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ORIGINAL ARTICLE

SEASONAL VARIATION IN MILK COMPOSITION AND PHYSIOLOGICAL INDICATORS IN DAIRY COWS: A ONE-YEAR STUDY FROM EASTERN SLOVAKIA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study aimed to evaluate seasonal variations in the main components of raw cow's milk and to assess the relationships between milk composition and selected physiological indicators, including milk urea nitrogen (MUN), lactoferrin (LF), and somatic cell count (SCC). A total of 3000 milk samples from 250 clinically healthy dairy cows were collected across four seasons on a commercial farm in eastern Slovakia. Milk fat and protein exhibited significant seasonal differences ($p < 0.001$), with the lowest values in summer and the highest in autumn. Lactose concentrations remained relatively stable throughout the year, showing no significant seasonal variation. MUN showed notable seasonal variation, with significantly lower levels in winter compared with other seasons, reflecting changes in feeding regime and nitrogen metabolism. Lactoferrin levels were lowest in summer and highest in autumn, likely reflecting physiological changes associated with the lactation stage. Seasonal changes in SCC were not statistically significant, although a trend toward higher values in summer was observed. These findings highlight the influence of nutrition, physiological state, and environmental conditions on milk composition and udder health. Understanding seasonal dynamics is essential for optimizing dairy herd management, mastitis monitoring, and technological quality of raw milk.

Key words: lactoferrin; milk; milk composition; milk fat; season; somatic cell count

INTRODUCTION

The composition of bovine milk is a complex biological matrix determined by a wide range of genetic, physiological, environmental, and technological factors, including its fat, protein, lactose, mineral, and bioactive component profile. Among the most influential variables are season, nutritional status, udder health, stage and number of lactations, and overall herd management conditions. Numerous studies in Europe and beyond have confirmed that seasonal factors are among the key determinants of milk composition, particularly affecting fat, protein, lactose, urea nitrogen, and somatic cell count [1, 2, 3, 4].

Seasonal variation in milk composition has been observed not only in Europe but also in other continents with different climatic and production conditions. In Western Europe, long-term monitoring in the Netherlands confirmed significant seasonal changes in milk fat and protein, with the lowest values during summer and the highest in winter [5]. Similar trends were reported in Mediterranean systems, where high summer temperatures strongly reduced milk solids [2].

In Asia, studies from China demonstrated pronounced seasonal fluctuations in milk urea nitrogen and protein content related to feeding regime and climate [6], while Indian research highlighted the role of heat stress and metabolic load in shaping seasonal milk composition and SCC [7]. Outside Europe, seasonal changes in milk composition have also been described in intensive and semi-intensive systems in North America and other temperate regions, where summer heat stress and pasture quality play a major role [1].

These international findings indicate that although the direction of seasonal changes in milk composition is largely consistent worldwide, the magnitude of these changes depends strongly on regional climate, feeding strategies, and management systems. Therefore, region-specific data, such as those from eastern Slovakia, are essential for understanding local production conditions and for designing appropriate nutritional and health-management strategies.

Milk fat and protein levels typically decline during the summer due to elevated temperatures, heat stress, and seasonal changes in feeding, particularly the increased proportion of succulent feeds (green forage, pasture herbage) in the ration, whereas during the winter these parameters tend to be more stable and reach higher values [5, 8]. Lactose, the most stable component of milk, exhibits minimal seasonal variation; however, its concentration may be affected by metabolic stress, udder infections, and the energy balance of dairy cows [7]. The urea nitrogen content of milk (MUN) is an essential indicator of nitrogen balance and dietary protein utilization efficiency. It is well known that MUN rises primarily during the grazing season when the intake of rapidly degradable nitrogen is higher [9, 6], and excessive levels may negatively affect fertility and the metabolic load of dairy cows.

Somatic cell count (SCC) is a key indicator of udder health, with elevated values closely linked to subclinical mastitis, alterations in milk composition, and deteriorated technological properties, particularly in cheese production [10, 7]. An increased SCC is associated with reduced casein content, proteolytic degradation of milk proteins, and lower cheese yield and quality. Seasonal fluctuations in SCC have been reported, with higher levels in summer, mainly due to heat stress and increased environmental bacterial load [8].

