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ORIGINAL PAPER

Effect of milk performance and udder health on the zinc content of cow's milk*

Zenon Nogalski[1], Anna Nogalska[2]

[1] Department of Animal Nutrition, Feed Science and Cattle Breeding
[2] Department of Agricultural and Environmental Chemistry
University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

Abstract

Milk is an important source of zinc (Zn), which performs various catalytic, structural, and regulatory functions in the human body. Milk yield, composition, and quality are considerably affected by the incidence of mastitis. The aim of this study was to determine the effect of milk performance and udder health on the Zn content of cow's milk. A total of 605 milk samples were collected from 32 Polish Holstein-Friesian (HO) cows (8 animals in lactations 1, 2, 3, and 4 each). Milk samples were taken once a week, between lactation days 7 and 90, and then once a month until the 10th month of lactation. The proximate composition of milk and the somatic cell count (SCC) were determined. The Zn content of milk was determined by atomic absorption spectrometry (AAS). In order to evaluate udder health, milk samples were classified as follows: ≤ 200 ths – healthy udder, 201 - 400 ths – risk of mastitis, 401 - 1 000 ths – subclinical mastitis, and > 1 000 ths somatic cells mL^{-1} – severe subclinical mastitis. Two models of analysis of variance (ANOVA) were applied. The effect of lactation number (LN) and udder health on the Zn content of milk was evaluated in the first model. The effect of daily milk yield and the fat/protein ratio on the Zn content of milk was evaluated in the second model. The average Zn content of milk was 4.6 mg dm^{-3}. The concentration of Zn in milk was highest in the second week of lactation, and it decreased in subsequent weeks. Milk from the oldest cows (lactation 4) and the most productive cows had the lowest (P<0.05) Zn content. The fat/protein ratio, which is an indicator of energy balance in cows, was positively correlated with the Zn content of milk. The Zn content of milk increased (P<0.05) with increasing SCC. A high content of Zn in milk can be an additional indicator of mastitis in cows.

Keywords: zinc, daily milk yield, mastitis, somatic cell count, fat/protein ratio

Anna Nogalska, Ph.D, Assoc. Prof., Department of Agricultural and Environmental Chemistry, Faculty of Agriculture and Forestry, University of Warmia and Mazury in Olsztyn, Oczapowskiego 8, 10-719 Olsztyn, Poland, anna.nogalska@uwm.edu.pl
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INTRODUCTION

Milk and milk products (e.g., butter, cheese, yogurt) are primary sources of micronutrients and essential nutrients in the human diet, which are required to maintain normal bodily function, health and wellness, and prevent nutrient deficiencies (Gernand et al. 2016). This has prompted producers to improve milk quality by enriching the products with health-promoting ingredients (Ataro et al. 2008). This group of ingredients includes zinc (Zn), an abundant intracellular micronutrient that performs various catalytic, structural and regulatory functions in the human body (Pechová et al. 2006). Zinc concentration in milk is determined mostly by the diet and health status of dairy cows (Wang et al. 2014, Nogalska et al. 2020). Mastitis is the second, after infertility, most economically important disease in dairy cattle, which adversely affects milk yield and quality (Japertiene et al. 2007, Sawa et al. 2007). Somatic cell count (SCC) is an indicator of udder health in cows (Mollenhorst et al. 2010). Somatic cells are mostly cells of the immune system, which participate in the innate immune response. This group of cells includes lymphocytes, macrophages, polymorphonuclear cells, and selected types of epithelial cells (Schukken et al. 2003). An increase in SCC is indicative of udder infection, leading to compromised milk performance and undesirable changes in milk composition (Schukken et al. 2003, Costa et al. 2020). The relationships between SCC, cow productivity, and the content of major milk components have been extensively researched, whereas only a few studies have investigated the relationship between SCC and the mineral content of milk. According to Singh et al. (2017), udder infections resulting in elevated SCC may alter the mineral profile of milk, and the magnitude of the observed changes can have a diagnostic and prognostic value. A trend towards a positive effect of Zn supplementation on the health of the mammary gland was identified in association with SCC (Pechowá et al. 2006). In terms of the effect of Zn on health performance in dairy cattle, some studies reported that dietary Zn supplementation decreased SCC (Cope et al. 2009, Chandra et al. 2015). In contrast, Whitaker et al. (1997) recorded no effect of dietary Zn supplementation on SCC or mastitis.

