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Effects of Thermal Variability on Milk Production Traits in Dairy Cattle Under Temperate Continental Conditions in Serbia

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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) as an indicator of thermal variability, on daily milk yield (MY), milk fat content (MF), and milk protein content (MP) was assessed. This 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 ($p < 0.0001$). Milk production was highest within the THI range of 51–60, corresponding to thermoneutral conditions, whereas higher THI values (>60) reflect increasing thermal load and were associated with measurable reductions in milk yield, as confirmed by statistical analysis. These findings demonstrate that thermal variability and heat stress significantly influence milk production and composition and highlight the importance of integrating microclimatic indicators into sustainability-oriented dairy management and breeding strategies.

Keywords: heat stress; temperature–humidity index (THI); milk yield; climate adaptation; dairy systems; production resilience

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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

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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 [1]. In dairy farming, heat stress has emerged as one of the most critical consequences of climate change, particularly in high-producing systems. Recent evidence indicates that the impacts of climate change on dairy production are increasingly evident not only in traditionally warm regions but also across Europe, including Southeast Europe, where rising temperatures and more frequent heatwaves are altering the thermal environment of livestock systems. These changes are particularly relevant for temperate and Mediterranean regions, where heat stress is no longer limited to extreme conditions but occurs under moderate thermal loads, affecting animal behaviour, welfare, and productivity [2,3].

Stress encompasses all environmental changes that initially trigger behavioural responses, 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 [4]. 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 [5]. When combined with nutritional stress, heat stress exerts a particularly detrimental impact on animal physiology in livestock production systems [6]. 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 [7]. In addition, heat stress induces a range of interconnected physiological mechanisms, including reduced feed intake [8], increased metabolic heat load, endocrine alterations (e.g., changes in cortisol and thyroid hormone regulation) [9], and oxidative stress [10].

In dairy cattle, prolonged exposure to heat stress negatively affects milk production, milk composition, reproductive performance, and animal welfare [11]. Heat load presents a major challenge due to its combined negative effects on both productivity and welfare [12]. 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 [13]. 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 [14]. To quantify heat stress under practical production conditions, various mathematical indicators have been proposed; however, the Temperature–Humidity Index (THI) remains the simplest and most widely used due to its ease of calculation and practical applicability [15,16]. THI is calculated based on ambient air temperature and relative humidity [17], and numerous formulas have been developed since the mid-20th century [18]. As such, THI is widely applied to assess the impact of heat stress on daily milk yield and milk composition in lactating cows under field conditions [19,20].

Given its widespread application, the relevance of THI is further supported by observed climatic trends in Serbia. Long-term meteorological data indicate a consistent increase in average air temperatures, with several recent years classified as extremely warm and deviations exceeding +2.0 °C compared to the reference period [21]. These changes are accompanied by an increased number of tropical days, indicating more frequent exposure of livestock to elevated thermal conditions. Under such circumstances, THI values in Serbia commonly reach or exceed the heat stress threshold during the summer months, with values around 72 from May to September and exceeding 80 during peak summer periods [20]. Building on these quantified climatic trends in Serbia, recent observations further indicate a shift towards more frequent and prolonged periods of thermal load, particularly during the summer months [21]. Under such conditions, dairy production systems are increasingly exposed to sustained thermal challenges rather than isolated extreme events. 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 [22]. As a result, numerous studies have focused on improving thermotolerance in dairy cattle without compromising production performance [23]; however, reported heat stress thresholds and production responses vary widely among studies, reflecting differences in climate, housing systems, genetic background, and methodological approaches [24]. 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 [25,26]. 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 [20]. 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. This gap highlights the need for a comprehensive, field-based evaluation of moderate but persistent thermal variability under temperate continental conditions in Serbia. Based on the presented evidence, it is hypothesised that under temperate continental conditions, moderate but persistent thermal variability, quantified using the Temperature–Humidity Index (THI), significantly affects milk yield and composition in dairy cattle. It is further assumed that these effects vary depending on environmental conditions and animal-related factors. In this context, sustainability in dairy production is primarily understood as the capacity to maintain stable productivity under variable thermal conditions.

