.66 b	0.76 a	0.69	0.0172	0.0505	0.0221	0.0371	0.8768	0.3927
AST (IU/L)	77.0	77.5	79.1	76.7	77.7	83.2	3.05	0.0212	0.0076	0.8205	0.4442	0.9735	0.9538
ALT (IU/L)	18.6	21.1	23.9	21.7	21.9	23.1	1.33	0.0001	0.0011	0.8837	0.2890	0.4722	0.5141
Cholesterol (mg/dL)	142	136	133	138	135	136	3.03	0.0038	0.2268	0.0769	0.5308	0.4292	0.0027
LDL (mg/dL)	102	96.6	96.4	101	97.2	96.8	2.55	0.0011	0.0892	0.1983	0.6793	0.7378	0.0002
HDL (mg/dL)	30.1	27.2	30.1	27.6	30.0	29.4	0.94	0.6166	0.4326	0.3067	0.9439	0.8656	0.8592
ADP (μg/mL)	5.49 a	5.46 a	4.95 b	5.89 a	5.26 ab	5.19 b	0.16	0.5331	0.5331	0.0413	0.0396	0.0387	0.9407
LEP (ng/mL)	14.0 b	17.8 ab	19.5 a	13.2 b	17.4 ab	19.9 a	0.06	0.0531	0.6359	0.0239	0.0376	0.0148	0.5816
BUN (mg/dL)	9.18	9.85	11.1	9.10	10.3	10.7	0.36	0.0620	0.4033	0.5931	0.1339	0.9363	0.6575
FSH (ng/mL)	7.48 a	6.90 ab	6.31 b	7.50 a	6.79 ab	6.33 b	0.14	0.0090	0.5439	0.0398	0.0319	0.0111	0.2328
LH (ng/mL)	7.68 a	6.54 ab	6.23 b	7.33 a	6.42 ab	6.32 b	0.14	0.0013	0.1834	0.0149	0.0164	0.0490	0.1615
TSP (g/dL)	7.11 b	7.74 ab	7.97 a	7.27 b	7.66 ab	8.42 a	0.13	0.5880	0.2996	0.0222	0.0298	0.5281	0.8405
Albumin (g/L)	3.13	3.11	3.06	3.06	3.16	3.02	0.04	0.0464	0.2021	0.1810	0.1740	0.2298	0.0725
TNF-α (ng/L)	437 b	464 ab	564 a	392 b	457 ab	541 a	12.6	0.0023	0.7592	0.0301	0.0347	0.3024	0.4594
IL-1 (ng/L)	11.7 b	14.8 ab	15.5 a	11.5 a	13.1 ab	16.5 a	0.59	0.0185	0.9731	0.0452	0.0360	0.0117	0.6806
IL-6 (ng/L)	543 b	544 b	659 a	535 b	544 b	681 a	10.5	0.2758	0.8534	0.0399	0.0218	0.5533	0.3030
SAA (μg/mL)	2.55	2.73	2.78	2.72	2.67	2.80	0.12	0.4453	0.6311	0.7372	0.3686	0.0284	0.6813
Hpt (μg/mL)	52.3	55.5	58.6	52.9	57.9	59.7	1.66	0.5403	0.0562	0.4288	0.9777	0.1256	0.6014
HSP70 (ng/mL)	4.13 b	4.41 b	5.78 a	4.17	4.58	4.92	0.25	0.6860	0.3531	0.0163	0.1141	0.0714	0.7191
Ca (mg/dL)	9.39	9.26	9.06	9.43	9.33	9.13	0.10	0.7609	0.7707	0.7711	0.7531	0.4603	0.5468

¹AFC = age at first calving.²BSD = blood sampling day.

IGF-I – insulin-like growth factor I, TG – triglycerides; NEFA – non-esterified fatty acids; BHBA – β-hydroxybutyrate; AST – aspartate aminotransferase; ALT – alanine aminotransferase; LDL – low-density lipoproteins; HDL – high-density lipoproteins; ADP – adiponectin; LEP – leptin; BUN – blood urea nitrogen; FSH – follicle-stimulating hormone; LH – luteinizing hormone; TSP – total serum protein; TNF-α – tumor necrosis factor α; IL-1 – interleukin 1; IL-6 – interleukin 6; SAA – serum amyloid A; Hpt – haptoglobin; HSP70 – heat shock 70 kDa protein 1A; Ca – calcium.

a, b – means of the explanatory variable within the category of T or RH on the calving day (0 d) within the row with different letters differed significantly (P≤0.05).