Most researchers have focused on the impact of dairy cow diets on the Zn content of milk and the incidence of mastitis, whereas little attention has been paid to the influence exerted by increased SCC and mastitis, in particular subclinical mastitis, on micronutrient concentrations in milk. The aim of this study was to determine the effect of milk performance and udder health on the Zn content of cow's milk.

MATERIALS AND METHODS

The experimental materials comprised 605 milk samples collected from 32 Polish Holstein-Friesian (HO) Black-and-White cows. Eight cows in their 1st, 2nd, 3rd, and 4th lactation were selected from a herd of 230 cows kept on a farm in the Region of Warmia and Mazury (north-eastern Poland). Cows with similar expected calving dates (February – March) were selected for the experiment to eliminate the effect of calving season on the analyzed parameters. The cows were housed in free-stall barns. Cows were fed according to the cattle nutrition standards (IZPIB, 2009). A total mixed ration (TMR) was fed year-round. The TMR was composed of maize silage, haylage, alfalfa silage, and concentrate feed supplemented with protein concentrate, a vitamin-mineral premix, and feed additives that improve productivity and help balance complete diets (protein, rumen-protected fat, active yeast cultures and other energy supplements). Warmix Rumen, containing 6000 mg of zinc oxide per kg, was used as a premix. The daily dose was 250 g of the mixture per head until the 200th day of lactation and 200 g of the mixture per head for cows > 200 days of lactation. Milk samples were taken once a week, between lactation days 7 and 90, and then once a month until the 10th month of lactation. Fresh milk samples were analyzed to determine their chemical composition (content of crude protein, casein, fat, lactose, and dry matter) by infrared spectrophotometry using the MilkoScan FT 120 (FossElectric A/S, Hilleroed, Denmark), and SCC was determined by flow cytometry using the Bentley BactoCount instrument. 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 (ISO 8070:2007). 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 Zn in mineralized samples was determined in an atomic absorption spectrometer (AAS) with flame atomization (SpectrAA240FS, Candela, USA), with the use of hollow-cathode lamps (Varian). In order to evaluate udder health, milk samples were classified by the modified method of Sawa and Piwczyński (2003), as follows: ≤200 ths somatic cells per mL of milk – healthy udder; 201 - 400 ths somatic cells per mL of milk – risk of mastitis; 401 - 1 000 ths somatic cells per mL of milk – subclinical mastitis; >1 000 ths somatic cells per mL of milk – severe subclinical mastitis. None of the cows showed clear symptoms of clinical mastitis or was treated for mastitis.

Based on the amount of milk milked in the morning and evening, daily milk yield was determined for each cow, and it was converted to energy-corrected milk (ECM). ECM: milk with standardized energy content (Sjaunja et al. 1991).

$$\text{ECO (kg)} = \text{milk (kg)} \cdot \frac{(0.383 \cdot \text{fat (\%)} + 0.242 \cdot \text{protein (\%)} + 0.7832)}{3.140}$$

Based on the results of the herd's milk assessment, the productivity of cows in standard lactation (305 days) was determined, which was then converted into kg of ECM milk.

Data analysis

The results were analyzed statistically in the Statistica 13.3 program (2020). In order to obtain the normal distribution of variables, SCC was log-transformed according to the formula:

$$Y = L_n (x)$$

where x represents SCC determined in milk samples.

The least squares method was applied to determine whether the Zn content of milk was affected by cow's age (lactations 1-4) or SCC class (≤200 ths, 201 - 400 ths, 401 - 1 000 ths, and >1 000 ths). The following model of analysis of variance (ANOVA) was used:

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

where: Y_{ijk} – the value of the analyzed parameter, μ is population mean, A_i – the effect of cow's age (1-4), B_j – the effect of SCC class (1-4), $(AB)_{ij}$ – the cow's age x SCC class interaction, and e_{ijk} – random error.

The least squares method was also applied to determine whether the Zn content of milk was affected by daily milk yield (≤30, 30.1-45, >45) or the fat/protein ratio (<1.1, 1.1-1.5, >1.5). The following ANOVA model was used:

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

where: Y_{ijk} – the value of the analyzed parameter, μ is population mean, A_i – the effect of daily milk yield (1-3), B_j – the effect of the fat/protein ratio (1-3), $(AB)_{ij}$ – the daily milk yield x fat/protein ratio interaction, and e_{ijk} – random error.

The Pearson correlation coefficients were calculated to evaluate the relationships between the Zn content of milk vs. cow productivity and milk composition.

