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Effects of Thermal Variability on the Sustainability of Milk Production in Dairy Cattle

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Abstract

Milk production in dairy cattle is increasingly challenged by thermal variability. This underscores the need for reliable assessment of microclimatic conditions and their interaction with animal- and management-related factors to ensure sustainable dairy production. The aim of this study was to evaluate the effects of thermal variability and selected environmental and biological factors on key milk production traits in dairy cattle. The influence of fixed factors related to production conditions and microclimatic variability, including the Temperature–Humidity Index (THI), on daily milk yield (MY), milk fat content (MF), and milk protein content (MP) was assessed. The study used a dataset covering two observation periods of daily milk production traits in cows of different breeds (Simmental, Holstein-Friesian, Red Holstein, and Brown Swiss) reared in three regions of the Republic of Serbia (Mačva, Podunavlje, and Šumadija), enabling an assessment of thermal variability under diverse production and microclimatic conditions. The expression and variability of the investigated traits were determined using the PROC FREQ and PROC MEANS procedures, while the effects of individual factors were analysed using general linear and regression models, with results expressed as least squares means. All examined factors showed a highly significant effect on MY, MF, and MP ($p < 0.0001$). Although the overall level of heat stress was moderate, milk production was highest within the THI range of 51–60. These findings demonstrate that thermal variability significantly influences milk production and composition and highlight the importance of integrating microclimatic indicators into sustainability-oriented dairy management and breeding strategies.

Keywords: dairy cattle; thermal variability; milk production traits; heat stress; Temperature–Humidity Index (THI); sustainable milk production

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1. Introduction

Climate change represents one of the most significant global challenges affecting agricultural and livestock production systems worldwide. Rising average air temperatures, increasing frequency of heat waves, prolonged periods of elevated ambient temperatures, and the growing occurrence of tropical days and nights have been widely documented over recent decades. These climatic changes pose a serious threat to the sustainability of animal production systems by intensifying environmental stressors that impair animal health, welfare, and productivity. In dairy farming, heat stress has emerged as one of the most critical consequences of climate change, particularly in high-producing systems.

Stress encompasses all environmental changes that initially trigger behavioural reponses, followed by physiological reactions in animals. The primary mechanisms underlying stress involve alterations in pituitary hormone secretion, leading to changes in metabolism, reproductive and productive insufficiency, reduced immune competence, and behavioural disturbances (Baumgard and Rhoads, 2013). Heat stress is defined as the combined effect of environmental factors acting on an animal, resulting in an increase in body temperature and the activation of physiological responses aimed at maintaining thermal balance (Dikmen and Hansen, 2009). When combined with nutritional stress, heat stress exerts a particularly detrimental impact on animal physiology in livestock production systems (Sejian et al., 2012). Excessively high air temperatures and elevated humidity levels may directly disrupt cellular functions and cause damage to various tissues and organs in domestic animals, including dairy cows and bulls (Das et al., 2016).

In dairy cattle, prolonged exposure to heat stress negatively affects milk production, milk composition, reproductive performance, and animal welfare. Heat load presents a major challenge due to its combined negative effects on both productivity and welfare (Nordlund et al., 2019). Long-term genetic selection for increased milk yield has further intensified this problem, as selection for high production has been associated with reduced fertility and diminished resilience to heat stress. Owing to the negative correlation between milk production traits and thermotolerance, genetic progress in milk yield has inadvertently reduced the ability of modern dairy cows to adapt to elevated temperatures (Carabaño et al., 2019). Consequently, heat stress represents a multifactorial constraint on the sustainability of dairy production systems.

The severity of heat stress effects depends on the interaction between environmental conditions, animal-related characteristics, and management practices. To quantify heat stress under practical production conditions, various mathematical indicators have been proposed; however, the Temperature–Humidity Index (THI), also referred to as the Thermo-Hygrometric Index, remains the simplest and most widely used indicator due to its ease of calculation and practical applicability (Maggiolino et al., 2020; Dimov et al., 2020). THI is determined using only ambient air temperature and relative humidity (Ouellet et al., 2019), and numerous formulas for its calculation have been developed since the mid-20th century (Sahin and Ugurlu, 2017). The application of THI enables the estimation of heat stress impacts on daily milk yield and milk composition in lactating cows during test-day measurements (Ekine-Dzivenu et al., 2020; Mićić et al., 2022).

In recent years, the effects of climate change have become increasingly evident in Southeast Europe, including the Republic of Serbia, where the past decade has been marked by a higher frequency of hot days, tropical nights, and prolonged periods of elevated summer temperatures. Although extreme heat stress events are generally less pronounced than in subtropical regions, moderate but persistent thermal variability under temperate continental conditions may cumulatively impair dairy cow health, productivity, and welfare (Pinto et al., 2020). As a result, numerous studies have focused on improving thermotolerance in dairy cattle without compromising production performance (Amamou et al., 2019); however, reported heat stress thresholds and production responses vary widely among studies, reflecting differences in climate, housing systems, genetic background, and methodological approaches (Jeon et al., 2023). Studies conducted under production conditions in Serbia have confirmed that heat stress represents a relevant constraint to dairy cattle performance, leading to reduced milk yield, altered milk composition, and compromised animal welfare during periods of increased thermal load (Joksimović-Todorović et al., 2011; Cincović et al., 2023). More recent analyses based on test-day records have demonstrated significant effects of breed, region, and lactation characteristics on milk production traits under high Temperature–Humidity Index (THI) values (Mićić et al., 2022). However, existing research is largely limited to seasonal comparisons, specific breeds, or extreme heat stress thresholds, while comprehensive evaluations addressing moderate but persistent thermal variability across multiple breeds, regions, and extended observation periods under field conditions in Serbia remain scarce.

