



Article The Mineral Composition of Milk from High-Yielding Dairy Cows Depending on the Month of Lactation and Udder Health

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Abstract: The aim of this study was to determine the effects of the month of lactation and udder health of high-yielding Polish Holstein-Friesian (PHF) cows on daily milk production and the content of K, Ca, Na, Mg and Zn in milk. The experimental materials comprised 380 milk samples collected from 38 cows. The highest average daily milk yield (49.1 kg) was noted in cows in the second month of lactation and, considering udder health, in cows whose milk contained 201,000 to 400,000 somatic cells per mL on average (denoting risk of mastitis). The K content of milk increased (p < 0.05) in successive months of lactation, whereas the opposite trend was observed in the Zn content of milk. The average content of Ca (842 mg·dm⁻³), Na (344.5 mg dm⁻³) and Mg (98.5 mg dm⁻³) in milk was below the lower limits of the normal physiological ranges. Milk from cows with healthy udders (\leq 200,000 somatic cells per mL) had the highest concentration of K, whereas the levels of Na and Zn were highest in milk from cows with clinical mastitis (> 1 mln somatic cells per mL). Udder inflammation was accompanied by an increase in the levels of Na and Zn in milk. The high content of Na and Zn in milk can be an additional indicator of mastitis in cows.

Keywords: potassium; calcium; sodium; magnesium; zinc; daily milk yield; primiparous cows; multiparous cows; somatic cell count (SCC)

1. Introduction

Dairy products are popular due to their unique flavor, aroma and health-promoting properties, which are determined by the physicochemical composition of milk [1]. A glass (250 mL) of cow's milk contains on average 250 mg of phosphorus (P), 380 mg of potassium (K), 300 mg of calcium (Ca), 125 mg of sodium (Na), 32 mg of magnesium (Mg) and 0.90 mg of zinc (Zn) [2,3]. The chemical composition of milk may vary depending on various genetic (cattle breed, genotype), environmental (management system, diet, season) and physiological (cow's age, lactation stage, udder health, yield) factors. The profitability of dairy cattle production is largely dependent on milk performance. Selective breeding for increased productivity has compromised the health status of dairy cattle herds, including reducing fertility and an increasing incidences of metabolic diseases, locomotor disorders and inflammations of the mammary gland and udder tissue (mastitis) [4]. The focus on food safety and hygiene, and the quality of dairy products has contributed to the development of milk payment systems where quantity payment (kg) is combined with quality payment based on the chemical composition, cytological and microbiological quality of milk [5,6]. According to Polish Standard (PN-A-86002),

the maximum allowable somatic cell count (SCC) per ml of extra class milk is 400,000 and the total bacterial count cannot exceed 100,000. However, udder inflammations remain the greatest problem in herds of high-yielding dairy cows. Mastitis is the second, after infertility, most economically significant disease worldwide [7], which reduces the yield and quality of milk [8,9]. The relationships between SCC and major milk components have been extensively researched, whereas few studies have investigated the relationships between SCC and the content of mineral elements in milk. In addition, milk from high-producing Polish Holstein-Friesian (PHF) cows may contain insufficient concentrations of selected macronutrients, in particular Ca and Mg, relative to the relevant standards [10,11].

The aim of this study was to determine the effects of the month of lactation and udder health of high-yielding PHF cows on daily milk production and the content of K, Ca, Na, Mg and Zn in milk.

2. Materials and Methods

The experimental materials comprised 380 milk samples collected from 38 Black-and-White PHF cows. The cows were in their first lactation (18 animals) and in their second, third and fourth lactation (20 animals). Milk samples were collected at two-week intervals, from the first week postpartum to the fifth month of lactation. Udder health was evaluated based on SCC, in thousands of cells per mL of milk: \leq 200—healthy udder, 201–400—risk of mastitis (due to a low number of samples in the 400–500 group, these samples were included in the 500–1000 group), 401–1000—subclinical mastitis, >1000—clinical mastitis [12].

