

Contents of Zn, Cu, Mn and Se in Milk in Relation to their Concentrations in Blood, Milk Yield and Stage of Lactation in Dairy Cattle

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Abstract

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The objective of the study was to assess the effect of actual daily milk production and lactation stage on concentrations of Zn, Mn, Cu and Se in milk, and monitor correlations between milk and blood concentrations of these microelements.

The study was performed in a herd of Holstein cattle with the average milk yield of 8,562 kg. Thirty-five dairy cows housed in one group were included in the study. Blood and milk samples were taken during two separate milk yield checks done 4 weeks apart. Actual milk production of monitored cows ranged from 19.6 to 62.6 l daily. For lactation stages we evaluated results of examinations performed from 7 to 188 days of lactation.

Blood examinations showed that the cows included in our study had good supplementation with the microelements in question. Milk concentrations of individual microelements were as follows: 3855.2 ± 814.7 µg/l of Zn; 36.3 ± 14.4 µg/l of Cu; 20.1 ± 8.3 µg/l of Mn, and 28.6 ± 7.1 µg/l of Se. The effect of daily milk production on milk concentrations of the microelements was identified only for copper ($r = -0.302, p \leq 0.05$). The variable of days of lactation (not considering days of the colostrum period) showed a positive correlation in manganese ($r = 0.419, p \leq 0.01$); copper and selenium showed negative correlations (Cu: $r = -0.258, p \leq 0.05$; Se: $r = -0.277, p \leq 0.05$). The daily milk production influenced negatively only Cu concentration in milk, but Se, Zn and Mn was not influenced. With advancing lactation after colostrum period the concentration of Mn in milk raised, the concentration of Cu and Se declined and the concentration of Zn was unchanged.

Cows, microelements, blood plasma

Today, selenium, copper, zinc, manganese and iodine are the most often analyzed microelements. Although veterinary studies focus mainly on their effect on the health of farm animals, increasing emphasis has been lately put also on their impact on the quality of food. Moreover, dietary supplementation with microelements enhances the nutritive value of food. Cows' milk concentration of zinc ranges from 2–6 mg/l, copper 0.1–0.6 mg/l, manganese 20–50 µg/l and selenium 2–60 µg/l (Knowles et al. 2006). Comparison of these values with the daily requirements of 4- to 6-month-old children (i.e., 5 mg of Zn; 0.4–0.6 mg of Cu; 0.01 mg of Se and 0.3–0.6 mg of Mn [Milner 1990]) shows that milk might be an important source of these micronutrients. The scope for increasing concentration of micronutrients in milk is limited by the complex and co-coordinated biochemical mechanisms of animal homeostasis. The transition element cations (Cu, Mn, Zn) have concentrations in blood, tissues and milk that are largely independent of the intake, as they relate to regulation of gut absorption and changing metabolic demands (Windisch 2002). In contrast, small anion such as Se is readily transported across membranes of the digestive tract. For this reason, selenium concentration in milk is greatly influenced by its dietary intake. Knowles et al. (2006) summarize the results of some reports of research in

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dairy cows concerning the responsiveness of milk to dietary supplementation as follows: $Se \approx I \gg Zn \approx Cu > Ca \approx Fe \approx Mn$.

The issue of micronutrients contained in milk is generally neglected and the factors affecting their concentrations are not known. Although many authors confirmed the variability of milk concentration of individual microelements, specific factors have not been satisfactorily identified. The majority of experiments have focused on selenium; less attention has been paid to zinc and copper. Milk concentration of manganese has been studied rarely.

The aim of our study was therefore to assess the effect of actual daily milk production and lactation stage on concentrations of Zn, Mn, Cu and Se in milk, and monitor the correlations between milk and blood concentrations of these microelements.