Therefore, the aim of this study was to evaluate the impact of thermal variability on the sustainability of milk production in dairy cattle under temperate continental conditions. The specific objectives were to: (i) quantify the effects of thermal variability, expressed through the THI, on daily milk yield, milk fat content, and milk protein content; (ii) assess the influence of key biological and environmental factors, including breed, region, lactation number, lactation stage, calving season, and month of test day, on milk production traits; and (iii) identify THI ranges associated with optimal

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and reduced milk production performance, providing evidence relevant for sustainability-oriented dairy management and breeding strategies in the context of ongoing climate change.

2. Materials and Methods

2.1. Study Area and Climatic Characteristics

The study was conducted under production conditions in the Republic of Serbia, located in Southeast Europe and characterized by a temperate continental climate. The geographic coordinates of the country range from 42°14′ to 46°11′ north latitude and from 18°49′ to 23°01′ east longitude. According to official data from the Republic Hydrometeorological Service of Serbia (RHMS, 2024), 2024 was recorded as the warmest year, or among the warmest years, in the history of meteorological observations in Serbia, with a mean annual air temperature approximately 2 °C above the 1991–2020 reference period. Furthermore, all ten warmest years on record in Serbia have occurred since 2000, indicating a strong and accelerated warming trend over the past two decades. These climatic conditions suggest increased exposure of dairy production systems to sustained thermal load under temperate continental conditions, providing an appropriate environmental framework for evaluating the effects of thermal variability on milk production traits.

2.2. Study Design and Data Collection

Data were collected within the framework of routine monthly milk recording carried out in accordance with the Main Cattle Breeding Programme for the Central Serbia region, implemented by the Institute for Animal Husbandry (Zemun, Belgrade), using the AT4 method. The analysed milk production traits included daily milk yield (MY, kg/day), milk fat content (MF, %) and milk protein content (MP, %).

The dataset comprised test-day records from cows of different dairy breeds (Simmental, Holstein-Friesian, Red Holstein and Brown Swiss) reared in three regions of the Republic of Serbia (Mačva, Podunavlje and Šumadija) (Figure 1). These regions represent distinct agro-climatic environments relevant to heat-stress exposure in dairy production systems: Mačva is a predominantly lowland area (<200 m a.s.l.) on the Pannonian Plain; Podunavlje encompasses low-elevation Danube river-valley and terrace zones with warmer and more humid summer conditions; whereas Šumadija is a moderately hilly region (>200 m a.s.l.) characterised by greater diurnal temperature variability compared with surrounding lowlands.

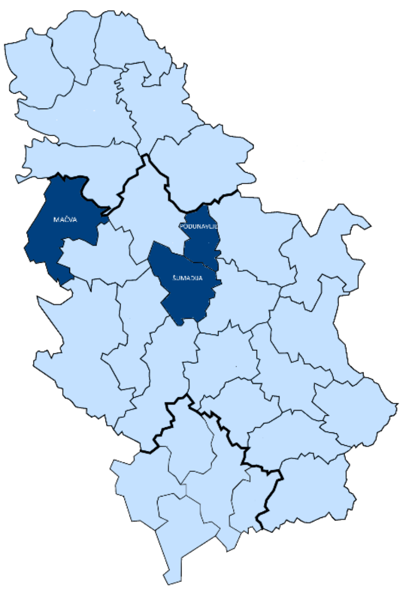

Figure 1. Map of the administrative districts of Serbia.

Air temperature and relative humidity inside the barns were recorded hourly using a data logger (AMTAST AMT116, Qingdao, Shandong, China), in parallel with milk sampling and the recording of production traits. Based on calving dates (start of lactation), milk production records were classified into four calving seasons: winter (December–February), spring (March–May), summer (June–August) and autumn (September–November). According to lactation number, cows were grouped into six classes (1st, 2nd, …, 6th and subsequent lactations). Lactations were further divided into five stages: I (≤60 days), II (61–120 days), III (121–200 days), IV (201–300 days) and V (>300 days).

2.3. Calculation of the THI

Based on the measured microclimatic parameters, the THI was calculated using the following equation (Dunn et al., 2014):

$$THI = (1{,}8 \times T + 32) - (0{,}55 - 0{,}0055 \times RH) \times (T - 26{,}8),$$

where T = air temperature (°C), and RH = relative humidity (%).