While many international studies have explored seasonal variations in milk quality, there is still limited information on conditions in Slovakia, particularly on large-scale farms in mountainous areas. Therefore, this study aims to analyse seasonal changes in the physicochemical composition of milk, urea nitrogen concentration, and SCC under intensive dairy production conditions in eastern Slovakia over a 1-year period. The study also includes an assessment of relationships among individual parameters, which may contribute to a better understanding of the interactions among nutrition, udder health, and milk technological quality.

Unlike most previous studies that focus mainly on traditional milk quality indicators (fat, protein, lactose, urea nitrogen, SCC), this work also integrates lactoferrin as an additional functional and immunological marker. The combined evaluation of lactoferrin with classical technological and physiological indicators represents a novel approach under Slovak production conditions and allows a more complex assessment of mammary gland status and milk quality across seasons.

MATERIALS AND METHODS

Farm Characteristics and Housing Conditions

The study was conducted at an agricultural cooperative located in the eastern Slovak region, situated on the northern foothills of the Levoča Mountains (49.2761° N, 20.6834° E) at an altitude of approximately 550–570 m above sea level. The area lies within a mountainous production zone characterised by a continental climate with warm summers and cold winters typical of central Europe (Slovakia has a primarily humid continental climate with warm summers and cold winters).

The annual average temperature in Slovakia generally ranges from about 9°C to 11°C, with January averages below 0°C and July averages around 18–21°C. Annual precipitation in mountainous and highland regions of Slovakia is relatively high, often about 700–800 mm or more per year, with monthly maxima during summer months and lower totals in winter [11, 12, 13].

The herd comprised approximately 700 livestock units, including 250 Slovak Spotted dairy cows. The cows were kept in a free-stall system with solid bedding.

The animals were fed a total mixed ration (TMR) prepared directly on the farm, with its composition adjusted according to season. During winter, the ration was based mainly on maize silage, grass silage, hay, concentrate mixture, and mineral–vitamin supplements. In spring and summer, fresh green forage or pasture herbage formed an important part of the ration, supplemented with silage and concentrates to ensure adequate energy and protein supply. In autumn, the diet gradually shifted back to a higher proportion of conserved forages.

Cows had continuous ad libitum access to fresh drinking water from automatic water troughs throughout the year. Milking was carried out twice daily in a parallel milking parlour (Agromont, Slovakia) equipped with twelve fixed stalls arranged in two opposing rows. The average annual milk yield of the dairy cows exceeded 7,000 L per cow.

Animal Group

A total of n = 250 clinically healthy dairy cows in different parities (1st to 8th lactation; average parity 2.67) were included in the study. The cows were at various stages of lactation, with days in milk ranging from 2 to 625 (mean 119). Including the full physiological spectrum of lactation stages enabled an objective assessment of seasonal effects without selection bias.

Milk Sampling

Individual milk samples were collected once per month from each lactating cow over a 1-year period (March 2024 – February 2025). Cows in the dry period were not sampled. Because not all cows were in milk throughout the entire year, the total number of samples was slightly lower than the theoretical maximum (12 × 250 = 3000). In total, 3000 raw milk samples were obtained from approximately 250 cows, corresponding to about 2500–3000 samples depending on lactation stage and dry periods.

Sampling was always performed during the morning milking to minimise technological variability and ensure comparability between samples.

Before sampling, a clinical examination of the udder and a milk assessment using the California Mastitis Test (CMT) were performed. Only CMT-negative composite samples (mixed milk from all four quarters of the udder) were collected, thereby eliminating intra-udder quarter variability. In total, 3000 CMT-negative composite milk samples were included in the final analysis.

Sample Transport and Processing

Samples were collected into sterile containers and immediately cooled to 5°C. Within 6 hours of collection, they were transported to the Laboratory of the Department of

Hygiene, Technology and Food Safety at the University of Veterinary Medicine and Pharmacy in Košice (Slovakia) for analysis.