Materials and Methods

The study was performed at a farm with 161 Holstein cows located in Northern Bohemia. The average milk production of this herd was 8,562 kg of milk per lactation, with 3.61% of fat (310 kg of fat per lactation) and 3.17% of protein (272 kg of protein per lactation) on average. Dairy cows were divided into groups and kept in loose housing. Thirty-five dairy cows housed in one group were included in our study. Blood and milk samples were taken during two separate milk production checks. During the study, the animals were fed the same total mixed ration. The content of individual microelements per 1 kg of dry matter was as follows: 105 mg/kg of Zn, 17 mg/kg of Cu, 66 mg/kg of Mn and 0.4 mg/kg of Se. Nutrient composition of this feed ration is specified in Table 1. Average consumption of dry matter (DM) was 20.5 kg. The cows included in the study were in the first half of their lactation; their average milk production was 36.61 daily. Mixed milk samples from morning milking were taken from each dairy cow. The samples were put in plastic containers and analyzed for the microelement content. On the same day, blood samples from vena/arteria coccygea were taken using Hemos sampling kits. Coagulable blood samples for serum and non-coagulable blood samples with lithium heparinate were collected. After precipitation and centrifugation, blood serum and whole blood were kept frozen until they were analyzed in the laboratory.

Table 1. The composition of daily feed ration (650 kg, milk yield 35 l)

Dry matter	g	23190
Metabolisable energy	Mcal	39.32
Crude protein	g	4 300
Acid detergent fibre	g	4 500
Neutral detergent fibre	g	7 600
Starch	g	4 300
Fat	g	1 300
Ash	g	1 420
Copper	mg	394
Manganese	mg	1530
Zinc	mg	2435
Selenium	mg	9.28

Blood serum concentrations of Zn and Cu were identified by atomic absorption spectrophotometry using Solaar M6 appliance (Unicam, Great Britain). Before measuring, serum samples were deproteinized by supplementing trichloroacetic acid at a 1 : 1 ratio. After centrifugation, we measured the content of Zn and Cu in the supernatant.

In order to measure the concentration of Mn in whole blood and Zn, Cu and Mn in milk, we had to mineralize the samples via wet-processing treatment with the supplement of hydrogen peroxide and nitric acid (2 ml of milk [blood] + 1 ml H₂O₂ + 2 ml HNO₃). We applied the microwave digestion technique using microwave ashing system MLS-1200 (Milestone, Italy). Zn was identified in the mineralisate by flame atomic absorption spectrophotometry using Solaar M6 (Unicam, Great Britain). Cu and Mn contents were measured via electrothermic atomic absorption spectrophotometry using Solaar 939 (Unicam, Great Britain).

Milk and whole blood concentration of Se was determined after mineralization of samples by hydride technique using Solaar M6 (Unicam, Great Britain) with electrothermically heated cuvette as per methodology designed by Pechová et al. (2005). All analyses were performed by the laboratory of the Clinic of Ruminant Diseases of the University of Veterinary and Pharmaceutical Sciences Brno.

Each set of samples was subjected to basic statistical evaluations (mean, standard deviation, median, quartiles, maximum, and minimum). Correlations between milk production, lactation stage, and concentration of individual microelements in blood and milk were identified by correlation analysis. The assessment was carried out using Microsoft Windows^{XP} Excel software.

Results and Discussion

The effect of actual daily milk production and lactation stage (days of lactation) on the concentration of microelements in milk was evaluated (a total of 70 data for each

element). The actual daily milk production of monitored cows ranged from 19.6 to 62.61. The average daily milk production was 36.61. For lactation stages we evaluated the results of examinations performed from 7 to 188 days after parturition (105.4 days at the average). A rather wide dispersion of milk production and lactation stage indicators was chosen intentionally in order to acquire the broadest possible spectrum at the same feed ration.