RESULTS AND DISCUSSION

The analyzed cows were characterized by an average 305-day lactation yield of 11 064 kg milk and an average daily yield of 39.8 kg milk (Table 1). The percentages of major milk components were typical of HO cows in Poland (Catle assessment and breeding, 2023). SCC ranged from 5 to 1 795 ths, with an average of 242 ths per mL of milk. The average Zn content of milk was

Table 1

Characteristics of cow's milk samples

Specification	Number of milk samples	Mean	Minimum	Maximum	Standard error of mean
SCC (ths mL^{-1})	605	242 445	5000	1 795 000	16089
SCC L_n	605	11.5	8.5	14.4	0.06
Daily milk yield (kg)	605	39.8	7.8	65.2	0.48
ECM yield during 305-day lactation (kg)	605	11 064	7026	15 467	85.82
Fat content (%)	605	4.3	2.0	8.5	0.05
Protein content (%)	605	3.3	1.3	4.4	0.02
Fat/protein ratio	605	1.3	0.6	3.7	0.02
Lactose content (%)	605	4.8	4.0	6.7	0.01
Dry matter content (%)	605	13.1	10.9	18.7	0.05
Zinc (mg dm^{-3})	605	4.6	2.2	9.1	0.05

SCC – somatic cell count, ECM – energy-corrected milk

4.6 mg·dm^{-3}, and it varied widely from 2.2 to 9.1 mg dm^{-3}, which indicates that it exceeded the natural upper limit for Zn concentration in milk of up to 6.0 mg dm^{-3}, determined by Knowles et al. (2006). A similar Zn content in milk was obtained in the study of Rodríguez-Rodríguez et al. (2001). However, Pérez-Carrera et al. (2016) obtained 2.3 mg dm^{-3}, which corresponded to half the content obtained in the present study. In the present study, cow's age, which was expressed as lactation number (LN), significantly affected the Zn content of milk (Table 2).

Milk from cows in their 4th lactation had the lowest (P<0.05) concentration of Zn. The Zn content of milk increased with increasing SCC (Table 2). The difference in the Zn content of milk between cows with SCC ≤200 ths per mL and SCC >1 000 ths mL^{-1} was 0.3 mg dm^{-3} (P<0.05). Olsson et al. (2001) demonstrated that Zn concentration in mammary tissue was positively correlated with the milk performance and age of dairy cows. However, such correlations were not observed in this study, where the Zn content of milk was negatively correlated with milk yield and cow's age (LN). In the work of Pechowá et al. (2008), the average Zn content of milk was 3.8 mg dm^{-3} and the average milk yield was 8 560 kg, compared with 4.6 mg dm^{-3} and 11 064 kg, respectively in the current experiment, which testifies to a minor effect of milk yield on Zn concentration. According to many authors, the relationship between cow productivity and the Zn content of milk results from higher Zn supplementation levels in large dairy cattle farms (Hosnedlová et al. 2005, Król et al. 2016, Libera et al. 2021).

The Zn content of milk was also significantly differentiated by daily milk yield (Table 3), and it was highest in milk from cows that produced

Table 2

Effect of lactation number and udder health on the zinc content of cow's milk

Specification	Lactation number (LN)				Somatic Cell Count (SCC), (ths mL^{-1})				SE	P-value		
	1	2	3	4	≤200	201-400	401-1000	>1000		LN	SCC	LN× SCC
Number of milk samples	152	152	152	149	361	127	78	39				
Zinc (mg dm^{-3})	4.62^{a}	4.51^{ab}	4.58^{a}	4.24^{b}	4.43^{b}	4.59^{b}	4.62^{ab}	4.73^{a}	0.048	0.037	0.046	0.691

Means followed by different letters (within the factor) are significantly different: *a*, *b* at $P<0.05$.

Table 3

Effect of daily milk yield and the fat/protein ratio on the zinc content of cow's milk

Specification	Daily milk yield (DMY, kg)			Fat/protein ratio (FPR)			SE	P-value		
	≤30	30.1-45	>45	<1.1	1.1-1.5	>1.5		DMY	FPR	DMY×FPR
Number of milk samples	156	271	178	139	295	171				
Zinc (mg dm^{-3})	4.69^{a}	4.76^{a}	4.32^{b}	4.35^{B}	4.58^{B}	5.02^{A}	0.047	0.023	0.000	0.040

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

30.1-45 kg of milk on the sampling day, and lowest in milk from the highest-yielding cows. The fat/protein ratio had a significant (P<0.01) influence on Zn levels in milk, which increased with increasing values of this ratio. An interaction between daily milk yield and the fat/protein ratio was noted for Zn concentration in milk (Figure 1).