Laboratory Analyses

The analysis included the determination of the following basic physicochemical parameters:

- fat (%)
- protein (%)
- lactose (%)
- milk urea nitrogen (MUN; mg/dL)
- somatic cell count (SCC; $\times 10^3$ cells/mL)

In the laboratory, samples were analysed using a LactoScan MCCV analyser from Milkotronic Ltd., Bulgaria. All analyses were conducted in accordance with the relevant international standards ISO 21543 and STN EN ISO 13366-2 [14, 15].

MUN concentration was assessed in relation to the dynamics of milk composition and milk yield. Samples for MUN determination were analysed at the Central Dairy Laboratory, which is accredited according to ISO/IEC 17025, in cooperation with the State Breeding Services of the Slovak Republic, using data from the national herd-recording information system [16].

Because lactoferrin content cannot be routinely determined with LactoScan instruments, samples were processed separately at the Central Dairy Laboratory. Milk was first centrifuged (1,400 × g, 45 min), and the fat layer was removed. Lactoferrin in the skimmed milk fraction was then quantified using an immunochemical method – a quantitative ELISA assay based on antibodies specific to bovine lactoferrin.

Before analysis, milk samples were diluted 1:10,000 following the protocol [17] to ensure that measured values fell within the calibration curve range. The ELISA assay utilised goat anti-bovine lactoferrin antibodies coated onto microtiter plates and a secondary antibody conjugated with peroxidase for detection. After the enzymatic reaction and absorbance measurement, lactoferrin concentrations were calculated from the calibration curve and converted back to the original (undiluted) sample.

Statistical Analysis

Data were divided according to four seasons: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February). For each season, mean values and standard deviations (SD) were calculated for all monitored parameters (fat, protein, lactose, urea nitrogen, lactoferrin, SCC). Differences between seasons were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $\alpha = 0.05$. Statistically significant differences between means are reported together with the corresponding p-values.

In addition, relationships between variables were assessed using Pearson's correlation and linear regression across the entire dataset and within each season. Particular attention was given to correlations between the basic milk components (fat, protein, lactose) and urea nitrogen, lactoferrin, and SCC, as these relationships can reveal interactions between nutrition, udder health, and milk composition. Statistical analyses were performed using IBM SPSS Statistics version 20.

RESULTS

The results from monthly monitoring over 1 year indicate marked seasonal variations in the biochemical composition of raw cow's milk, as well as in selected physiological indicators reflecting nutritional status and udder health. Data are shown as mean, median, standard deviation, minimum, and maximum for each season.

Seasonal Changes in the Basic Milk Components

Fat

Milk fat content showed a clear seasonal pattern. The lowest average values were recorded during the summer (3.83%), whereas the highest values were observed in the autumn (4.20%). The difference between summer and autumn was highly significant ($p < 0.001$). Fat content in autumn was also significantly higher compared with spring and winter (by approximately 0.2 percentage points, $p < 0.01$). In contrast, no significant difference was found between winter (3.98%) and spring (3.95%) (Table 1).

Protein

Protein content exhibited a seasonal trend similar to that of fat. The lowest mean value was observed in summer (3.49%), while the highest was recorded in autumn (3.68%). Autumn and winter values were significantly

Table 1. Seasonal average values of basic milk components (Mean ± SD)

Season	Autumn	Winter	Spring	Summer
Fat (%)	4.20 ± 0.58[a]	3.98 ± 0.55[a,b]	3.95 ± 0.54[a,b]	3.83 ± 0.57[b]
Protein (%)	3.68 ± 0.28[a]	3.60 ± 0.27[a,b]	3.52 ± 0.26[a,b]	3.49 ± 0.29[b]
Lactose (%)	4.94 ± 0.28	4.87 ± 0.33	4.87 ± 0.29	4.93 ± 0.28
SCC (10^3 cells/mL)	274.99 ± 58.44	366.60 ± 10.70	364.79 ± 76.64	423.44 ± 86.72
MUN (mg/dL)	35.29 ± 8.49[b]	26.57 ± 5.58[a]	35.81 ± 7.74[b]	34.39 ± 6.84[b]
Lactoferrin (mg/dL)	171.96 ± 40.05[b]	157.96 ± 36.46[b]	158.20 ± 37.73[b]	153.79 ± 52.40[a]

