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ORIGINAL ARTICLE

ANALYSIS OF FACTORS INFLUENCING SOMATIC CELL COUNT IN DAIRY COW MILK

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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

The aim of this study was to evaluate the effects of parity, days in milk (DIM), and season on somatic cell count (SCC) in milk of Holstein dairy cows. The study was conducted on a single dairy farm in eastern Slovakia on 600 cows with an average annual milk yield of 10,317 ± 653 kg, fed a total mixed ration. Cows were grouped according to parity, DIM, and season. Milk composition (protein, fat, lactose) was determined using near-infrared spectrophotometry (MilkoScan FT+), and SCC was measured using Fossomatic FC and Bentley FCM. Parity significantly affected SCC, with the lowest values observed in primiparous cows and a significant increase ($P < 0.001$) with advancing lactation number; the highest somatic cell count was recorded in the 4th and higher lactations. The effect of days in milk showed increased somatic cell count during early (0–30 days) and peak lactation (31–100 days), followed by the lowest values ($P < 0.05$). As lactation progressed and milk yield declined, somatic cell count increased, reaching maximum levels after 305 days. Seasonal variation was also significant, with the highest somatic cell count observed in summer ($P < 0.001$) and the lowest in winter months.

Keywords: cow milk; days in milk; mastitis; parity; season

INTRODUCTION

The somatic cell count (SCC) in milk is one of the most important indicators of hygienic quality and mammary gland health in dairy cows. Somatic cells (SC) are a natural component of cow's milk and consist mainly of leukocytes (white blood cells) and epithelial cells originating from the lining of the mammary gland. Their primary function is to provide immune protection against microbial infections in the udder. An increased SCC is generally associated with inflammatory processes in the mammary gland, particularly mastitis, which is one of the most common and economically significant diseases in dairy cows [1, 2].

According to Regulation (EC) No. 853/2004, European food legislation has established limit values for the average somatic cell count (SCC) in raw cow's milk of 400,000 cells.ml^{-1} in European Union countries [3]. In the United States, the maximum permissible SCC limit for raw milk is 750,000 cells.ml^{-1} [4]. Milk from healthy cows typically contains a low SCC (< 200,000 cells.ml^{-1}), whereas elevated values indicate an immune response associated with inflammation or the presence of subclinical mastitis [5].

In practice, the SCC is widely used as an important tool for monitoring herd health status, enabling early detection of subclinical mastitis, and evaluating the effectiveness of management measures in dairy herds. In addition to its relevance to animal health, SCC has a substantial impact on the technological properties of milk. Elevated somatic cell counts adversely affect milk composition, particularly protein, fat, and lactose contents, impair milk processing characteristics, and may reduce both the yield and quality of dairy products, especially cheese [6, 7].

Factors influencing the SCC in the milk of dairy cows include the stage of lactation, parity, season, time of milk sampling, milk yield, body condition, herd management, genetics, and the health status of the mammary gland [8].

Parity, days in milk (DIM), and season are major biological and environmental determinants of SCC variability. Parity reflects cumulative exposure to pathogens and physiological aging of the mammary gland, DIM represents changes associated with different stages of lactation and metabolic status, and season reflects environmental influences such as temperature stress. These factors are among the most frequently reported determinants of SCC in dairy production systems.

The aim of this study was to evaluate the effects of parity, days in milk, and season on the SCC in the milk of dairy cows. We hypothesized that parity, DIM, and season significantly influence SCC, with higher SCC expected in cows of higher parity, at the end of lactation, and during the summer season due to environmental and physiological stress.

MATERIALS AND METHODS

Data collection

The study was conducted on a single dairy farm in the east of Slovakia, which is situated at 48°34′ north latitude and 20°53′ east latitude, an analysis of Holstein dairy cows (n = 600), whose annual milk production was 10,317 ± 653 kg and which were fed a total mixed ration (TMR) formed from corn, clover, and grass silage supplemented with a concentrate component based on corn, wheat, barley, oats, soybean, and rapeseed extracted meal.