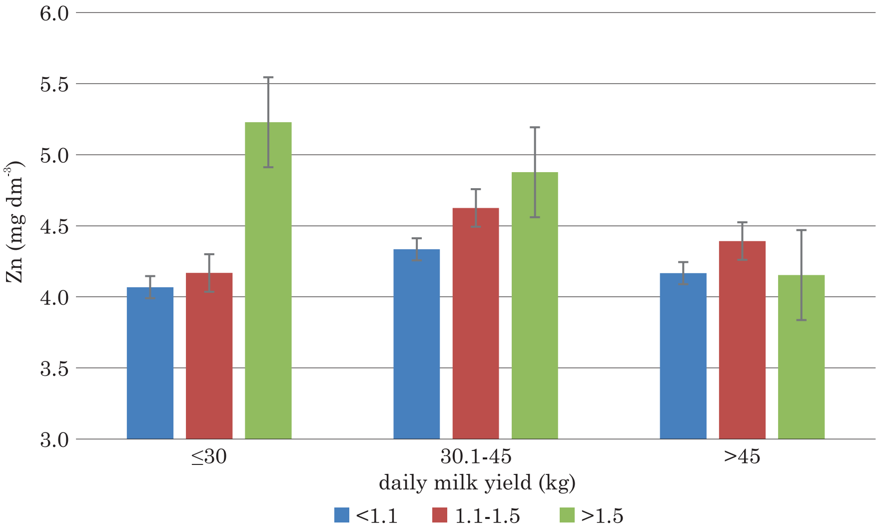

Fig. 1. The interaction between daily milk yield and the fat/protein ratio for the Zn content of cow's milk

The Zn content of milk was positively correlated (P<0.05) with milk fat content, SCC, and the fat/protein ratio (Table 4). Negative correlations were found between the Zn content of milk vs. daily milk yield, the content of lac-

Table 4

Correlation between zinc content and other parameters of cow's milk

Specification	Zinc (mg dm^{-3})
Daily milk yield (kg)	-0.15*
ECM yield during 305-day lactation (kg)	-0.11
Fat content (%)	0.20*
Protein content (%)	0.01
Fat/protein ratio	0.23*
Lactose content (%)	-0.14*
Dry matter content (%)	-0.14*
SCC L_n	0.16*
Lactation number	-0.36*

SCC – somatic cell count, ECM – energy-corrected milk, * P≤0.05

tose and dry matter in milk, and cow's age (LN), which confirms the differences presented in Tables 2 and 3. The correlation coefficient between Zn content and cow age (LN) was quite high, and the remaining values were lower, although significant.

Figure 2 illustrates changes in the Zn content of milk during lactation, taking into account 305-day ECM yield. Milk produced in the second week of lactation had the highest Zn content. The concentration of Zn in milk

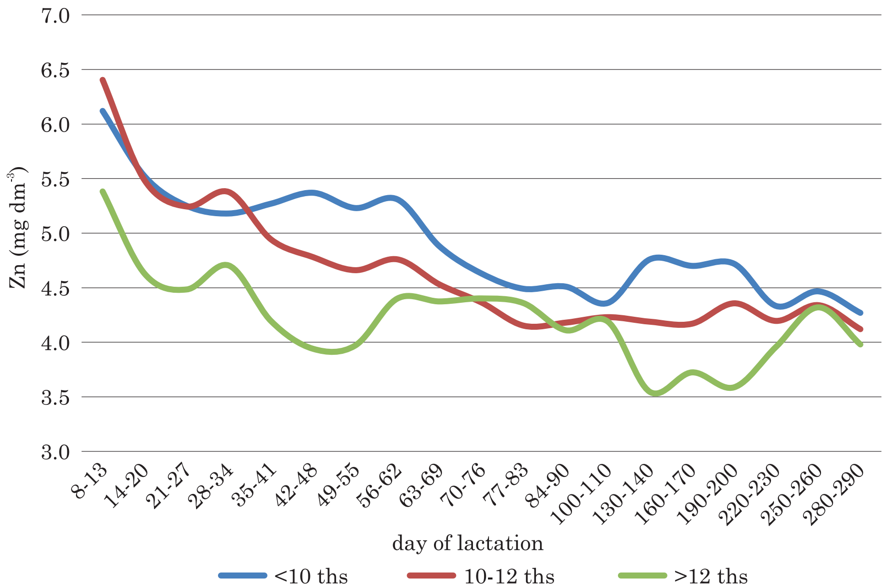

Fig. 2. Zinc content of milk on subsequent days of lactation depending on cow productivity (ECM) during 305-day lactation

decreased in subsequent weeks, and only minor fluctuations were observed. Between the first and last sampling, the average Zn content decreased by approximately 2 mg.