HS not only decreases milk yield but also affects milk composition. In this research, we observed a decrease in protein content in the milk of cows exposed to higher T and RH conditions. On the other hand, increasing T from the calving day up to +21 d during early lactation was associated with higher milk casein, but increasing RH was associated with decreasing that milk compound. Also, the milk lactose concentration was associated with barn microclimate indices during early lactation. With an increasing T on the +14 d and RH on the calving day and the +14 d during early lactation, we recorded decreased milk lactose content. Although observed effects are not entirely consistent, several other studies also reported reduced milk protein and casein percentage during hot compared to temperate seasons (Heck et al., 2009; Bernabucci et al., 2015; Quellet et al., 2019) and in cows housed in controlled environments under HS conditions compared to those under conditions of thermal neutrality (Rhoads et al., 2009; Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017; Ruiz-Gonzalez et al., 2023). The mechanisms underlying HS-induced reduction in milk protein similar to casein contents are not fully understood. Increased temperature and relative humidity can reduce the ability of cattle to dissipate excess heat which can ultimately lead to heat stress occurrence and associated physiological changes (Wheelock et al., 2010). Still, they may include various metabolic changes, both related and unrelated to a lower feed intake. For example, HS is often associated with decreased glucose levels in the blood resulting from decreased DMI and limited propionate supply for hepatic gluconeogenesis. Heat stress causes a decline in hepatic glucose leading to a reduced supply of glucose to the mammary glands which in turn negatively affects milk lactose synthesis (Wheelock et al., 2010). Also, Gao et al. (2017) hypothesized that in these conditions, an enhanced utilization of amino acid (AA) for glucose production could occur in the liver, leading to an insufficient supply of milk protein precursors to the mammary gland. Also, Nardone et al. (1997) proposed that a supposed reduction of mammary blood flow, as blood distribution is altered in an increased temperature and heat stress occurrence, may decrease the supply of milk protein precursors to the mammary gland, causing a downturn in the production of casein and possibly all mammary-synthesized milk constituents. On the other hand, Cowley et al. (2015) suggested that a reduction in milk protein and also milk casein concentrations and altered milk protein composition found in cows affected by HS were attributed to specific downregulation of mammary casein synthesis, which, however, had no residual effect that continued beyond the period of HS. Considering the HS effect on milk fat percentage, there is considerable inconsistency in reported results, suggesting that various factors, including severity and duration of HS, influence a cow's response to environmental conditions (Tao et al., 2018). In observational studies, fat concentration in milk was either increased (Smith et al., 2013) or reduced (Heck et al., 2009; Bernabucci et al.,

2015; Quellet et al., 2019) by excessive heat. Similarly, cows exposed to HS in climate-controlled experiments had either increased (Baumgard et al., 2011), not changed (Wheelock et al., 2010; Cowley et al., 2015; Gao et al., 2017), or decreased (Rhoads et al., 2009; Ruiz-Gonzalez et al., 2023) milk fat content than cows under thermal neutrality. In this study, the average milk fat percentage until 150 DIM increased with rising ambient T and RH during the postpartum transition period. This increase was associated with lower BCS, plasma glucose, and IGF-I and higher TG, NEFA, and BHBA in blood at 21 d after calving, indicating energy deficiency and enhanced adipose tissue (AT) mobilization. Around parturition, most high-producing cows fall into NEB due to rising energy requirements for milk production and a restricted feed intake capacity, and lipolysis of adipose tissue is a typical response to the NEB postpartum (Stefanska et al., 2016 a, b; Baumgard et al., 2017). Fat released to circulation in the form of NEFA can then be taken up and utilized by the liver, but it can also be used by the mammary gland for milk fat synthesis, proportionally to their blood concentration. That results in a higher milk fat percentage in early lactation, which could, to some extent, contribute to the increase that we observed until 150 DIM. However, mechanisms underlying the long-term effects of postpartum exposure to increased T and RH on fat, protein, casein, and lactose concentrations remain unclear.