Dairy cows were fed uniformly in groups of primiparous and multiparous cows at the beginning of lactation and in the 2nd and 3rd lactation phases. The cows were fed separately in the 1st lactation phase (31 – 100 DIM). The influence of parity, DIM, and season on the SCC in milk was evaluated as average, with milk samples divided into the following groups according to:

- lactation order: primiparous cows (number of lactations = 1), multiparous cows (number of lactations 2, 3, and 4+)
- days in milk (DIM): 0 – 30, 31 – 100, 101 – 200, 201 – 305, > 305
- seasons: spring (March – May), summer (June – August), autumn (September – November), winter (December – February)

The cows were maintained in free-stall housing. The dairy cow beds were designed as mattress areas covered with a thin layer of straw. The beds were regularly groomed and supplemented with bedding as needed, with occasional liming used to reduce microbial growth and odors. The dairy cows were fed a total mixed ration (TMR) *ad libitum,* provided in the feed trough. The TMR was formed monthly, depending on the nutrient requirements according to the milk yield and capacity of dry matter intake (DMI). The average daily DMI was 21.75 ± 2.27 kg and did not differ significantly between groups. The samples of the TMR were taken from the feed manager on the control day and were analysed for dry matter (DM), which consisted of crude protein (CP), fat, acid, and neutral detergent fibre (ADF, NDF), and starch analysed by conventional methods [9]. The DM was determined by weight upon drying the sample at 105 °C under the prescribed conditions. The CP content was determined by the Kjeldahl method using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hoganas, Sweden). The fat was determined by the device Det-Gras (JP SELECTA, Spain). The ADF and NDF were determined using a Dosi-Fibre Analyser (JP SELECTA, Spain), and the content of starch was determined by polarimetry. The net energy of lactation (NEL) and non-fibre carbohydrates (NFC) were calculated using regression equations according to the National Research Council [10].

The cows were milked twice a day at 06:00 a.m. and 04:00 p.m. in a parallel milking parlour (BouMatic, Sweden), and individual milk samples were analysed once per month. On the farm, a basic milking hygiene program was implemented. After milking, teats and udder were treated with a disinfectant solution, typically iodine-based. The samples were cooled to 4 °C and immediately transported to the Central Analytical Laboratory of Milk with accreditation under the registration number 096/5878/2015/2 in collaboration with The Breeding Services of Slovakia, using the breeding information system. A total of approximately 4,000 milk samples were included in the analysis.

Milk samples were analysed for milk protein, fat, and lactose by a near-infrared spectrophotometric assay using MilkoScan FT+ (Foss Electric, Hillerød, Denmark) according to STN 57 0536 [11], and SCC was analysed using a Fossomatic FC (Foss Electric, Hillerød, Denmark) and Bentley FCM (Bentley Instruments Inc., Chaska, USA) according to STN EN ISO 13366-2 [12].

Statistical analysis

The results were processed using the statistical program GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) and expressed as mean (x), standard deviation (SD), minimum and maximum values. To evaluate differences in SCC depending on parity, DIM, and season, we used one-way analysis of variance (ANOVA) and Tukey's test with a 95% confidence interval for multiple comparisons of means. Only animals that remained in the milk recording system throughout the entire lactation were selected for the final statistical dataset, ensuring consistency of observations across the study period. A total of 600 unique dairy cows were included in the analysis, each contributing on average 7 test-day measurements. Since individual cows contributed observations to multiple DIM categories and seasonal groups, a linear mixed-effects model with cow ID as a random effect was employed to appropriately account for the dependence of repeated measurements.

RESULTS

The average nutrient concentration in TMR and production indicators for the farm are shown in Table 1.

The SCC, depending on the parity, is shown in Table 2. The lowest SCC was confirmed in the 1st lactation (110.96 ± 52.33 x 10^3.ml^{-1}), which increased significantly ($P < 0.001$) with increasing lactation number (in the 2nd lactation, 213.56 x 10^3.ml^{-1} and in the 3rd lactation, 313.22 x 10^3.ml^{-1}), while the highest SCC was found in dairy cows in the 4th and higher lactations (417.95 ± 97.85 x 10^3.ml^{-1}).