Values in rows labeled with different letters (a,b) exhibit statistical significance ($P < 0.001$).

higher than those in spring and summer ($p < 0.001$). The difference between autumn and summer (approximately 0.19 percentage points) was highly significant ($p < 0.001$). However, no significant difference was observed between autumn and winter, suggesting that both seasons represent periods with peak milk protein concentration. The lower protein content in summer is linked to an imbalance in the energy-to-protein ratio during grazing. In contrast, the higher intake of concentrated feed in winter supports increased milk protein synthesis. Seasonal minimum and maximum values of all monitored parameters are summarized in Table 2.

Table 2. Seasonal minimum and maximum of the monitored parameters

Parameter	Lowest Value (Season)	Highest Value (Season)
Fat (%)	Summer (3.83)	Autumn (4.20)
Protein (%)	Summer (3.49)	Autumn (3.68)
Lactose (%)	Spring (4.87)	Autumn (4.94)
Milk urea nitrogen (mg/dL)	Winter (26.57)	Spring (35.81)
Lactoferrin (mg/L)	Summer (153.79)	Autumn (171.96)

Lactose

Lactose was the most stable of all monitored milk components. Its values fluctuated only minimally, within a narrow range of approximately 4.87–4.94%. The lowest concentrations were recorded during winter and spring, whereas summer and autumn showed slightly elevated levels. No significant seasonal differences were observed.

Seasonal Differences in Milk Urea Nitrogen

Milk urea nitrogen (MUN) exhibited one of the most pronounced seasonal dynamics among the evaluated parameters. The lowest concentrations were observed in winter, with an average value of approximately 27 mg/dL, which was significantly lower than those in all other seasons ($p < 0.001$). In spring, summer, and autumn, MUN concentrations were markedly higher, ranging from 34 to 36 mg/dl, with the highest levels recorded in spring. No significant differences were found among spring, summer, and autumn, indicating that these periods shared similarly elevated urea nitrogen concentrations (Figure 1).

The increased MUN levels in spring and summer likely reflect a higher intake of rapidly degradable nitrogen during the grazing period, along with a less balanced dietary energy-to-protein ratio. The spring peak may be associated with the lush, nitrogen-rich pasture at the onset of the vegetation season, while summer levels reflect the continued presence of fresh forage. Conversely, the winter minimum suggests a more balanced ratio with a lower proportion of rapidly degradable protein.

Values exceeding 35 mg/dL are considered borderline in terms of reproductive performance, as chronically elevated MUN has been associated with a 5–10% reduction in conception rates. In our dataset, spring values most closely approached this threshold, suggesting a potential excess of dietary protein relative to energy early in the grazing season. Extremely low MUN values (<15 mg/dL), which would indicate protein deficiency in the ration, were not observed.

Seasonal Differences in Lactoferrin Content

The lowest mean values of lactoferrin were observed in summer (154 ± 52 mg/L) and were significantly lower than in all other seasons ($p < 0.001$). Higher values were recorded in autumn (172 ± 40 mg/L), while winter and spring exhibited intermediate concentrations. No significant differences were detected among spring, winter, and autumn values ($p > 0.05$), indicating that the observed seasonal variation was primarily due to lower lactoferrin concentrations in summer.

The reduction in lactoferrin during summer is likely related to heat stress, which can decrease feed intake, milk production, and mammary protein synthesis. The higher