Results of blood examinations showed a good supplementation with microelements in the cows under study. The concentration of Cu in blood serum of animals was $826.75 \pm 138.08 \mu\text{g/l}$ ($13.0 \pm 2.17 \mu\text{mol/l}$), and no dairy cow showed values lower than $572 \mu\text{g/l}$ (Underwood and Suttle 1999), which is the threshold indicating satisfactory copper supplementation. Selenium contents were very good too, as no cow showed values lower than $100 \mu\text{g/l}$ (Pavlata et al. 2000). Blood concentration of Se was $183.75 \pm 29.63 \mu\text{g/l}$ ($2.33 \pm 0.38 \mu\text{mol/l}$). Blood serum concentration of Zn was $1063.92 \pm 181.13 \mu\text{g/l}$ ($16.27 \pm 2.77 \mu\text{mol/l}$). Only two samples showed slightly decreased Zn concentrations that dropped below $600 \mu\text{g/l}$ (Underwood and Suttle 1999). This was probably due to certain individual specifications or redistribution of Zn in the organism. Blood concentration of Zn can drop by up to 50% due to high stress, trauma or inflammatory processes (Zadák 2002).

The average concentration of Mn in blood was $14.03 \pm 3.24 \mu\text{g/l}$ ($0.26 \pm 0.06 \mu\text{mol/l}$), which is lower than the reference range for cattle (20–70 $\mu\text{g/l}$) reported by Underwood and Suttle (1999). However, Underwood's concentrations are relatively high and we noted lower values. For example, Gehrke and Lachowski (1997) found that the concentration of Mn in lactating cows ranged from 1.55 to 4.85 $\mu\text{g/l}$ in blood serum and from 5.10 to 12.35 $\mu\text{g/l}$ in blood. These values are comparable with our findings. According to Gelfert and Staufenbiel (2000), the values for diagnosing the deficiency disease reported in various studies (6 $\mu\text{g/l}$ in blood serum) are too high, as the screening carried out at 70 farms in Germany showed lower values, which would, in fact, point to Mn deficiency disease. Considering the rather high content of Mn in the feed ration of cows included in our study, we do not believe they suffered from the Mn deficiency disease. Hansen et al. (2006) reported that 15.8 mg of Mn per 1 kg of dry matter (DM) is sufficient. In fact, this value is several-fold lower than the concentrations in our study. Results of various studies indicate that their authors have different views on the role of blood concentration of Mn as indicator of overall Mn supplementation. Legleiter et al. (2005) suggest that serum concentration of Mn does not reflect the level of Mn supplementation. The distribution of the concentrations of monitored microelements in blood and blood serum are specified in Fig. 1, in milk in Fig. 2 and Fig. 3.

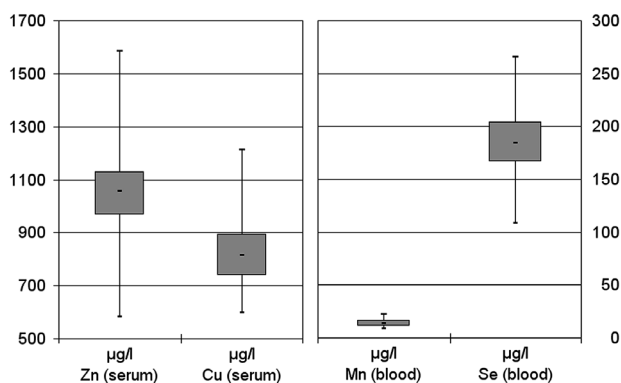


Fig. 1. Distribution (maximum, quartile 75%, median, quartile 25%, minimum) of the concentration of microelements in serum/blood of dairy cows (n = 70)