An analysis of milk performance revealed that milk from cows producing more than 12 000 L of milk during lactation had the lowest Zn content. The only exception were cows producing 10 000-12 000 L of milk, whose milk contained less Zn over a two-week period in the third month of lactation. The concentrations of micronutrients in milk during lactation were analyzed by Bedo et al. (1995), who found that the Zn content of milk tended to decrease during lactation. In the present study, a tendency to decreasing Zn concentration in milk with the progress of lactation was most pronounced in lower-producing cows (Figure 2). In milk from the highest-producing cows (>12 000 L of milk), Zn concentration was very low in the second month of lactation, i.e. during maximum milk production (peak lactation). According to Zadák (2002), the blood concentration of Zn can drop by up to 50% due to high stress, high burden placed on high-producing cows, or disease. Enjalbert et al. (2006) reported that low Zn levels in the blood serum and milk of cows led to the loss of appetite, reduced bioavailability and absorption of feed nutrients and, in consequence, lower milk performance. Denholm et al. (2022) found negative genetic correlations between Zn concentration in milk vs. milk, fat, and protein yields; serum Zn levels were also strongly negatively correlated with milk and fat yields. According to the cited authors, breeding strategies aimed at improving cow productivity have a negative impact on the levels of beneficial nutrients such as Zn in milk intended for human consumption. In the current study, a positive correlation was observed between SCC and the Zn content of milk. The findings of other authors are inconclusive. Some of them reported a slight decrease in Zn levels (Grigorian, Manasian 1982) in the milk of cows with clinical and subclinical mastitis, others (Kalorey et al. 2001) noted no effect of SCC, whereas Banga et al. (1989), Yildiz and Kaygusuzoglu (2005), and Nogalska et al. (2020) demonstrated that Zn content increased in milk from inflamed tissue.

The levels of trace nutrients in blood serum and milk are considered as robust bioindicators of both agricultural pollution and certain diseases in dairy cows (Zhang et al. 2010). Serum zinc concentration decreased in dairy cows with subclinical hypocalcemia, compared with healthy animals (Wang 2014). In a study by Zhang et al. (2010), serum Zn concentration was significantly decreased in subclinically ketotic dairy cows. The results of the present study suggest that Zn levels in milk can be useful in the diagnosis of subclinical mastitis, in addition to other methods. The fat/protein ratio, which is an indicator of energy balance in cows (Nogalski et al. 2015), was positively correlated with the Zn content of milk. When the energy balance is negative, cows mobilize their body fat reserves (postpartum lipolysis), which increases milk fat content and decreases milk protein content (Kessel et al. 2008). Thus, the fat/protein ratio is negatively correlated with energy balance in dairy cows, at least during early lactation (Buttchereit et al. 2011. A fat/protein ratio of >1.5 indicates an abnormally high lipolysis and has proven to be a good phenotypic predictor of ketosis and other metabolic disorders (Heuer et al. 1999). In the current study, negative energy balance was observed mostly at the early stage of lactation, when Zn levels in milk were highest. The interaction between daily milk yield and the fat/protein ratio for the Zn content of milk resulted from the fact that in the highest-producing cows (>45 kg of milk), Zn concentration in milk was highest at the fat/protein ratio of 1.1–1.5, and not >1.5, which was observed at lower daily milk yield

CONCLUSIONS

The concentration of Zn in milk was highest in the second week of lactation, and it decreased in subsequent weeks. Milk from the oldest cows (lactation 4) and the most productive cows had the lowest Zn content. The concentration of Zn in milk increased with increasing SCC. A high content of Zn in milk can be an additional indicator of mastitis in cows.

Author contributions

Z.N. – conceptualization, Z.N. – funding acquisition, Z.N. – investigation, Z.N. – methodology, Z.N., A.N.– visualization, Z.N, A.N. – writing, Z.N. – original draft preparation, Z.N., A.N. – writing – review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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