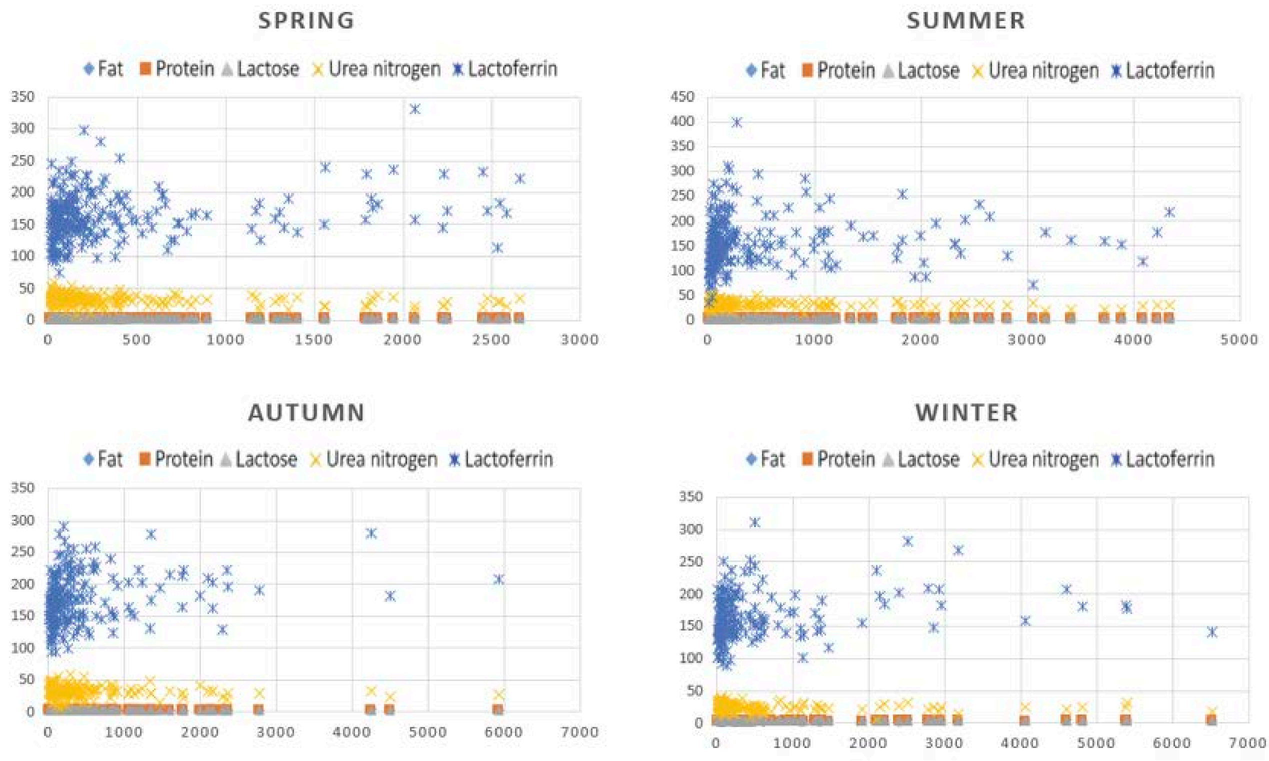

Fig. 1. Seasonal scatter distribution of milk composition parameters (fat, protein, lactose, urea nitrogen, lactoferrin)

concentrations observed in autumn likely reflect physiological changes associated with late lactation and the dry-off period, when lactoferrin naturally increases in milk as part of normal mammary gland function. Although lactoferrin, as an acute-phase protein, can reach considerably higher concentrations during inflammation or in colostrum, the seasonal variation observed in milk from healthy cows (approximately 150–170 mg/L) indicates predominantly physiological rather than pathological influences.

Somatic Cell Count

Somatic cell count (SCC) fluctuated between seasons; however, despite numerical differences, no statistically significant seasonal effect was detected ($p > 0.05$; $p = 0.0653$). The high between-cow variability, reflected in large standard deviations, likely masked more subtle seasonal patterns (Figure 2).

Geometric means showed that the lowest SCC values occurred in autumn (approximately 2.75×10^5 cells/mL). In contrast, higher values were recorded in winter and spring (around 3.6×10^5 cells/mL), with the highest levels observed during the summer months (approximately 4.2×10^5 cells/mL). These trends suggest that summer is the most critical period for milk hygiene, likely due to increased environmental stress and a higher bacterial load associated with warm, humid conditions. Median SCC values likewise confirmed that the cleanest milk was produced in autumn, whereas the highest SCC occurred in summer.