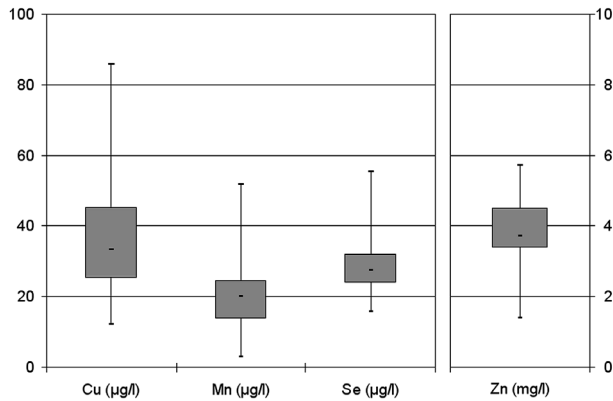


Fig. 2. Distribution (maximum, quartile 75%, median, quartile 25%, minimum) of the concentration of microelements in milk of dairy cows (n = 70)

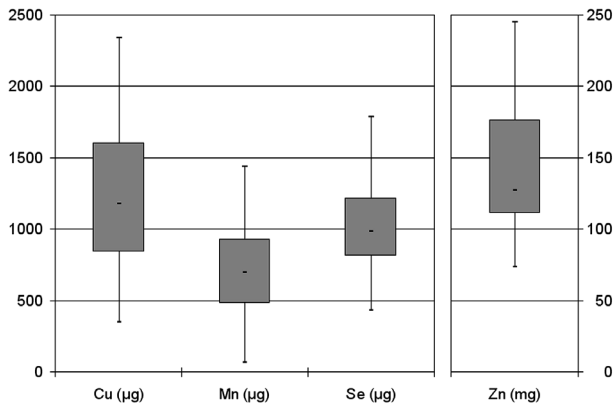


Fig. 3. Distribution (maximum, quartile 75%, median, quartile 25%, minimum) of the total amounts of individual microelements excreted in milk in 1 day (n = 70)

Table 2. Comparison of the concentration of microelements in raw cow milk based on literature

Zn in milk (µg/l)	Cu in milk (µg/l)	Mn in milk (µg/l)	Se in milk (µg/l)	Reference
3855.2 ± 814.7	36.25 ± 14.36	20.07 ± 8.26	28.59 ± 7.12	This work
3750.8 ± 689.21				Pechová et al. (2006)
3960.0 ± 149.1	52.0 ± 5.0	21.0		Anderson (1992)
4409.2 ± 667.0	76.0 ± 32.0		16.44 ± 4.41	Rodriguez et al. (2001)
3419.2 ± 18.96	95.0 ± 70.0			Martin-Hernandez et al. (1992)
3699.8 ± 219.7	160.0 ± 20.0			Zurera et al. (1994)
4209.2 ± 392.3	155.0 ± 35.0			Rondon et al. (1994)
4209.8 ± 410.0	16.0 ± 3.0			Moreno et al. (1993)
4400 ± 300	100 ± 10		25.9 ± 5.4	Benemariya et al. (1993)
3290 ± 210	160 ± 30	56 ± 9		Rojas et al. (1994)
3900	130	22	40–1270	Abollino et al. (1998)
2016	1.98		13.24	Licata et al. (2004)
3950 ± 380	70 ± 16	30 ± 7		O'Brien et al. (1999)
1978–10890	15–178	1.09–49.1	0.05–223	Hermansen et al. (2005)

Our results are comparable with values identified by other authors. Table 2 presents a comparison of our values with concentrations established in other studies.

The zinc concentration in milk is higher than its blood serum concentration. In our study it was 3855.2 ± 814.7 $\mu\text{g/l}$ and the total volume of Zn discharged through milk was 142.87 ± 42.42 mg. Only a few studies on the content of Zn in cow's milk have been published to date, and the factors affecting its concentration in milk have not been described thoroughly. In humans, 0.5–1.0 mg of Zn per day is transported through the mammary gland into milk (King 2002) and Zn transportation into milk is hypothesized to be an active process (Kelleher and Lonnerdal 2003). Zinc in cow's milk primarily binds to casein and, to a small extent, citrate. Almost 90% of Zn binds to casein in mature milk, in contrast to just 60% in the colostrum (Kincaid and Cronrath 1992). In casein, Zn binds primarily to colloid calcium phosphate in casein micelles (Silva et al. 2001).