The cows were kept on a farm located in the Region of Warmia and Mazury (NE Poland). They were housed in free-stall barns. To eliminate the effect of the calving season on milk composition, cows with similar expected calving dates were selected for the experiment. A total mixed ration (TMR) was fed year-round. The TMR was composed of maize silage and haylage, supplemented with farm-made concentrates (protein concentrates, vitamin-mineral premixes) and feed additives that improve productivity and help balance complete diets (protein, rumen-protected fat, active yeast cultures and other energy supplements).

Samples of fresh milk were analyzed for SCC by flow cytometry using the Bentley BactoCount instrument [13]. Milk samples were subjected to high-pressure wet mineralization involving microwave-assisted digestion (Mars Xpress 5, Candela, USA), with the use of a mixture of 6 mL of 65% Suprapur nitric acid (Merck, Germany) and 1 mL of 30% Suprapur hydrochloric acid (Merck, Germany), in accordance with the relevant standards. Each sample was analyzed in duplicate. Every mineralization procedure involved 2 blank samples and 2 samples of certified reference material (BCR-063R). The content of elements (K, Na, Ca, Mg, Zn) in the mineralized samples was determined in an atomic absorption spectrometer with flame atomization (SpectrAA240FS, Candela, USA) [14,15]. Mineralized milk samples were assayed for the content of Zn, Ca and Mg by atomic absorption spectrometry (AAS) with the use of hollow-cathode lamps (Varian), and for the content of K and Na by atomic emission spectroscopy (AES) with the use of the relevant standards (Sigma-Aldrich, USA).

The results were processed statistically using Statistica 13.3 software. Milk samples collected at two-week intervals were averaged and pooled into composite monthly samples. The effects of the month of lactation and udder health on daily milk production and the content of K, Ca, Na, Mg and Zn in milk were determined by the least squares method, using the following formula:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ij}$$

where: Y_{ijk} —value of the analyzed parameter, μ —population mean, A_i —effect of the month of lactation (1–5), B_j —effect of udder health (1–4), $(AB)_{ij}$ —month of lactation x udder health interaction, e_{ij} —random error.

The significance of differences between means was determined by a Tukey's range test at p < 0.05 and p < 0.01. Since the empirical distribution of SCC was not normal, the data were log-transformed

(the natural logarithm was applied). The Pearson correlation coefficient between the natural logarithm of SCC (ln_{scc}) and the mineral content of milk was calculated.

3. Results and Discussion

The average SCC per mL of milk from high-yielding PHF cows varied throughout lactation (Table 1). The highest SCC was noted in the second month of lactation, and it was significantly (p < 0.05) higher than in the fifth month of lactation. The month of lactation of high-yielding PHF cows had a significant effect on milk production. The highest daily milk yield (peak of lactation) was achieved in the second month after calving (49.1 kg), and it decreased in subsequent months to reach 37.9 kg of milk on average in the fifth month of lactation. A significant (p < 0.01) difference was found between daily yields in the firsts three months of lactations vs. the fourth and fifth month. In a study by Kapusta et al. [16], milk yield peaked in high-producing cows on day 70 after calving. Other authors who investigated PHF cows also demonstrated that milk yield continued to increase until day 60 of lactation, and it remained stable until day 120 [17]. Unfortunately, high productivity may adversely affect the performance traits of PHF cows, such as fertility and productive lifetime, and contribute to metabolic and locomotor disorders [18].

Mastitis is the most common disease in high-yielding cows [19]. This disease reduces milk production and causes undesirable changes in the chemical composition of milk, thus decreasing its processing suitability. The number of somatic cells per ml of milk (SCC) is the most popular indicator of udder health status during lactation. In the present study, udder health had a significant influence on the average daily milk yield in high-producing PHF cows (Table 1). The highest average daily milk yield (48.4 kg) was noted in cows whose milk contained 201,000 to 400,000 somatic cells per ml (denoting risk of mastitis), and the difference relative to cows with clinical mastitis (6.0 kg) was highly significant. According to some authors [20], SCC of 250,000 to 300,000 per mL of milk may mark the beginning of infection. In the current study, cows with an increased risk of developing mastitis (201,000 to 400,000 somatic cells per ml of milk) produced the largest amounts of milk. Franzoi et al. [21] found that an increase in SCC impaired milk productivity, profitability and quality, and that very low SCC had a similar or even more detrimental effect on milk yield and quality than high SCC. This implies that the relationship between SCC vs. milk yield and composition is not linear.