DISCUSSION

The results of this study confirm that season is a significant factor influencing the elemental composition of cow's milk as well as selected physiological indicators. The identified differences correspond with long-established trends described in the literature; however, the data obtained under large-scale farming conditions in eastern

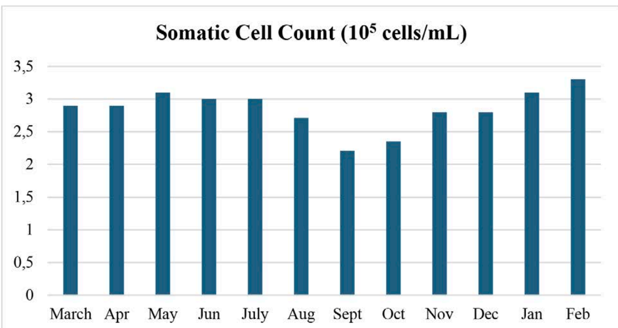

Fig. 2. Monthly and seasonal changes in Somatic Cell Count ($\times 10^5$ cells/mL)

Slovakia provide an important contribution to knowledge derived from other climatic and production systems.

The lowest fat and protein contents were detected during the summer, which agrees with several studies reporting the negative effect of heat stress on milk solids concentration [5, 8]. Elevated temperatures, reduced feed intake, and metabolic adjustments reduce fatty acid synthesis, resulting in lower milk fat concentration. At the same time, altered degradation and utilisation of nitrogenous compounds may contribute to reduced protein content. Conversely, the highest values of these parameters were recorded in autumn, when environmental conditions are more stable, and cows are often in later stages of lactation, which may positively influence milk composition.

Comparable seasonal patterns have also been described in Central and Eastern European production systems. Studies from Poland, the Czech Republic, Hungary, and the Balkans report a similar decline of fat and protein during summer and their increase in autumn and winter, largely attributed to heat stress and changes in feeding strategies [18]. Similar autumn–winter increases in milk fat and protein were documented in Poland under grazing/feeding-season conditions [19], while Hungarian monitoring data likewise confirmed clear within-year seasonal shifts in raw milk fat and protein [20]. Consistent results have also been reported from the Balkan region, where multi-year datasets showed the lowest fat and protein values during summer and the highest during winter [18]. Evidence from Slovakia further supports this regional pattern, with the lowest fat and protein contents repeatedly observed in summer [21]. The magnitude of seasonal differences observed in the present study therefore corresponds well with data from neighbouring countries, indicating that climatic and nutritional drivers of milk composition are highly comparable across this region.

Lactose concentration remained relatively stable throughout the year, reflecting its fundamental biological function and its strong link to milk's osmotic pressure [7]. Lactose is less sensitive to nutritional or environmental fluctuations, and therefore its variability is generally lower than that of fat and protein.

Milk urea nitrogen showed one of the most pronounced seasonal fluctuations. The highest values in spring and summer are typical for the grazing period, when the intake of rapidly degradable nitrogen is increased [9, 6]. Excessive MUN levels may indicate an imbalance between dietary nitrogen and available energy. This can negatively influence not only milk composition but also fertility and metabolic load in dairy cows. The low MUN values observed during winter reflect a more stable ratio based on conserved forage.

Lactoferrin concentrations were lowest in summer and highest in autumn. Lactoferrin is an immunologically active mammary gland protein [10]. The summer decrease likely reflects physiological factors such as reduced protein synthesis during late lactation, whereas the autumn increase is associated with the late stage of lactation and the dry-off period.

However, the magnitude of seasonal variation is strongly influenced by local environmental and production conditions. The studied farm is located in a mountainous area of eastern Slovakia, characterised by cooler winters, relatively mild summers, and a shorter vegetation period compared with lowland regions. These conditions influence forage availability, grazing duration, and the structure of the ration during the year. In such areas, the transition between pasture-based and conserved-forage feeding is more pronounced, which may explain the clear seasonal differences observed in fat, protein, MUN, and lactoferrin. Unlike lowland intensive systems, where feeding regimes are often more uniform throughout the year, mountain production zones show stronger contrasts between winter and summer diets, as well as greater sensitivity to climatic stress. Therefore, although the general effect of season on milk composition is well known, region-specific studies such as this one are necessary to understand how global trends are modified by local climate, altitude, and feeding systems.