Copper concentration in cow's milk is rather low and, unlike zinc, its blood serum concentration is several-fold higher. In our group, it was 36.25 ± 14.36 $\mu\text{g/l}$ and a total volume of Cu discharged through milk was 1246.67 ± 472.13 μg . Abollino et al. (1998) used speciation analysis to study distribution of Cu in cow's milk. Their results show that 53.7% of copper binds to casein and 40.8% of Cu is in cationic fraction, but no copper was identified in anionic fraction. No other fractions were specified in that study. It should be noted that 44.7% of copper in milk is in a soluble form.

Only a few data on the milk concentration of Mn have been reported. Milk Mn concentration is rather low but comparable with its concentration in blood. In our group, milk Mn concentration was 20.07 ± 8.26 $\mu\text{g/l}$ and a total volume of Mn discharged through milk was 716.83 ± 316.68 μg . Distribution of Mn in cow's milk was studied by Abollino et al. (1998) who used speciation analysis. Their results show that only 10.4% of Mn binds to casein, 47.7% is in cationic fraction and 10.8% was identified in anionic fraction. Abollino et al. specified no other fractions. Out of a total amount of Mn in milk, 84.3% is in a soluble form.

The concentration of selenium in milk is lower compared to Se concentration in whole blood. In our group, milk Se concentration was 28.59 ± 7.12 $\mu\text{g/l}$ and a total volume of Se discharged through milk was 1007.15 ± 280.45 μg . Michalke (2006) used speciation analysis to study individual fractions of Se in human milk. The selenium concentrations per species were determined in the low molecular weight (LMW) range as 2.5 ± 0.2 $\mu\text{g/l}$ (Se-carrying glutathione), 3.1 ± 0.3 $\mu\text{g/l}$ (Se-cystamine), 5.2 ± 0.4 $\mu\text{g/l}$ (Se-cystine) and 1.1 ± 0.1 $\mu\text{g/l}$ (Se-methionine). It is known that milk Se concentration varies depending on the geographic location and natural Se content in soil. Selenium is introduced into the food chain by plants that absorb inorganic selenium salts from the soil and convert them into organic forms of the element (mainly as selenomethionine); these are then incorporated into proteins. The concentration of selenium in plants varies widely and depends on the selenium content and characteristics of the soil. It has been found that supplementation of cattle's feed with organic forms of Se (as selenomethionine) increases Se concentration in milk (Knowles et al. 2006; Muniz-Naveiro et al. 2006; Givens et al. 2004; Heard et al. 2004; Juniper et al. 2006). We studied supplementation of different forms of Se in dairy goats, comparing sodium selenite, selenium proteinate, selenium yeast and lactate-protein complex. Pechová et al. (2008ab) found higher Se concentration in milk only after supplementation of selenium yeast, containing 63% Se as selenomethionine. Another possibility of increasing milk Se concentration is to apply inorganic selenium forms parenterally (Grace et al. 1997).

Correlations between concentrations of individual microelements in milk and other factors were evaluated using correlation analysis of the acquired data (Table 3). We did not find any significant correlations between milk and blood concentrations of the microelements. These results could be to some extent influenced by the fact that supplementation of dairy

Table 3. Correlation coefficients between the concentration of individual microelements in serum/blood, daily milk yield, day of lactation and concentration of microelements in milk

Correlation coefficients	Concentration in milk			
	Zn	Cu	Mn	Se
Daily milk yield	-0.221	-0.302*	0.097	-0.205
Day of lactation	0.153	-0.258*	0.419**	-0.277*
Concentration of microelements in serum/blood	-0.145	-0.185	-0.096	0.181

* $p < 0.05$ ** $p < 0.01$

cows with Zn, Cu, Mn and Se was good. Final verification of these correlations would require more extensive monitoring that would involve dairy cows from different herds, including herds showing signs of the deficiency disease. Wasowicz et al. (2001) found a significant linear correlation between concentrations of Zn in blood plasma and milk in the first stage of lactation and weak, but also significant linear correlations between Se concentration in the plasma and milk of women.