2.4. THI Classification and Time Lag

Daily THI values were classified into five categories for inclusion as a fixed factor in the statistical model: I ≤ 40; II 41–50; III 51–60; IV 61–70; and V ≥ 71. To account for delayed physiological responses to heat stress, THI values recorded three days prior to test-day milk recording were used, following established methodological approaches (Bohmanova et al., 2008; Brügemann et al., 2011; Wildridge et al., 2018).

2.5. Observation Periods

Two observation periods were included in the same research model: the full-year period (12 months), referred to as Period I, and the period from 1 April to 30 November (8 months), referred to as Period II. Period II excludes December, January, February, and March, when low temperatures are expected and unfavourable THI values do not occur.

2.5. Statistical Analyses and Models

The data were analysed using the statistical software package SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). Descriptive statistics and variability of milk production traits were calculated using the PROC FREQ and PROC MEANS procedures. The effects of environmental, biological, and management-related factors on milk traits were evaluated using the PROC GLM procedure, while the impact of THI values was analysed using the PROC GLM and PROC REG procedures. Least squares means (LSM) were estimated within the PROC GLM framework.

The fixed model used to examine the effects of factors on the analysed milk traits is as follows:

$$Yijklmno = \mu + Oi + Rj + Gk + Sl + Lm + Mn + Fo + Tp + eijklmnop$$

where:

- Yijklmno: the observed trait, represents the observed trait
- μ: is the overall mean of the population for the given trait,
- Oi: is the fixed effect of the i-th region (i=1,…,3),
- Rj: is the fixed effect of the j-th breed (j=1,…,4),
- Gk: is the fixed effect of the k-th calving year (k=1,…,8),
- Sl: is the fixed effect of the l-th calving season (l=1,…,4),
- Lm: is the fixed effect of the m-th lactation number (m=1,…,6),
- Mn: is the fixed effect of the n-th month of test day (n=1,…,12),
- Fo: is the fixed effect of the o-th lactation stage (o=1,…,5)
- Tp: is the fixed effect of the p-th THI level (p=1,…,5) i
- eijklmnop: is the random residual error, assumed to be normally distributed with mean zero and variance N (0, σ2).

Scheffé's multiple comparisons were used in PROC GLM (SAS/STAT) to test the significance ($p < 0.001$) of differences in daily milk traits attributable to the analysed effects.

Differences among LSM were evaluated using Scheffé's multiple comparison test, with statistical significance declared at $p < 0.001$.

3. Results

The results of the study are presented in Tables 1–5 and include descriptive statistics of the dataset, followed by model-based evaluations of the effects of breed, region, lactation characteristics, and thermal conditions on test-day milk production traits.

Table 1 presents the descriptive statistics of daily milk yield (MY, kg/day), milk fat content (MF, %), milk protein content (MP, %), and THI values across dairy breeds included in the study. The values presented are overall averages, while the model-derived results will later be shown as LSM for the respective observation periods. The total dataset comprised 734,492 test-day records. Across all breeds, the overall average daily milk yield was 15.99 kg/day, with a standard deviation of 5.07 kg and a coefficient of variation of 31.71%, indicating substantial variability in milk yield at the population level. In contrast, milk composition traits exhibited markedly lower variability. The overall mean milk fat content was 4.03% ($\sigma = 0.19$; CV = 4.84%), while the mean milk protein content was 3.23% ($\sigma = 0.14$; CV = 4.46%). These descriptive indicators confirm that daily milk yield represents the most variable production parameter within the analysed dataset.

At the breed level, Holstein cows achieved the highest daily milk yield (17.79 kg/day), followed by Red Holstein (17.70 kg/day), Brown Swiss (15.86 kg/day), and Simmental (15.80 kg/day). Regarding milk fat content, the highest average value was recorded in Brown Swiss cows (4.12%), while the lowest was recorded in Holstein cows (3.97%). The highest milk protein content was produced by Brown Swiss cows (3.25%), while the lowest was observed in Holstein cows (3.21%). These breed-related differences in milk yield contribute to the overall variability observed for MY within the analysed dataset (Table 1).

Table 1. Descriptive statistics of daily milk yield (MY, kg/day), milk fat content (MF, %), milk protein content (MP, %) and THI values by dairy breed.

Breeds	Traits	N	Overall mean $\bar{x}$	Min	Max	σ^2	σ	Cv (%)
Simmental	**MY, kg/day**	661990	15.80	3.60	44.00	24.20	4.92	31.13
	MF, %		4.04	2.04	5.98	0.04	0.19	4.73
	MP, %		3.23	2.01	3.99	0.03	0.14	4.47
Holstein	**MY, kg/day**	50983	17.79	3.60	55.00	36.99	6.08	34.18
	MF, %		3.98	2.72	5.93	0.05	0.22	5.50
	MP, %		3.22	2.03	3.99	0.02	0.14	4.31
Holstein red	**MY, kg/day**	21345	17.70	3.60	52.80	34.07	5.84	32.99
	MF, %		3.97	2.71	5.43	0.05	0.23	5.75
	MP, %		3.21	2.07	3.99	0.02	0.14	4.45
Brown Swiss	**MY, kg/day**	174	15.86	4.00	26.00	24.85	4.98	31.43
	MF, %		4.12	3.67	4.77	0.04	0.20	4.86
	MP, %		3.25	2.71	3.68	0.02	0.13	3.88
Total for all test-day records	**MY, kg/day**	734492	15.99	3.60	55.00	25.71	5.07	31.71
	MF, %		4.03	2.04	5.98	0.04	0.19	4.84
	MP, %		3.23	2.01	3.99	0.02	0.14	4.46
Total for THI	**THI**	**734492**	63.19	36.17	86.25	125.44		17.72

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Legend: N - number of test-day records used in the analysis; σ² - variance; σ standard deviation; Cv – coefficient of variation.