The SCC, depending on DIM, is shown in Fig. 1. The detected SCC fluctuated significantly with increasing lactation. In 0 – 30 DIM, an increased SCC (210.01 x 10^3.ml^{-1}) was detected, which subsequently decreased with increasing milk production and days in milk; when at the peak of lactation (1st phase of lactation), it reached the lowest values (140.94 x 10^3.ml^{-1}). With a gradual decrease in milk production from the peak of lactation, SCC

Table 1. Analysed nutrient content of TMR and milk composition

Nutrients in TMR (% DM)	x	SD	Minimum	Maximum
DM (%)	44.12	3.54	34.7	51.4
CP (g.kg^{-1} DM)	153.7	11.0	105.0	174.0
NEL (MJ.kg^{-1} DM)	6.44	0.2	5.7	6.7
NDF (g.kg^{-1} DM)	373.1	40.0	280.0	450.0
ADF (g.kg^{-1} DM)	229.9	28.0	187.0	342.0
Starch (g.kg^{-1} DM)	229.3	50.0	103.0	301.0
NFC (g.kg^{-1} DM)	358.0	37.0	206.0	409.0
Fat (g.kg^{-1} DM)	41.8	5.0	31.0	60.0
Milk production and composition				
Milk yield (kg.d^{-1})	34.00	10.0	10.00	79.13
Fat (%)	3.72	0.7	1.17	8.17
Protein (%)	3.28	0.4	1.40	5.70
Lactose (%)	4.86	0.2	2.43	5.40
SCC (10^3.ml^{-1})	266.94	82.53	2.00	485.22

DM – dry matter; CP – crude protein; NEL – net energy of lactation; NDF – neutral detergent fibre; ADF – acid detergent fibre; NFC – non-fibre carbohydrates; TMR – total mixed ration; d – day; SCC – somatic cell count

Table 2. SCC ($10^3.ml^{-1}$) in milk depending on parity

Parity	x	SD	Minimum	Maximum
1st lactation	110.96[a]	52.33	3.00	150.85
2nd lactation	213.56[b]	78.59	2.00	270.52
3rd lactation	313.22[c]	95.78	2.00	420.89
4th and ↑ lactation	417.95[d]	97.85	2.00	659.87

Means with different superscripts are significantly different (P < 0.001)

increased, reaching the highest values (464.43 x $10^3.ml^{-1}$) after the 305th DIM.

Means with different superscripts are statistically significant (P < 0.05)

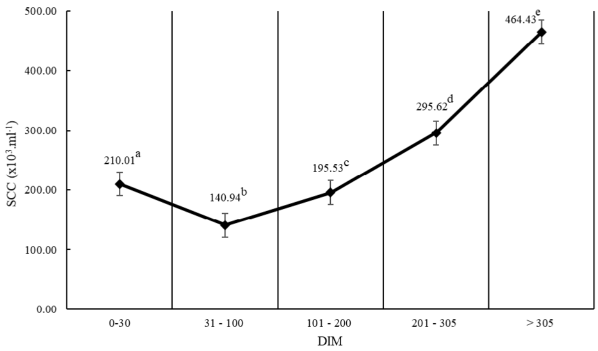

Fig 1. SCC in milk ($10^3.ml^{-1}$) depending on DIM

The SCC, depending on the season, is shown in Fig. 2. The highest SCC was confirmed in the summer (387.29 x $10^3.ml^{-1}$) and the lowest in the winter (218.88 x $10^3.ml^{-1}$) months, with confirmed statistical significance (P < 0.05).

Means with different superscripts are significantly significant (P < 0.05)

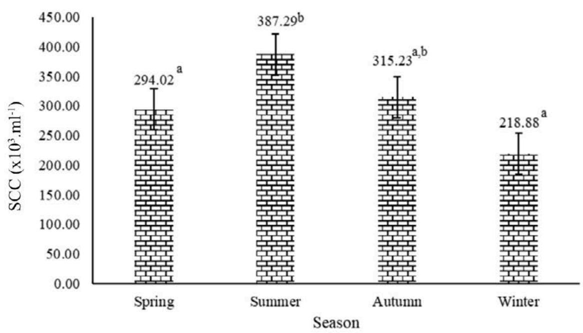

Fig. 2. SCC in milk ($10^3.ml^{-1}$) depending on season

DISCUSSION

Fluctuations in production parameters in milk indicate different composition and nutrient content of TMR for individual groups of dairy cows due to different genetic potential of the herd, order, and lactation phase of dairy cows. Nutrition, particularly the composition and balance of the TMR, plays an important role in modulating immune function and SCC levels. Adequate energy and protein supply supports immune competence, while imbalances (negative energy balance, insufficient fibre, or excess rapidly fermentable carbohydrates) may predispose cows to metabolic stress and increased susceptibility to mastitis, which is reflected in elevated SCC. Although the TMR in this study met recommended nutrient requirements, variations in nutrient intake across lactation phases may have contributed to fluctuations in SCC.