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 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

This study was conducted under production conditions in the Republic of Serbia, located in Southeast Europe and characterised 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 [21], 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 from the beginning of 2013 to the end of 2019 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, Serbia), using the AT4 method [27] which is a recognised international standard for milk recording and performance testing. The analysed milk production traits included daily milk yield (MY, kg/day), milk fat content (MF, %) and milk protein content (MP, %).

The dataset comprised 734,492 test-day records from cows of different dairy breeds (Simmental—661,990; Holstein-Friesian—50,983; Red Holstein—21,345; Brown Swiss—174) reared in three regions of the Republic of Serbia (Mačva, Podunavlje, and Šumadija). However, the markedly lower number of Brown Swiss records should be considered when interpreting breed-related results (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.

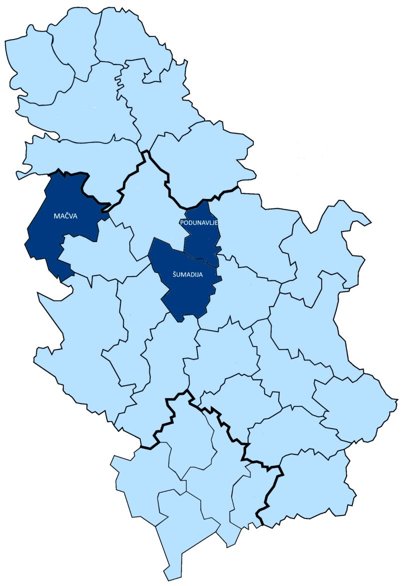

Figure 1. Map of the administrative districts of Serbia. The dark blue area indicates the study region, and the lines represent administrative boundaries.

Air temperature and relative humidity inside the barns were recorded hourly using a data logger (AMTAST AMT116, Qingdao, 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 ($\leq$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 [28]:

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (T - 26.8), \quad (1)$$

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 $\leq$ 40; II 41–50; III 51–60; IV 61–70; and V $\geq$ 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 [29–31].

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.

2.6. 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 (LSMs) 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:

$$Y_{ijklmnop} = \mu + O_i + R_j + G_k + S_l + L_m + M_n + F_o + T_p + e_{ijklmnop} \quad (2)$$

where

- $Y_{ijklmnop}$: The observed trait.
- μ: The overall mean of the population for the given trait.
- O_i: The fixed effect of the i-th region (i = 1, . . ., 3).
- R_j: The fixed effect of the j-th breed (j = 1, . . ., 4).
- G_k: The fixed effect of the k-th calving year (k = 1, . . ., 7).
- S_l: The fixed effect of the l-th calving season (l = 1, . . ., 4).
- L_m: The fixed effect of the m-th lactation number (m = 1, . . ., 6).
- M_n: The fixed effect of the n-th month of test day (n = 1, . . ., 12).
- F_o: The fixed effect of the o-th lactation stage (o = 1, . . ., 5).
- T_p: The fixed effect of the p-th THI level (p = 1, . . ., 5).
- $e_{ijklmnop}$: 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

Descriptive statistics of milk production traits (MY, MF, MP) and THI across dairy breeds are presented in Table 1. The values presented are overall averages, while the model-derived results will later be shown as LSMs 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% (σ = 0.19; CV = 4.84%), while the mean milk protein content was 3.23% (σ = 0.14; CV = 4.46%). These descriptive indicators confirm that daily milk yield represents the most variable production parameter within the analysed dataset.

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	661,990	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	50,983	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	21,345	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	734,492	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	734,492	63.19	36.17	86.25	125.44		17.72

Legend: N—number of test-day records used in the analysis; Min—minimum; Max—maximum; σ^2—variance; σ standard deviation; Cv—coefficient of variation (%); MY—milk yield (kg/day); MF—milk fat (%); MP—milk protein (%); and THI—Temperature–Humidity Index.