Table 2. LSM values of milk traits by region and breed.

Period	Factor	Region			Breed			
	Traits	**Mačva**	**Podunavlje**	**Šumadija**	**Simmental**	**Holstein**	**Holstein red**	**Brown Swiss**
I	**MY, kg/day**	17.12^{a}	18.81^{b}	17.71^{c}	16.72^{a}	19.05^{b}	18.99^{c}	16.75^{d}
	MF, %	3.97^{a}	3.97^{a}	4.01^{b}	4.00^{a}	3.95^{b}	3.93^{c}	4.05^{d}
	MP, %	3.17^{a}	3.24^{b}	3.23^{c}	3.21^{a}	3.21^{a}	3.20^{b}	3.21^{a}
II	**MY, kg/day**	16.94^{a}	18.62^{b}	17.53^{c}	16.59^{a}	18.97^{b}	18.80^{c}	16.42^{d}
	MF, %	3.96^{a}	3.96^{a}	4.01^{b}	3.99^{a}	3.93^{b}	3.92^{c}	4.04^{d}
	MP, %	3.17^{a}	3.23^{b}	3.23^{b}	3.21^{a}	3.21^{a}	3.20^{b}	3.21^{a}

Notes: Different superscript letters (a,b,c) within the same row indicate statistically significant differences ($p < 0.001$) according to Scheffé's multiple comparison test.

Table 3. LSM values by calving season, lactation number, and lactation stage.

Period	**Factor**	**Calving season**				**Lactation number**						**Lactation stage**				
	Traits	**W**	**Sp**	**Sum**	**A**	**1**	**2**	**3**	**4**	**5**	**6**	**≤60 days**	**61-120**	**121-200**	**201-300**	**>300 days**
I	**MY, kg/day**	17.90^{a}	17.81^{b}	17.83^{c}	17.97^{d}	17.11^{a}	17.97^{b}	18.60^{c}	18.23^{d}	17.86^{e}	17.51^{f}	21.16^{a}	22.57^{b}	19.62^{c}	14.75^{d}	11.30^{e}
	MF, %	3.98^{a}	3.98^{a}	3.98^{a}	3.99^{b}	3.97^{a}	3.98^{b}	3.98^{b}	3.99^{c}	3.99^{c}	3.99^{c}	3.85^{a}	3.89^{b}	3.97^{c}	4.07^{d}	4.14^{e}
	MP, %	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.14^{a}	3.16^{b}	3.21^{c}	3.25^{d}	3.28^{e}
II	**MY, kg/day**	17.93^{a}	17.91^{b}	17.38^{c}	17.57^{d}	16.94^{a}	17.79^{b}	18.40^{c}	18.04^{d}	17.67^{e}	17.34^{f}	21.04^{a}	22.29^{b}	19.27^{c}	14.57^{d}	11.31^{e}
	MF, %	3.97^{a}	3.96^{b}	3.98^{c}	3.98^{c}	3.96^{a}	3.97^{b}	3.97^{b}	3.98^{c}	3.98^{c}	3.98^{c}	3.84^{a}	3.88^{b}	3.96^{c}	4.06^{d}	4.13^{e}
	MP, %	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.14^{a}	3.16^{b}	3.21^{c}	3.25^{d}	3.28^{e}

Notes: Different superscript letters (a,b,c,d,e,f) within the same row indicate statistically significant differences ($p < 0.001$) according to Scheffé's multiple comparison test. W- **Winter; Sp-Spring; Sum- Summer; A- Autumn.**

Table 4. Seasonal trends of least squares means (LSM) for daily milk yield (MY), milk fat content (MF), and milk protein content (MP).

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Period	Factor	Month of Test Day											
	Traits	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
I	**MY, kg/day**	17.95^{a}	18.05^{b}	17.92^{c}	17.88^{d}	17.93^{e}	17.87^{f}	17.86^{g}	17.89^{h}	17.80^{i}	17.73^{j}	17.82^{k}	17.84^{l}
	MF, %	3.99^{a}	3.98^{b}	3.99^{a}	3.98^{b}	3.98^{b}	3.98^{b}	3.98^{b}	3.98^{b}	3.98^{b}	3.98^{b}	3.99^{a}	3.99^{a}
	MP, %	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.22^{b}	3.21^{a}	3.21^{a}	3.21^{a}
II	**MY, kg/day**	.	.	.	17.79^{a}	17.82^{b}	17.73^{c}	17.71^{d}	17.74^{e}	17.66^{f}	17.57^{g}	17.53^{h}	.
	MF, %	.	.	.	3.98^{a}	3.98^{a}	3.98^{a}	3.97^{b}	3.97^{b}	3.98^{a}	3.98^{a}	3.96^{c}	.
	MP, %	.	.	.	3.21^{a}	3.20^{b}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.20^{b}	.