NEFAs mobilized from adipose tissue in cattle mainly comprise C16:0, C18:0, and cis9-C18:1 (Hostens et al., 2012). In our study, environmental conditions were not related to the individual concentrations of these FA in milk fat, except C14:0. However, we observed an increased proportion of total LCFA at the expense of SCFA and MCFA in cows exposed to increased T and RH. A similar trend was reported in other observational (Hammami et al., 2015; Heck et al., 2009) and climate-controlled studies (Liu et al., 2017; Ruiz-Gonzalez et al., 2023). Ruiz-Gonzalez et al. (2023) showed, however, that in contrast to the relative percentage of LCFA (>C16) in milk fat, the yield of these fatty acids did not increase in cows under HS conditions, and the reduction in >C16 FA proportion was due to decreased yields of <C16 FA and C16 FA. In ruminants, while most LCFA originate from feed and body adipose tissue, all SCFA and MCFA, and approximately half of C16 fatty acids are synthesized de novo in the mammalian epithelial cells from rumen-derived acetate and butyrate (Shingfield et al., 2013). The decrease in SCFA and MCFA in milk produced by cows exposed to HS could be, therefore, attributed to reduced feed intake and, consequently, lower ruminal volatile fatty acids production. Additionally, as Shahzad et al. (2015) reported, high ambient temperatures can alter liver transcriptome, possibly leading to decreased expression of genes (e.g. *FASN*) necessary for fatty acid synthesis. In this study, rising ambient T and RH from d 0 to d 21 after calving were also associated with increasing SFA and declining UFA concentrations in milk fat.

This result is in line with the assumption of enhanced mobilization of adipose tissue in animals exposed to hot environments because NEFAs released from abdominal fat in cows suffering from NEB are mostly SFA (Hostens et al., 2012). Alternatively, as reported by Wang et al. (2020), the underlying mechanism might involve the altered expression of stearoyl-CoA desaturase 1 (SCD1), catalyzing the transformation of SFA into MUFA in the mammary gland. Contrary to our findings, Heck et al. (2009) and Hammami et al. (2015) found a higher proportion of UFA in milk produced in hot environments, which could, however, reflect different feeding patterns across seasons. Trans FA are produced as intermediates of ruminal biohydrogenation in which the dietary UFA are converted by rumen microbiota to SFA (Shingfield et al., 2013). The higher concentrations of trans-UFA in cows exposed to increased ambient temperatures observed by Heck et al. (2009) and in our study, possibly associated with an incomplete biohydrogenation process, suggest that rumen metabolism contributes to HS effects on milk fat composition.

The elevated level of NEFA in the blood at 21 d after calving observed in this study in cows exposed to increased T and RH on the calving day contradicts the results reported in the literature. Several studies analyzed metabolic profiles in dairy cows under experimentally induced HS or thermoneutral pair-fed (PF) conditions (studies in which the amount of feed offered to thermoneutral animals is adjusted to be similar to the amount consumed by HS animals) in both established (>80 DIM; Rhoads et al., 2009; Wheelock et al., 2010; Gao et al., 2017; Ruiz-Gonzalez et al., 2023) and early lactation (<35 DIM; Lamp et al., 2015; Koch et al., 2016). In these experiments, both HS and PF animals were in the state of NEB and had lower plasma glucose concentrations. However, cows' response to NEB caused by hyperthermia differed from that to NEB resulting from a lower nutrition plane. NEFA levels in blood increased in PF but not in HS cows (Rhoads et al. 2009; Wheelock et al., 2010; Lamp et al., 2015; Koch et al., 2016; Gao et al., 2017; Ruiz-Gonzalez et al., 2023), which in HS animals coincided with increased plasma insulin (Wheelock et al., 2010; Ruiz-Gonzalez et al., 2023) and urea levels (Wheelock et al., 2010; Lamp et al., 2015; Gao et al., 2017). One of the important functions of insulin in the body is to inhibit lipolysis in adipocytes. Several authors, including Wheelock et al. (2010) and Lamp et al. (2015), proposed that insulin-mediated decrease in AT mobilization under HS conditions is an adaptive mechanism to minimize metabolic heat production, as oxidation of NEFA generates more heat than oxidation of carbohydrates. However, blocking the release of NEFA makes lactating cows more dependent on glucose as a major energy source. When propionate delivery to the liver is decreased due to reduced DMI, cows may mobilize skeletal muscle protein and use AA as alternative substrates for hepatic gluconeogenesis (Koch et al., 2016; Gao et al., 2017). The increased plasma urea levels observed in HS

cows supported this hypothesis because the deamination of AA before their use for glucose synthesis results in the liberation of ammonia, which is then converted to urea.

In the current study, cows exposed to increased ambient T and RH environments on the calving day were characterized by lower plasma glucose and increased plasma NEFA and BHBA concentrations concurrent with unchanged insulin and urea levels. This indicates that the previously reported anti-lipolytic effect of HS was not observed under our experimental conditions. The differences in the severity of HS between studies might have contributed to the inconsistency of the results. In previous climate-controlled research, HS cows were exposed to temperatures of 28°C (THI 76; Lamp et al., 2015; Koch et al., 2016), 29–39°C (THI varying between 72 and 83; Rhoads et al., 2009; Wheelock et al., 2010; Ruiz-Gonzalez et al., 2023) and 32–36°C (THI 84; Gao et al., 2017). In our study, we set temperature thresholds at 16°C (which, according to the formula recommended by the National Research Council (NRC, 1971), corresponds to THI of approximately 60 independently from RH) and 20°C (corresponding to THI of 63, 65 and 68 at 0%, 50% and 100% RH, respectively). It cannot, therefore, be excluded that environmental conditions experienced by HS animals in this experiment were not severe enough to trigger all metabolic responses previously observed in lactating dairy cows.