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 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, including frequent exposure to thermal conditions exceeding the lower heat stress threshold reported for temperate climates (THI ≈ 55–60) [2,32,33]. The heat load on cows in Serbia is generally lower than in tropical regions, where THI values are persistently high. However, within the analysed dataset, THI values frequently exceeded levels associated with the onset of production and physiological responses to heat stress, indicating that dairy cows were not operating predominantly under thermoneutral conditions. Oliveira et al. (2025) [34] reported that the thermoneutral zone in dairy cows is the temperature range between the lower and upper critical ambient temperatures. The optimal thermoneutral comfort zone for cows corresponds to a THI value of 57, while a value of 72 induces heat stress [35,36]. Bernabucci et al. (2010) [37] categorised THI stress ranges as mild (THI 68–71), moderate (72–79), moderate to severe (80–89), and severe (>90). Vučković et al. (2020) [38] also reported that lactating cows begin to physiologically adapt to the detrimental effects of heat stress when THI reaches 68; thus, THI values of 68 and 72 were defined as thresholds for heat stress in lactating cows.

Overall, the descriptive statistics presented in Table 1 characterise 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 Appendix A Table A1

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), 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 provides 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.

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 (N = 734,492)	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 (N = 442,772)	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}

Legend: N = number of test-day records; MY—milk yield (kg/day); MF—milk fat (%); MP—milk protein (%); and LSMean—least squares mean. Results are presented as LSMeans obtained using a general linear model (GLM). Different superscripts (a,b,c,d) within columns indicate significant differences ($p < 0.001$) according to Scheffé's multiple comparison test.

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.

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 (N = 734,492)	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 (N = 442,772)	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}

Legend: N = number of test-day records; MY—milk yield (kg/day); MF—milk fat (%); MP—milk protein (%); and LSMean—least squares mean. Results are presented as LSMeans obtained using a general linear model (GLM). Different superscripts (a,b,c,d,e,f) within columns indicate significant differences ($p < 0.001$) according to Scheffé's multiple comparison test. W—Winter; Sp—Spring; Sum—Summer; and A—Autumn.

The most pronounced differences were associated with the 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% ($\leq$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 the 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.

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

Period	Factor	Month of Test Day											
	Traits	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
I (N = 734,492)	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 (N = 442,772)	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}	

Legend: N = number of test-day records; MY—milk yield (kg/day); MF—milk fat (%); MP—milk protein (%); LSMean—least squares mean. Results are presented as LSMeans obtained using a general linear model (GLM). Different superscripts (a,b,c,d,e,f,g,h,i,j,k,l) within columns indicate significant differences ($p < 0.001$) according to Scheffé's multiple comparison test.

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.

Table 5. LSM values by THI interval levels.

Period	Factor	THI Levels				
	Traits	**≤40**	**41–50**	**51–60**	**61–70**	**≥71**
I (N = 734,492)	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 (N = 442,772)	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]

Legend: N = number of test-day records; MY—milk yield (kg/day); MF—milk fat (%); MP—milk protein (%); LSMean—least squares mean. Results are presented as LSMeans obtained using a general linear model (GLM). Different superscripts (a,b,c,d) within columns indicate significant differences ($p < 0.001$) according to Scheffé's multiple comparison test.

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 varied across THI classes, remaining within a range of 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 dataset's seasonal restriction to warmer months. In both periods, the best production performance was observed within the THI interval 51–60, while deviations towards lower or higher THI levels were associated with reductions in daily milk yield. These results indicate that milk production begins to decline when THI exceeds 60, suggesting the onset of heat stress effects under the studied conditions.

4. Discussion

This study provides an integrated interpretation of how thermal variability due to heat stress, along with biological and management factors, influences the sustainability of milk production in dairy cattle in the temperate-continental climate of the Republic of Serbia. The results indicate that cumulative exposure of cows to thermal variations throughout the year, particularly during the warmer months, can affect productivity even below classical heat stress thresholds [1,9,10,39].

The relative contribution of the explanatory variables varied among the analysed production traits, with daily milk yield showing greater sensitivity and a significantly higher proportion of explained variability (R^2 of 0.6206 in the first period and 0.6172 in the second period) to the analysed environmental and management factors compared to milk fat and milk protein content. This pattern aligns with the structure of the data set, where breeds with higher daily milk yield tend to have lower milk fat content and only moderate differences in milk protein content. This observation is consistent with the physiological regulation of milk synthesis in cows, whereby milk yield responds rapidly to changes in the external environment, while milk composition and quality are more strictly regulated and therefore less sensitive to short-term climate variability [19,40]. Similar differences in sensitivity between traits have been consistently reported in studies based on test-day records in various production systems, supporting the interpretation that milk yield is the most sensitive production indicator of heat stress in the dairy industry [9,31].