Notes: Period I (12 months) and Period II (April–November). Different superscript letters (a,b,c,d,e,f,g,h,i,j,k,l) within the same row indicate statistically significant differences ($p < 0.001$) according to Scheffé's multiple comparison test.

Table 5. LSM values by THI interval levels.

Period	FACTOR	THI levels				
	Traits	**≤40**	**41-50**	**51-60**	**61-70**	**≥71**
I (Whole year)	**MY, kg/day**	17.81^{a}	17.88^{b}	17.93^{c}	17.89^{d}	17.89^{d}
	MF, %	4.00^{a}	3.98^{b}	3.98^{b}	3.98^{b}	3.98^{b}
	MP, %	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}	3.21^{a}
II (Apr-Nov)	**MY, kg/day**	.	17.34^{a}	17.86^{a}	17.78^{c}	17.79^{d}
	MF, %	.	3.97^{a}	3.98^{b}	3.97^{b}	3.97^{b}
	MP, %	.	3.20^{a}	3.21^{b}	3.21^{b}	3.21^{b}

Notes: Different superscript letters (a,b,c,d) within the same row indicate statistically significant differences ($p < 0.001$) according to Scheffé's multiple comparison test.

Table 1 also provides descriptive statistics for the THI across all observations. The overall mean THI value was 63.19, with recorded values ranging from 36.17 to 86.25 and a variance of 125.44. The corresponding coefficient of variation was 17.72 %. These data indicate that the analysed production records encompassed a broad spectrum of microclimatic conditions, from low to moderately elevated thermal load. The heat load on cows in Serbia is lower than in countries closer to the equatorial regions. Within the analysed dataset, the average THI value was substantially below the threshold of 72; the mean was 63.19, with a minimum of 36.17 and a maximum of 86.25. This leads to the conclusion that even the highest recorded value, according to the classification by McDowell et al. (1976), falls within the moderate stress level (THI 79–88), while the annual average remains below the mild stress level (THI 72–78).

Overall, the descriptive statistics presented in Table 1 characterize the production level and variability structure of the dataset and provide the quantitative basis for subsequent model-based analyses of environmental and biological effects on milk production traits. Building upon this descriptive overview, the next analytical step involved quantifying the statistical relevance of the selected fixed effects included in the general linear model. The results of this analysis for daily milk yield (MY), milk fat content (MF), and milk protein content (MP) across the two observation periods are presented in **Supplementary Table S1 (see Supplementary File).**

Analysis of variance (ANOVA) showed that, for all examined traits – MY (kg/day), MF (%), and MP (%)—each of the eight factors was highly statistically significant ($p < 0.0001$) in both observation periods. Considering the full-year observation period (I), the coefficient of determination (R^2) was 0.6206 for MY, 0.2887 for MF, and 0.1665 for MP. When examining the second observation period (II),

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which was four months shorter than the first, the coefficients of determination (R^2) were slightly lower for MY (0.6172), and slightly higher for MF (0.2998) and MP (0.1673). This finding confirms the stability of the model structure under differing seasonal data ranges as well as suggests that, when applying the same model specification to a shortened observation period, the proportion of explained variability for the MY trait decreased slightly, while the explanatory power of the model for MF and MP increased marginally.

Having established the overall statistical significance of the analysed fixed effects, LSM provide a more detailed insight into the direction and magnitude of these differences. Table 2 presents the LSM values of the analysed milk traits according to two factors: the region where the animals were reared and the breed of the cows. At the regional level, and consistently across both observation periods, the highest average daily milk yield across all breeds was recorded in the predominantly flatland region of Podunavlje, at 18.81 and 18.62 kg/day, respectively. In contrast, cows reared in Mačva achieved lower yields (17.12 and 16.94 kg/day, respectively), while intermediate values were observed in Šumadija (17.71 and 17.53 kg/day, respectively). Differences among regions were less pronounced for milk composition traits. Milk fat content was highest in Šumadija (4.01% in both periods), compared with Mačva and Podunavlje (3.97–3.96%). Milk protein content showed minimal regional variation, although the lowest values were observed in Mačva (3.17%), whereas slightly higher values were recorded in Podunavlje and Šumadija (3.23–3.24%).

Breed-related differences were more clearly expressed for daily milk yield. The LSM values for the four breeds show that Holstein-Friesian cows had the highest average daily milk yield, with 19.05 kg/day in Period I and 18.97 kg/day in Period II. Red Holstein cows followed with 18.99 and 18.80 kg/day, Simmental cows with 16.72 and 16.59 kg/day, and Brown Swiss cows with 16.75 and 16.42 kg/day, respectively. In terms of milk fat content the highest milk fat content was observed in Brown Swiss cows (4.05% and 4.04%), followed by Simmental cows (4.00% and 3.99%), and the lowest in the two Holstein populations (3.95% to 3.92%), which is consistent with breed characteristics. Regarding milk protein content, the LSM values showed only marginal variation among breeds, with identical values of 3.21% for Simmental, Brown Swiss, and Holstein-Friesian cows, and 3.20% for Red Holstein cows, across both periods. Overall, the LSM estimates presented in Table 2 confirm that regional and breed effects are more strongly expressed for daily milk yield than for milk composition traits.