The month of lactation and udder health had significant effects on the mineral composition of milk (Table 2). The analyzed milk had high K content (1447.6 mg dm⁻³ on average), and similar values were reported by other authors [11,22,23]. In the current experiment, K concentration increased with the progress of lactation. The difference between the fourth and fifth month of lactation vs. the first three months was statistically significant. The milk of cows with healthy udders had the highest K content (1513.6 mg dm⁻³), which was significantly higher than in cows with an increased risk of developing mastitis (1385.4 mg dm⁻³) and cows with subclinical mastitis (1336.3 mg dm⁻³). Only in the subclinical mastitis group was the K content of milk below the lower reference limits of 1350 to 1550 mg K dm⁻³ [24]. A considerable decrease in the K content of milk from mastitic cows was also reported by other authors [20].

In the present study, Ca concentration in milk was low, the average Ca content reached 842 mg·dm⁻³, and it was approximately 16% below the lower limit of the normal physiological range (1000–1400 mg dm⁻³) [24] (Table 2). The Ca content of milk was highest (p < 0.01) in the first month after calving, compared with the next months. Milk from cows with subclinical mastitis contained 905.1 mg Ca dm³, and milk from healthy cows contained 815.8 mg Ca·dm³, but the noted difference was not significant. According to many authors, milk from cows suffering from mastitis has lower Ca content than milk of the highest cytological quality [20,22]. Stasiuk and Przybyłowski [25] compared milk from different animal species and found that Ca concentration in cow's milk was generally low, at 801 mg dm⁻³ on average, ranging widely from 684 to 1095 mg dm⁻³. The lower Ca content of milk in dairy cattle herds is probably related to the intensive production system based on conserved grass (silage, hay) and high

quantities of concentrates [10,26]. On the other hand, Gabryszuk et al. [27] demonstrated that the Ca content of milk from cows raised on an organic farm was only 637.4 mg/dm³.

In the current study, the Na content of milk was higher (p < 0.05) in the first month of lactation than in the third month. Considering udder health, the Na content of milk ranged from 401.3 mg dm⁻³ in cows with clinical mastitis to 322.5 mg dm⁻³ in cows with an increased risk of developing mastitis, and the observed differences were highly significant (Table 2). The Na content of milk from cows with subclinical and clinical mastitis was at the lower limit of the normal range, i.e., 350–600 mg dm⁻³ [24]. In the remaining animals, i.e., cows with healthy udders and with an increased risk of developing mastitis, Na concentration in milk was below the normal values. In a study by Górska and Mróz [22], the Na content of milk increased significantly with increasing SCC. Sodium chloride and lactose are responsible for maintaining the osmotic pressure of milk equal to the osmotic pressure of blood. During mammary gland inflammation, the lactose content of milk decreases, which increases the rate of NaCl diffusion from the bloodstream [20,24]. Since selected minerals and vitamins directly affect immune system function, they can also play a role in mastitis prevention.

Similarly to Ca concentration, the Mg content of milk from high-producing cows also often falls below the lower reference limits [10,23–25,28]. In the present experiment, the average Mg content of milk reached 98.5 mg dm⁻³ (Table 2), and it was slightly below the lower reference limits (100–150 mg dm⁻³) [24]. The highest concentration of this macronutrient (103.4 mg Mg dm⁻³) was observed in milk from cows in the first month of lactation. The difference relative to the third month of lactation reached 8.9 mg Mg dm⁻³, and it was statistically significant. Considering udder health, the highest concentration of this macronutrient (104.8 mg Mg dm⁻³) was observed in milk from cows with subclinical mastitis, and it was significantly higher than in milk from healthy cows and cows with clinical mastitis. Górska and Mróz [22] found that SCC had no significant influence on the content of Ca, K and Mg in milk, but their levels decreased with increasing SCC.