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 [41]. Results 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 [14,24]. 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. The relatively small number of Brown Swiss records in the present dataset reflects the population structure of dairy cattle under Serbian production conditions, where this breed is considerably less represented than Simmental and Holstein populations. 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 [41]. Comparable breed-related patterns have been documented in Central and Eastern European dairy systems and multi-breed evaluations under variable climatic conditions [39,42,43].

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 [44,45]. Peak performance during mid-lactation and the subsequent decline towards later stages are well-established characteristics of the lactation curve [40,46]. Importantly, the interaction between lactation dynamics and thermal conditions suggests that cows in early and peak lactation may be particularly susceptible to heat stress due to the combined burden of high metabolic heat production and environmental heat load [22]. Similar interactions between lactation stage and heat stress have been reported in both field studies and genotype-by-environment analyses [42,47,48]. 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 [10,39]. The results clearly indicate that heat stress is present under the studied conditions and exerts a measurable negative effect on milk production, even at THI levels below traditionally defined extreme thresholds, as evidenced by the decline in milk yield observed at THI values above 60 (Table 5). 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 [49]. Heat load, particularly when persistent, can induce cumulative physiological strain that compromises production efficiency without necessarily triggering acute stress responses [50]. This interpretation aligns with a growing body of the literature advocating a shift from single-threshold concepts towards a gradient-based understanding of thermal stress and its cumulative effects on dairy production systems [9,37]. Heat stress, depending on its intensity and duration, can negatively affect animal health by causing metabolic changes, oxidative stress, immune suppression, reduced production and appetite, health deterioration, and ultimately even death. The animal's organism responds to stress first behaviourally, then physiologically, and finally with a combination of both responses. The animal's reactions to stress vary based on its experience with stressors, their intensity and duration, physiological status,

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and other factors [51]. Low antioxidant status in cows is observed during the summer in both the pre- and post-partum periods [52].

The identification of THI ranges associated with favourable production performance under Serbian conditions carries important implications for sustainability-oriented decision-making [26,53]. 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 [25,54]. 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 [26]. 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 [20]. 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 [55]. From Table 5, the results for both observation periods show that the highest milk production was achieved in the 51–60 THI interval, consistent with other reports [35,36]. The differences in milk yield within the intervals are not biologically large, but they are statistically significant. This statistical significance indicates the certain presence of heat stress effects in this study. Although the observed daily differences in milk yield across THI classes were numerically small, they should not be interpreted as negligible. In large datasets, statistical significance may reflect high power rather than biological magnitude; however, even modest daily differences may accumulate over time. For example, compared with the most favourable THI interval (51–60), the observed reduction of 0.04–0.12 kg/day corresponds approximately to 12.2–36.6 kg of milk per standard 305-day lactation, or 14.6–43.8 kg on an annual basis per cow.

From a practical perspective, such differences may become relevant at the herd level, particularly in intensive production systems, where even small reductions in individual animal performance can translate into measurable economic losses when aggregated across the entire herd. Therefore, the observed effects, although modest at the individual level, support the importance of managing thermal conditions even under moderate THI levels.

In this context, sustainability should be understood as the capacity of dairy production systems to maintain stable productivity under variable and changing thermal conditions. The present findings demonstrate that even moderate but persistent thermal load can lead to measurable reductions in milk yield, which, when accumulated over time and across herds, may affect production efficiency and economic viability. Therefore, managing thermal stress is not only a matter of animal welfare and short-term productivity, but also a key component of long-term sustainability in dairy systems under climate change [3].