Once liberated from adipose tissue, NEFAs are primarily taken up by the liver. They can either be oxidized or esterified and deposited in the hepatocytes as triglycerides, disturbing liver function and leading to hepatocyte damage. Consequently, cholesterol and albumin synthesis can be reduced, and hepatic enzymes such as ALT and AST can be released into the blood (Yang et al., 2023). In this study, greater mobilization of NEFA was not associated with changes in plasma concentrations of these indices, suggesting that the function of the liver in cows exposed to increased T and RH was not substantially compromised.

Heat shock proteins (HSPs) are a highly conserved family of proteins that bind to their cellular protein substrates and stabilize them against denaturation or misfolding caused by high temperatures or other stressful conditions, thereby improving cell viability. Among them, the HSPs with a molecular weight of 70 kDa (HSP70) are the most sensitive to changes in environmental temperature, and their plasma levels have been recognized as a marker for monitoring stress induced by heat exposure in animals (Hassan et al., 2019). The increased concentrations of HSP70 that we observed in the blood of cows exposed to ambient T >20°C on a calving day indicate that this temperature was high enough for cows to be in a state of hyperthermia. In line with our findings, Shahzad et al. (2015) showed higher expression of the *HSP70L1A1* gene in postpartum dairy cows during the summer compared to the spring season. Although HSP70 proteins help to protect cells from damage, they can also, when released from cells, stimulate the production of pro-inflammatory

cytokines (Bradford et al., 2015; Lemal et al., 2023). In this research, we observed increased plasma levels of IL-1, IL-6, and TNF- α cytokines in cows under $T > 20^{\circ}\text{C}$ and RH $> 75\%$ conditions, which is consistent with the results of other studies analyzing HS effects on cytokine levels in the blood (Chen et al., 2018) or cytokine genes expression (Gao et al., 2019). This inflammatory response could also be triggered by altered levels of adipokines secreted from adipose tissue, such as leptin and adiponectin. It is well known that although the primary function of AT is energy storage for the body, it can also release various humoral factors, playing an important role in regulating energy balance, reproduction, and immunity. LEP is considered mostly a pro-inflammatory factor, while ADP has anti-inflammatory effects (Frühbeck et al., 2017). We found increased LEP and lower ADP levels in the blood of cows exposed to the increased T and RH, suggesting that AT remodeling in early lactation stimulates inflammation. As reviewed by Bradford et al. (2015) and Lemal et al. (2023), strong links exist between inflammatory markers and periparturient diseases, as well as decreased milk production. Although mechanisms associating HS with immune response have not been fully explained, these results indicate that HS-induced inflammation during the transition period may contribute to compromised performance of cows in early lactation.

It is well recognized that environmentally induced hyperthermia compromises reproductive physiology, and mechanisms underlying impaired fertility of dairy cows exposed to HS are complex (Cartwright et al., 2023; Dovolou et al., 2023). When considering the association of exposure to a hot environment early after calving with subsequent reproductive performance, HS effects on the development of ovarian follicles are of particular interest. There is evidence that HS negatively alters the dynamics of follicular growth, steroidogenesis, and the quality of the oocyte and corpus luteum that forms following ovulation, therefore influencing postpartum interval to first ovulation, the intensity of external estrous signs and the maintenance of pregnancy (Ferguson, 2020; De Rensis et al., 2021; Dovolou et al., 2023). The estimated time needed for antral follicles to develop into pre-ovulatory follicles is 40 to 50 days (Negrón-Pérez et al., 2019). This means that HS conditions early postpartum can cause damage to follicles and oocytes that are destined to ovulate much later, during the time when most cows are beyond the voluntary waiting period and are eligible to be bred. Menta et al. (2022) recently showed that exposure to HS for 28 days after calving depressed the percentage of cows that became pregnant after the first insemination. Similarly, cows exposed in this study to ambient $T > 20^{\circ}\text{C}$ during the first three weeks postpartum were characterized by decreased reproductive efficiency as indicated by more days from calving to the first service and from the first service to conception, more services per conception, more days open and a longer calving interval. The decline in fertility observed in these cows was associated with altered metabolic and endocrinal profiles at 21 d af-