Somatic cell count did not exhibit statistically significant seasonality in this study, although a trend toward higher SCC in summer was observed. This trend is consistent with findings from other authors [22], who report elevated SCC at temperatures above 25°C due to increased bacterial load, reduced environmental hygiene, and decreased cow comfort [7]. It is important to note that the average SCC throughout the year was approximately 3.6×10^5 cells/mL, which is below the EU legislative limit for raw milk (400,000 cells/mL), indicating that the cows were clinically healthy throughout the study period. Milk with elevated SCC contains less lactose and casein and higher levels of proteolytic enzymes, which negatively affect cheese making properties. The high individual variability in SCC likely contributed to the lack of statistical significance, despite the summer trend.

Several biologically relevant relationships between milk composition and physiological indicators were observed. Milk protein content tended to be lower when MUN values were higher, which may reflect metabolic strain caused by an excess of rapidly degradable nitrogen without an adequate energy supply. Similar trends have been reported in other studies, where elevated MUN was associated with reduced milk protein and poorer fertility [9]. No consistent association between lactoferrin and SCC was observed in this study. SCC fluctuated seasonally, with the highest values during summer, likely due to increased environmental stress and higher bacterial load. Lactoferrin concentrations were within the physiological range (approximately 150–170 mg/L), reflecting normal seasonal changes associated with late lactation and the dry-off period rather than pathological processes. While lactoferrin can reach higher levels during subclinical or clinical mastitis, such conditions were not detected in this herd.

The inclusion of lactoferrin alongside traditional milk quality indicators provides a more comprehensive understanding of mammary gland physiology and its seasonal dynamics. In this context, the analysis confirms that nutritional management and udder health monitoring should take into account the seasonal dynamics of the parameters assessed. Regular assessment of indicators such as MUN, lactoferrin, and SCC can help optimize lactation strategy, improve reproductive and production performance, and increase technological milk quality, while also creating a solid foundation for further research into the interactions between nutrition, physiological status, and mammary gland defense mechanisms.

CONCLUSIONS

The results of the year-long monitoring of raw cow's milk quality under intensive dairy production conditions in eastern Slovakia confirmed that the season is a significant factor influencing the elemental composition of milk and selected physiological indicators. The lowest fat and protein contents were recorded during the summer, whereas the highest values of both components occurred in autumn. Lactose remained relatively stable throughout the year, showing the lowest seasonal variability of all monitored constituents.

Milk urea nitrogen displayed the most pronounced seasonal dynamics – low winter values reflected a stable ration, while elevated concentrations in spring and summer indicated an excess of rapidly degradable nitrogen during the vegetation period. Lactoferrin concentrations were lowest in summer and highest in autumn, likely reflecting physiological changes associated with the late stage of lactation and the dry-off period.

Somatic cell count (SCC) did not differ significantly among seasons, although a trend toward higher values was observed during the summer months, possibly reflecting increased mammary gland stress.

Overall, the analysis confirms that nutritional management and udder health monitoring should account for the seasonal dynamics of the parameters under evaluation. Regular assessment of indicators such as MUN, lactoferrin, and SCC can help optimise feeding strategies, improve reproductive and production performance, and enhance the technological quality of milk. These results provide a valuable basis for further research into the interactions between nutrition, physiological status, and the defence mechanisms of the mammary gland.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

Ethical approval was not necessary for this work.

Conflict of Interest

No conflict of interest.

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Generative AI Statement

Authors disclosed that no form of generative AI or AI-assisted technologies was used in the writing process.

Authors' Contributions

S.O. contributed to the study design, provided methodological supervision, assisted with data interpretation, and critically revised the manuscript, including statistical analysis.

J.Z. conceived and designed the study, performed the laboratory analyses, and prepared the manuscript draft.

F.Z. carried out sampling.

E.P.-K. supported data collection.

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