Although this study does not directly quantify specific sustainability indicators such as carbon footprint or resource-use efficiency, it addresses a critical underlying driver of system sustainability—thermal stress—by providing evidence on how microclimatic variability affects production stability. From a systems perspective, sustainable milk production should not be evaluated solely in terms of maximal output, but rather as the ability to maintain stable performance under fluctuating environmental conditions [48,55]. These findings support the integration of THI-based monitoring, microclimatic indicators into herd management, adaptive management practices, decision-support systems, and thermotolerance-oriented breeding strategies as practical approaches to improving climate resilience in dairy production systems [26,56]. Thermal variability thus represents a systemic challenge that interacts with genetics, physiology, housing design, and management practices, influencing the adaptive capacity of dairy systems under changing climatic conditions [48,56].

In temperate continental climates, where brief episodes of extreme heat alternate with longer periods of thermal exposure, maintaining cows within an optimal microclimatic range may be more important for long-term system stability than focusing solely on mitigating rare extreme events [39,57,58]. 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. Within this broader framework, the differential sensitivity of individual milk production traits provides further insight into how thermal variability is expressed at the production level.

The relatively limited variation observed in milk fat and protein content across THI classes is consistent with reports of weak or inconsistent associations between heat stress and milk composition traits. Several studies indicate 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 [19,59,60]. The limited 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 production data.

The results of this study indicate that heat stress effects are present even outside the traditionally defined periods of peak thermal load, highlighting the need for continuous monitoring and adaptive management. As heat stress causes production and economic losses in the dairy industry, any method of mitigating its effects can be very helpful. The most common recommendations for mitigating the negative effects of heat stress in the Republic of Serbia are managerial changes to production systems aimed at reducing total thermal radiation, reducing the number of animals per unit area of the farm, improving farm hygiene, introducing more free-range breeding systems, changing feeding times and meal composition, and similar measures.

Finally, several limitations should be acknowledged. THI indices serve as proxies for the complex microclimatic environment experienced by individual animals and may not fully capture the influence of additional factors such as solar radiation, air velocity, coat characteristics, or behavioural adaptation [32,40]. 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 [53]. 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 [55].

5. Conclusions

This study demonstrates that under temperate continental conditions in Serbia, dairy production systems are frequently exposed to thermal variability that significantly affects milk production. The results show that heat stress is not limited to extreme climatic conditions but occurs under moderate thermal loads, with milk yield declining when THI exceeds 60. This finding confirms that even non-extreme but persistent thermal exposure represents a relevant constraint for dairy production in temperate regions. Daily milk yield proved to be the most sensitive indicator of thermal stress, responding more clearly to changes in THI than milk fat and protein content. Although differences between THI classes were relatively small at the daily level, their cumulative effect over a standard lactation period highlights their practical importance, particularly at the herd level. The effects of thermal variability were influenced by breed, region, and physiological stage, indicating that responses to heat stress are context-dependent and shaped by both environmental and biological factors. These findings emphasise the need for a systems-based approach in evaluating dairy production under changing climatic conditions. From a sustainability perspective, milk production should be understood as the capacity of production systems to maintain stable performance under increasing thermal variability. The identification of optimal THI conditions (51–60) and the onset of production decline above this range provide a practical basis for improving climate resilience through targeted management strategies, including microclimate control, THI-based monitoring, and breeding for thermotolerance. The broader significance of this study lies in highlighting that moderate but persistent thermal stress represents an important and often underestimated challenge for dairy production in temperate climates. Future research should focus on incorporating additional climatic variables, improving modelling approaches through mixed models, and evaluating interaction effects between environmental and biological factors. Such approaches would enable a more precise assessment of thermal stress and support the development of more effective adaptation strategies for sustainable dairy production.

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Abbreviations

The following abbreviations are used in this manuscript:

THI	Temperature–Humidity Index
MY	Milk yield
MF	Milk fat
MP	Milk protein
RH	Relative humidity
LSMs	Least squares means

Appendix A

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.

Traits/Factors	N	Region	Breed	Calving Year	Calving Season	Lactation Number	Month of Test Day	Lactation Stage	THI	R^2
		df = 2	**df = 3**	**df = 7**	**df = 3**	**df = 5**	**df = 11**	**df = 4**	**df = 4**	
MY, kg/day	Period I (734,492)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6206
MF, %		<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	Period II (442,772)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.6172
MF, %		<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

Note: in the printed table, "p-Values" is a spanning header over the factor columns.

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