Having examined the spatial and breed-related variation in milk production traits, further insight into production dynamics requires consideration of physiological and reproductive determinants. In this context, Table 3 presents the LSM of daily milk yield, milk fat content, and milk protein content according to calving season, lactation number, and lactation stage. These factors collectively reflect the influence of reproductive and physiological status on milk production and allow evaluation of how productive performance evolves throughout the reproductive cycle.

With respect to calving season, moderate differences in daily milk yield were observed between seasons in both observation periods. During Period I (12 months), cows calving in autumn achieved the highest milk yield (17.97 kg/day), while in Period II (8 months), the highest yield was recorded in cows calving in winter (17.93 kg/day). The lowest milk yield during Period I was recorded in cows calving in the spring (17.81 kg/day), while in Period II it was observed in cows calving in the summer (17.38 kg/day), as expected. In contrast, milk composition traits showed limited seasonal variability. Milk fat content ranged from 3.96% to 3.99% across seasons and periods, while milk protein content remained stable at 3.21% irrespective of calving season.

A clearer production pattern was observed for lactation number. Daily milk yield increased from the first to the third lactation and subsequently declined in later lactations in both observation periods. In Period I, MY increased from 17.11 kg/day in the first lactation to 18.60 kg/day in the third, before decreasing to 17.51 kg/day in the sixth lactation. A similar trajectory was recorded in Period II (16.94, 18.40, and 17.34 kg/day, respectively). Variability in MF and MP across lactation numbers was comparatively limited.

The most pronounced differences were associated with lactation stage. In both periods, peak daily milk yield was recorded during days 61–120 of lactation (22.57 kg/day in Period I and 22.29

kg/day in Period II), whereas the lowest values occurred after 300 days in milk (11.30 and 11.31 kg/day, respectively). In parallel, milk fat and protein content exhibited a gradual increase from early to late lactation. In Period I, MF increased from 3.85% (≤60 days) to 4.14% (>300 days), while MP increased from 3.14% to 3.28%. Nearly identical trends were observed in Period II.

Overall, the LSM estimates presented in Table 3 demonstrate that physiological stage exerts a stronger influence on daily milk yield than on milk composition traits, with lactation stage representing the most dynamic source of variation among the analysed biological factors.

While the previous analysis addressed production dynamics in relation to reproductive and physiological factors, a more detailed temporal perspective is provided by examining monthly variation. Table 4 presents the LSM of daily milk yield, milk fat content, and milk protein content according to the month of test day for both observation periods.

In Period I (12 months), daily milk yield exhibited moderate monthly variation, ranging from 18.05 kg/day in February to 17.73 kg/day in October. The distribution of LSM values indicates relatively stable production levels during the cooler months, followed by a gradual decline toward late summer and early autumn. In Period II (April–November), which excludes winter months, MY values ranged from 17.82 kg/day in May to 17.53 kg/day in November. Compared to Period I, LSM estimates in Period II were consistently lower for corresponding months, reflecting differences in the temporal structure of the dataset.

Milk fat content showed minimal monthly fluctuation in both periods, with values remaining within a narrow range (3.96–3.99%). Similarly, milk protein content demonstrated high stability across months, varying only between 3.20% and 3.22%. Overall, the monthly LSM trends presented in Table 4 indicate that temporal variation at the month-of-test-day level is more clearly expressed for daily milk yield than for milk composition traits. These results provide a refined temporal framework for interpreting seasonal patterns of milk production prior to the evaluation of thermal indices.

Building upon the monthly variation described above, a direct assessment of thermal load was conducted using THI classes. The LSM values of daily milk yield, milk fat content, and milk protein content across predefined THI intervals are presented in Table 5.

Within the analysed dataset, THI values ranged from low to moderately elevated levels, with a mean of 63.19 and observed values spanning from 36.17 to 86.25. According to established THI classifications (McDowell et al., 1976), this range corresponds predominantly to thermoneutral and mild-to-moderate heat stress conditions, without frequent exposure to severe stress categories. In Period I (whole year), the highest daily milk yield was recorded within the THI interval of 51–60 (17.93 kg/day). Slightly lower values were observed in adjacent intervals (≤40: 17.81 kg/day; 41–50: 17.88 kg/day; 61–70: 17.89 kg/day; ≥71: 17.89 kg/day). A comparable pattern was observed in Period II (April–November), where MY reached 17.86 kg/day in the 51–60 interval, compared with 17.34 kg/day in the 41–50 interval and approximately 17.78–17.79 kg/day in higher THI categories.

Milk fat content exhibited minimal variation across THI classes, remaining within a narrow range around 3.97–4.00% in both observation periods. Similarly, milk protein content showed limited responsiveness to THI variation, maintaining values close to 3.20–3.21% across intervals. Notably, THI values below 40 were not recorded during Period II, reflecting the seasonal restriction of the dataset to warmer months. Across both periods, the most consistent production performance was observed within the intermediate THI interval (51–60), whereas deviations toward lower or higher THI levels were associated with modest reductions in daily milk yield.