Evaluation of SCC depending on the parity, we confirmed the lowest SCC in the 1st lactation, which increased significantly with increasing lactation number as a result of shorter exposure to pathogens and less wear and tear of the mammary gland tissue. The mammary gland of primiparous cows is less damaged by previous inflammatory processes and therefore responds more effectively to infectious stimuli [13]. Older dairy cows in higher lactation (4th and ↑) have a statistically significantly higher average SCC as a result of a more frequent occurrence of subclinical and chronic mastitis, cumulative damage to the mammary gland, and, due to age, a reduced effectiveness of the immune response [14]. In addition, older cows are often under production stress, and their physiological tissue regeneration capabilities are weakened. According to Olde Riekerink et al. (2008), SCC in multiparous cows can be 1.5 to 2 times higher compared to primiparous cows. These differences are important not only for herd management but also for decisions on selection and culling of animals [15].

By evaluating SCC depending on the DIM, we confirmed that immediately after calving, SCC increases due to physiological adaptation, metabolic stress after calving as a result of negative energy balance, and a weakened immune system [16]. SCC stabilizes and reaches its lowest values at the peak of lactation, with SCC often < 200 x 10^3.

ml^{-1} in healthy cows as an indicator of good health [17]. As lactation progresses (> 101 DIM), SCC increases as a result of decreased milk production, cell accumulation, increased permeability of the mammary gland epithelium, and a higher susceptibility to subclinical mastitis [18]. In > 305 DIM, values of SCC can exceed 300 – 600 x 10^3.ml^{-1} even in clinically healthy dairy cows [7]. According to Sordillo et al. (2002), the increase in SCC in the late phase of lactation is associated with poorer tissue regeneration and repeated inflammation of the mammary gland [19].

Evaluation of SCC in milk across different seasons revealed the highest values during summer, likely as a consequence of heat stress. High summer temperatures negatively affect the physiological functions of the animals, alter mammary gland cells at the molecular level, cells responsible for the synthesis of milk components, and increase the activity of certain microorganisms associated with mammary gland infections. These changes reduce the mammary gland's defense capacity and promote bacterial colonization [20, 21, 22]. When evaluating individual factors affecting SCC, we found that approximately 15% of cows exceeded the recommended limit of 400,000 cells.ml^{-1}.

CONCLUSIONS

Evaluation of SC in dairy cow milk serves as an important indicator of mammary gland health, particularly in subclinical forms of mastitis that do not present visible symptoms. Elevated SCC indicates inflammation and negatively affects milk quality, reducing its technological value, yield, and hygienic safety. Regular monitoring of SCC helps to minimize economic losses in milk production, optimize herd management, and ensure compliance with legislative milk quality standards. The results of our study confirmed that SCC in dairy cow milk is significantly influenced by parity, DIM, and season. These findings highlight the importance of targeted herd management that takes into account the physiological state of the animals, age, lactation, and climatic conditions to reduce the incidence of mastitis and maintain high hygienic quality of milk.

Ethical Statement

In this study, we used data obtained with the consent of the farm in collaboration with the Breeding Services of Slovakia, using the Breeding Information System.

Data Availability Statement

Data are contained within the article.

Conflict of Interest

The authors declare no conflicts of interest.

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Generative AI Statement

No generative AI and AI-assisted technologies were used in writing the manuscript.

Authors' Contributions

Conceptualization, P. T. L.; methodology, P. T. L., Z. F., I. M.; investigation, P. T. L., I. M.; data curation, P. T. L., M. H., T. M.; writing—original draft preparation, P. T. L.; writing—review and editing, M. H., T. M., I. M., Z. F.; funding acquisition, I. M., Z. F.

All authors have read and agreed to the published version of the manuscript.

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