Zinc is the most important micronutrient in cow's milk. A glass of cow's milk covers around 10% of the recommended daily intake of Zn in adults. The average Zn content of milk from the analyzed cows was high, at 4.76 mg dm⁻³ (Table 2). The concentration of Zn was highest in the first month of lactation (5.42 mg Zn dm⁻³), and it decreased in subsequent months. The difference between the first month of lactation vs. the fourth and fifth month was statistically significant. Zinc concentration in milk tended to increase steadily with increasing SCC. A significant increase in the Zn content of milk was noted in cows with subclinical and clinical mastitis, compared with cows with healthy udders and with an increased risk of developing mastitis. The highest Zn concentration was observed in milk containing more than 1 million somatic cells per ml (5.60 mg Zn dm⁻³). An interaction (p < 0.01) between the month of lactation and udder health was found for the Zn content of milk (Figure 1). The concentration of Zn in milk from cows with clinical mastitis was highest in the first month of lactation and udder health was found for the Zn content of milk (Figure 1).

Górska and Mróz [22] did not observe a significant relationship between SCC and the average Zn content of milk, which was determined at 3.49 mg dm⁻³. According to Skrzypek [29], Zn, copper and selenium contribute to the bacteriostatic properties of milk, and microelement supplementation in dairy cows has a beneficial influence on the udder immune system. Zinc facilitates the reconstruction of keratin. This mineral also activates numerous enzymes involved in immune system function, and Zn deficiency may increase the risk of bovine mastitis. Olsson et al. [30] demonstrated that Zn concentrations in mammary tissue were positively correlated with the milk performance and age of dairy cows. According to many authors, the above correlations result from higher Zn supplementation levels in large dairy cattle farms [31–34]. Zinc concentration in milk is also affected by environmental contamination, including atmospheric deposition [35]. In organic farms, dairy cattle are less exposed to heavy metals, but they can also be at greater risk of macronutrient and micronutrient deficiencies due to the limited use of mineral fertilizers and dietary mineral supplements, relative to conventional farming systems [28,36].

Parameter	Month of Lactation (ML)					Somatic Cell Count (SCC), ths mL^{-1}				SE	<i>p-</i> Value		
	1.	2.	3.	4.	5.	≤200	201–400	501-1000	≥1000		ML	SCC	$ML \times SCC$
Number of milk samples	76	76	76	76	76	261	42	50	27	-	-	-	-
SCC, ths mL^{-1}	193.6	349.2 ^a	307.8	333.5	149.8 ^b	52.3 ^A	279.6 ^B	650.5 ^C	1666.1 ^D	24.98	0.022	0.000	0.233
SCC, Ln	11.4	11.5	11.7	11.5	10.8	10.5 ^A	12.5 ^B	13.4 ^C	14.2 ^D	0.07	0.842	0.000	0.877
Daily milk yield, kg	46.3 ^A	49.1 ^A	45.8 ^A	41.9 ^B	37.9 ^B	45.2	48.4 ^A	44.3	42.4 ^B	0.49	0.001	0.000	0.172

Table 1. Effects of the month of lactation and the udder health of cows on the average daily milk yield.

Means followed by different letters (within the factor) are significantly different: a, b at p < 0.05; A, B, C, D at p < 0.01.

Table 2. Effects of the month of lactation and the udder health of cows on the content of potassium (K), calcium (Ca), sodium (Na), magnesium (Mg) and zinc (Zn) in milk, mg dm⁻³.