ter parturition, including lower levels of LH, FSH, IGF-1, and glucose and higher concentrations of NEFA and BHBA. IGF-1 is a growth factor that promotes growth and development within a variety of cell types, and its concentration in the blood largely depends on the nutritional status of an animal (Lucy, 2000). Previous research provided evidence that IGF-1 stimulates the gonadotropin-releasing hormone, and thereby, LH and FSH release, and acts synergistically with gonadotropins to stimulate follicle development (Zulu et al., 2002). Other studies reported that NEFA and BHBA, whose concentrations are elevated in follicular fluid during NEB, adversely affect oocyte quality (Leroy et al., 2005) and steroidogenesis (Garverick et al., 2013). Glucose, as the primary energy source for the ovary, was found to have a stimulating effect on follicular growth and LH secretion at the hypothalamic level (De Rensis et al., 2017). Taken together, these findings suggest that body energy balance is crucial for follicular growth and ovarian function. It is, therefore, likely that the exacerbated NEB that we observed in cows exposed to the hot environment early postpartum at least partly contributed to their decreased subsequent fertility. Other possible mechanisms by which HS impairs cows' reproductive function may be related to HS-induced inflammation (Chastant and Saint-Dizier, 2019) or the direct effect of hyperthermia on the endocrine and reproductive organs (Dovolou et al., 2023).

Levels of most traits associated with environmental conditions in the current study changed gradually with increasing ambient temperatures, starting from 16°C . Within the T and RH ranges used in this study, the humidity had a smaller effect than the temperature on milk yield and composition and did not affect fertility parameters. Previous research shows considerable differences in HS thresholds among breeds, traits studied, climate indices used, and climate regions (Vinet et al., 2023). Most studies used the THI index as an HS measure. THI thresholds reported for deterioration of Holstein cows' fertility traits ranged from 60 to 75 (García-Ispuerto et al., 2007; Morton et al., 2007; Schüller et al., 2014; Gernand et al., 2019). The onset of decline in milk yield has been declared at THI of 68–78 in various regions of the USA (Bohmanova et al., 2007; Dikmen and Hansen, 2009; Zimbelman et al., 2009), 73 in Spain (Carabaño et al., 2016), 69 in Belgium (Carabaño et al., 2016), 62 in Luxembourg (Hammami et al., 2013) and 60–62 in Germany (Brügemann et al., 2012; Gorniak et al., 2014). It has been proposed that cattle raised in temperate regions exhibit lower climatic adaptation and higher environmental sensitivity than those raised in warmer areas (Carabaño et al., 2016). Our results are not fully comparable with those of earlier studies as we analyzed the longer-term effects of cows' exposure to excessive heat during the early postpartum period and used T and RH parameters separately as HS measures. Although RH is an important factor for thermal comfort, it has a small effect on THI when air temperatures are relatively low. Following the equation developed by the NCR (1971), ambient T of 16°C

corresponds to THI of approximately 60 independently from RH (ranging from 59.3 at RH of 0% to 60.8 at RH of 100%). Thus, our results generally align with those obtained in Germany (Brügemann et al., 2012; Gorniak et al., 2014), located in the same region of Central Europe as Poland. They indicate that heat abatement strategies to minimize HS effects on high-producing dairy cows in temperate climates should be used even when air temperatures are still relatively low. Also, in these conditions, ambient T suffices to indicate HS.

Like all observational field research, this study is limited by the possibility that confounding factors could account for the observed associations. For example, we could not collect data on cows' individual DMI and physiological HS indicators, such as respiration rate and rectal temperature, which could help interpret our results. Also, even though the analyzed cows were clinically healthy, the occurrence of some subclinical disorders cannot be excluded. However, the results of this study contribute to the limited knowledge of the effects of cows' exposure to hot environments during the early postpartum period. Our findings indicate that housing cows under increased ambient T conditions during the first three weeks after calving was not only associated with altered immunometabolic blood parameters indicating more severe NEB and state of inflammation in these animals, but also had long-term negative effects on milk production and reproductive performance. Levels of these traits changed gradually with increasing ambient T, starting from 16°C. This suggests that dairy cows that calve during seasons when air temperatures exceed this threshold should be provided effective cooling during the early postpartum period starting on the day of calving. This would ensure optimal environmental conditions for high milk production and prevent economic losses associated with reduced milk yield and low fertility.

Acknowledgments

The authors thank the dairy cow breeders for their cooperation and the employees of the Polish Federation of Cattle Breeders and Dairy Farmers, especially cow nutrition managers, for this support, nutritional consultations, and laboratories for performing biochemical feed and milk analysis.

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Received: 10 VI 2024

Accepted: 4 X 2024