4. Discussion

The present study provides an integrated interpretation of how thermal variability, together with biological and management-related factors, shapes the sustainability of milk production in dairy cattle under temperate continental conditions. Rather than focusing exclusively on extreme heat stress events, the findings emphasise the relevance of moderate but persistent thermal load for productive stability in dairy production systems. This perspective is increasingly supported by studies conducted under non-extreme climatic conditions, which demonstrate that cumulative thermal exposure can affect productivity even below classical heat stress thresholds (Yan et al., 2021; Negri et al., 2021; Pinto et al., 2025).

The relative contribution of the applied explanatory variables differed among the analysed production traits, with daily milk yield showing a higher responsiveness (and a markedly higher proportion of explained variability) to the analysed environmental and management-related factors than milk fat and protein content. This pattern is consistent with the underlying structure of the dataset, where breeds with higher baseline milk yield tend to show lower baseline milk fat content (and only modest differences in baseline milk protein content), reflecting different production strategies and physiological demands. This observation aligns with the physiological regulation of milk synthesis, whereby milk yield responds rapidly to environmental perturbations, while milk composition traits are more tightly regulated and therefore less responsive to short-term climatic variability (Bohmanova et al., 2007; Ekine-Dzivenu et al., 2020). Similar trait-specific differences in responsiveness have been consistently reported in studies based on test-day records across diverse production systems, supporting the interpretation that milk yield represents the most sensitive production-level indicator of thermal stress under field conditions (Wildridge et al., 2018; Yan et al., 2021).

Breed-related effects observed in the present study can be interpreted within the broader context of genetic background, selection intensity, and production objectives characterising dairy systems in temperate regions (Cheruiyot et al., 2020). Model-adjusted LSM further indicate that regional effects were consistently expressed for daily milk yield across observation periods, whereas their influence on milk fat and protein content remained limited in magnitude, while breed-related differences followed stable and biologically expected patterns (Jeon et al., 2023). The relatively modest divergence in milk yield between specialised dairy breeds and the predominantly dual-purpose Simmental population reflects long-term adaptation to local production environments, as well as differences in breeding strategies and management intensity. In regions where dual-purpose breeds remain predominant, systematic genetic improvement combined with environmental adaptation can partially offset the productivity gap typically observed in highly specialised dairy populations (Cheruiyot et al., 2020). Comparable breed-related patterns have been documented in Central and Eastern European dairy systems and multi-breed evaluations under variable climatic conditions (Strączek et al., 2021; Knob et al., 2021; Negri et al., 2021).

The influence of lactation number and stage of lactation followed biologically expected trajectories and underscores the importance of accounting for physiological status when interpreting environmental effects on milk production (M'Hamdi et al., 2021; Soumri et al., 2025). Peak performance during mid-lactation and the subsequent decline towards later stages are well-established characteristics of the lactation curve (Stoop et al., 2009; Bohmanova et al., 2007). Importantly, the interaction between lactation dynamics and thermal conditions suggests that cows in early and peak lactation may be particularly susceptible to thermal variability due to the combined burden of high metabolic heat production and environmental heat load (Pinto et al., 2020). Similar interactions between lactation stage and thermal stress have been reported in both field studies and genotype-by-environment analyses (Strączek et al., 2021; Vinet et al., 2024; Mbuthia et al., 2022). This interaction has clear practical implications for sustainable herd management, as it identifies critical physiological windows during which targeted mitigation strategies may be most effective.

Thermal variability, characterised using THI classes, proved to be a meaningful descriptor of environmental pressure even in the absence of frequent extreme heat stress events (Negri et al., 2021; Pinto et al., 2025). While many earlier studies defined heat stress primarily through threshold values, more recent evidence indicates that production responses may occur at lower THI levels when exposure is sustained over time (Tamami et al., 2018). Moderate thermal load, particularly when persistent, can induce cumulative physiological strain that compromises production efficiency without necessarily triggering acute stress responses (Bernabucci et al., 2014). This interpretation aligns with a growing body of literature advocating a shift from single-threshold concepts towards a gradient-based understanding of thermal stress and its cumulative effects on dairy production systems (Bernabucci et al., 2010; Yan et al., 2021).

The identification of THI ranges associated with favourable production performance under Serbian conditions carries important implications for sustainability-oriented decision-making (Cincović et al., 2023; Samolovac et al., 2024). Previous studies conducted under Serbian production conditions provide important context for interpreting the identified THI ranges associated with favourable production performance. Research has consistently shown that variations in THI, even within moderate climatic conditions, can influence milk yield and selected quality traits, with the magnitude of response depending on lactation stage and physiological status (Cincović et al., 2010; Joksimović-Todorović et al., 2011). More recent analyses further emphasise that THI represents a practical and informative indicator of environmental pressure in Serbian dairy systems, supporting its use beyond the assessment of acute heat stress events (Cincović et al., 2023). Studies integrating animal-related and environmental factors have demonstrated that daily milk production responds measurably to THI variability under field conditions typical of Serbia, reinforcing the relevance of defining THI ranges linked to stable production performance (Mićić et al., 2022). In this context, the identification of favourable THI intervals aligns with broader evidence highlighting the importance of climate-aware management strategies for sustaining milk production and supporting long-term adaptation of dairy systems in Serbia (Mićić et al., 2025).