Mineral	Month of Lactation (ML)					Somatic Cell Count (SCC), ths mL ⁻¹				SE	<i>p</i> -Value		
	1.	2.	3.	4.	5.	≤200	201-400	501-1000	≥1000	02	ML	SCC	$\mathbf{ML}\times\mathbf{SCC}$
K	1376.1 ^b	1388.3 ^b	1439.2 ^b	1534.8 ^a	1628.3 ^a	1513.6 ^A	1385.4 ^B	1341.1 ^B	1450.4	13.95	0.044	0.002	0.053
Ca	993.1 ^A	799.3 ^B	811.8 ^B	760.8 ^B	829.9 ^B	815.8	874.5	910.1	875.8	13.12	0.000	0.113	0.624
Na	371.7 ^a	338.1	323.4 ^b	344.9	347.1	335.1 ^a	322.5 ^B	367.0 ^{a,b}	401.3 ^{A,a}	4.10	0.032	0.000	0.521
Mg	103.4 ^{Aa}	96.8 ^b	97.7	94.5 ^B	96.5 ^b	96.1 ^b	103.4 ^a	104.8 ^a	94.6 ^b	0.87	0.007	0.031	0.220
Zn	5.42 ^a	4.78	4.74	4.24 ^b	4.21 ^b	4.45 ^B	4.90	5.16 ^A	5.60 ^A	0.06	0.000	0.000	0.000

Means followed by different letters (within the factor) are significantly different: a, b at p < 0.05; A, B at p < 0.01.



Figure 1. The interaction between the month of lactation and the udder health of cows for the Zn content of milk.

The coefficients of correlation between the analyzed variables are presented in Table 3. Cow productivity, measured as the average daily milk yield, was negatively correlated with SCC (r = -0.20) and the K content of milk (r = -0.38) and positively correlated with the Ca (r = 0.11) and Zn content of milk (r = 0.14). Therefore, high-producing cows must rely on their own Ca reserves. In a previous study, cow productivity expressed as milk yield over 305-day lactation, was negatively correlated with the concentrations of K and Ca in milk [10]. Highly significant positive correlations were found between SCC vs. the content of Na (r = 0.256) and Zn (r = 0.30). This indicates that the levels of the above minerals increase or decrease during mammary gland inflammation, which may be an indicator of mastitis. Hussain et al. [37] reported that the levels of K, P, Ca, Mg, Zn and Fe were significantly higher in the milk of mastitic cows. According to Singh et al. [38], udder infections resulting in elevated SCC may alter the mineral and trace element profile of milk, and the magnitude of change may have a diagnostic and prognostic value. Stocco et al. [39] demonstrated that differential SCC could be a new informative tool for dairy farmers to monitor the quality of milk.

Table 3. Correlations between milk components.

Parameter	1	2	3	4	5	6	7
1. Daily milk yield	-	-0.20 **	-0.38 **	0.11 *	0.021	0.063	0.14 *
2. SCC		-	-0.08	0.11 *	0.256 **	0.13 *	0.30 **
3. Potassium			-	-0.116 *	0.178 **	-0.150 **	-0.10 *
4. Calcium				-	0.268 **	0.408 **	0.542 **
5. Sodium					-	0.166 **	0.249 **
6. Magnesium						-	0.349 **
7. Zinc							-

* p < 0.05; ** p < 0.01.

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The K content of milk was negatively correlated with Mg concentration, and positively correlated with Na concentration. The highest positive coefficients of correlation were calculated for the Ca content of milk vs. the levels of Zn (r = 0.542), Mg (r = 0.408) and Na (r = 0.268). It should be stressed that the Zn content of milk was bound by highly significant positive correlations with as many as four variables: Ca, Mg, Na and SCC. In contrast to the findings of Olsson et al. [30], a relationship between the Zn content of milk and milk production was not observed in this study.

4. Conclusions

The month of lactation and the udder health of high-yielding PHF cows affected milk production and mineral composition. The highest daily milk yield was noted in cows in the second month of lactation and, considering udder health, in cows whose milk contained 201,000 to 400,000 somatic cells per mL on average (denoting risk of mastitis). The content of Ca, Na and Mg in milk was below the lower limits of the normal physiological ranges, which could be related to the high production level. This suggests that milk from high-yielding cows can be deficient in some minerals, and supports the view that dairy products should be fortified with these minerals. Udder inflammation was accompanied by an increase in the levels of Na and Zn in milk. The high content of Na and Zn in milk can be an additional indicator of mastitis in cows.

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