In temperate continental climates, where short episodes of extreme heat are interspersed with longer periods of moderate thermal exposure, maintaining cows within an optimal microclimatic range may be more relevant for long-term system stability than mitigating rare extreme events alone (Renna et al., 2010; Negri et al., 2021). By linking THI-based environmental characterisation with production stability rather than isolated production losses, the present findings conceptually extend these observations by emphasising the importance of identifying thermally relevant conditions for sustainable dairy management under non-extreme climatic scenarios.

The relatively limited variation observed in milk fat and protein content across thermal classes is consistent with reports of weak or inconsistent associations between heat stress and milk composition traits. Several studies suggest that changes in milk fat and protein content under heat stress are often mediated indirectly through alterations in feed intake, nutrient partitioning, and metabolic prioritisation rather than through direct thermal effects (Abeni et al., 1993; Bernabucci et al., 2015; Ekine-Dzivenu et al., 2020). The muted response of milk protein content observed in the present study further supports the interpretation that milk yield remains the most responsive and informative trait for monitoring the impact of thermal variability in routine production data.

From a sustainability perspective, these findings highlight the importance of integrating microclimatic indicators into herd management, breeding strategies, and decision-support systems (Nardone et al., 2010; Cincović et al., 2023). Sustainable milk production should not be evaluated solely in terms of maximal output, but rather in terms of the capacity of production systems to maintain stable performance under fluctuating environmental conditions, an approach increasingly emphasised in both international and regional assessments of climate resilience in dairy systems (Mbuthia et al., 2022; Mićić et al., 2025). Thermal variability thus represents a systemic challenge that interacts with genetics, physiology, housing design, and management practices, as emphasised in integrative assessments of climate resilience in dairy systems (Nardone et al., 2010; Mbuthia et al., 2022).

Finally, several limitations should be acknowledged. Thermal indices serve as proxies for the complex microclimatic environment experienced by individual animals and may not fully capture the influence of factors such as solar radiation, air velocity, coat characteristics, or behavioural adaptation (Bohmanova et al., 2007; Herbut et al., 2012). In addition, the use of routine production data may obscure farm-level mitigation practices and microclimatic conditions that influence actual thermal exposure, as has been highlighted under Serbian production settings (Samolovac et al., 2024). Future research should therefore aim to combine index-based approaches with high-resolution microclimatic measurements and animal-based indicators to further refine the assessment of thermal variability and its implications for sustainable dairy production, particularly in temperate continental systems (Mićić et al., 2025).

5. Conclusions

This study demonstrates that milk production performance under temperate continental conditions is significantly shaped by moderate but persistent thermal variability. The highest production levels were observed in Holstein cows reared in the Podunavlje region, calving in autumn and in their third lactation, under THI values between 51 and 60, whereas reduced performance was associated with early lactation stages, spring calving, and lowland environments characterised by greater thermal exposure. These findings confirm that physiological stage and environmental context jointly determine production sensitivity to thermal load. The principal scientific contribution of this research lies in its comprehensive field-based evaluation of thermal variability across multiple breeds, regions, and physiological stages under real production conditions in Serbia. Unlike studies primarily focused on extreme heat stress thresholds, this work quantifies the production response to moderate THI fluctuations, thereby addressing an important research gap related to sustained thermal load in temperate dairy systems. The results highlight that sustainability in milk production should be interpreted as the capacity of production systems to maintain stable performance under fluctuating climatic conditions. Early lactation and first-calving cows appear particularly vulnerable, indicating critical windows for targeted management and breeding interventions.

Future research should integrate THI-based assessments with high-resolution microclimatic monitoring, animal-based welfare indicators, and long-term climate trend analyses in order to improve predictive modelling of production resilience. In addition, incorporating thermotolerance traits into breeding programmes and evaluating the economic implications of thermal variability represent essential steps toward strengthening the adaptive capacity of dairy systems under ongoing climate change.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Abbreviations

The following abbreviations are used in this manuscript:

THI	Temperature–Humidity Index
MY	Milk yield
MF	Milk fat

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MP Milk protein
RH Relative humidity
LSM Least squares means

Appendix B

Table A1. Statistical significance of factors (p values) on milk yield (MY, kg/day), milk fat content (MF, %) and milk protein content (MP, %) at period I and period II. Supplement files.

Traits/factors	N	Region	Breed	Calving Year	Calving Season	Lactation Number	Month of Test Day	Lactation Stage	THI	R^2
		p - values								
		df=2	df=3	df=7	df=3	df=5	df=11	df=4	df=4	
MY, kg/day		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6206
MF, %	Period I (734492)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2887
MP, %		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1665
		df=2	df=3	df=7	df=3	df=5	df=7	df=4	df=3	
MY, kg/day		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6172
MF, %	Period II (442772)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2998
MP, %		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1673

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