Similarly, we did not find a correlation between actual milk production and concentration of individual microelements in milk. Only the correlation between milk Cu concentration and daily milk production was significant ($r = -0.302$, $p \leq 0.05$). Negative correlation coefficients for Cu, Zn and Se indicate a possible effect of the supplementation level on milk concentration of these microelements: high milk production means that a total intake of microelements recalculated per 1 litre of milk is lower. Although all dairy cows were fed a feed ration with the same concentration of nutrients, there could be individual differences in the total intake of dry matter and thus also in the total intake of microelements. From this perspective, the results indicate a link to the intake of microelements through the feed ration. It is likely that more significant correlations concerning supplementation would be found during marginal or insufficient supplementation of dairy cows with microelements.

Assessment of the effect of the lactation stage on the concentration of microelements was limited to days of lactation 7 to 188, i.e. mature milk, as cows were not fed the same feed ration during the first week after parturition. Also, colostrum concentration of microelements was not the subject of this study. Some studies proved that concentrations of microelements are generally higher in colostrum than mature milk (Pavlata et al. 2004; Vaillancourt and Allen 1991). A significant correlation was found in milk Mn concentration that was increasing with days of lactation ($r = 0.419$, $p \leq 0.01$). On the contrary, negative correlations were found in selenium and copper (Cu: $r = -0.258$, $p \leq 0.05$; Se: $r = -0.277$, $p \leq 0.05$). These correlations could be caused by depletion of Se and Cu body reserves with days of lactation (they are gradually lost through milk). There was no significant correlation between milk Zn concentration and days of lactation. As for the level of supplementation evaluated according to blood Zn concentration, the highest concentration of Zn in the feed ration was 105 mg/kg of dry matter, which is almost twice as much as the recommended concentration. The unexpected tendency could be therefore caused by this high concentration of Zn in the feed ration. The concentration of microelements in milk during lactation was studied by Bedo et al. (1995). Their results show that the concentrations of Zn and Cu tended to decrease, while Mn showed non-significant variation during lactation.

Rodriguez et al. (2001) studied the effect of seasons on the concentration of microelements in pool milk samples. Their results show that Se concentration decreased from March to September, thus Se concentrations in cow's milk during winter and spring were significantly higher than those during summer and autumn. The content of Cu remained approximately constant during the year and the Zn concentration varied significantly, being lower in autumn than during winter and spring. On the other hand, O'Brien et al. (1999) found that the Cu content in milk was higher during the indoor period (81.5 µg/l) than

during the outdoor grazing period (55.1 $\mu\text{g/l}$) and that Zn and Mn concentration in milk showed little variation over the year. In fact, these seasonal differences were probably caused by different contents of microelements in the feed ration.

In the herd showing sufficient supplementation with individual microelements, no significant correlations between concentrations of the microelements in milk and blood were found. The daily milk production influenced negatively only Cu concentration in milk, but Se, Zn and Mn was not influenced by its. During lactation after colostrum period the concentration of Mn in milk raised, the concentration of Cu and Se declined and the concentration of Zn was unchanged.

Sledování obsahu Zn, Cu, Mn a Se v mléce ve vztahu k jejich koncentraci v krvi, nádoji a fázi laktace u dojnic

Cílem této práce bylo zhodnocení vlivu aktuálního nádoje a fáze laktace na koncentrace Zn, Mn, Cu a Se v mléce a sledování vztahů mezi koncentrací stopových prvků v mléce a v krvi.

Sledování bylo realizováno v chovu holštýnského skotu s průměrnou dojivostí 8 562 kg mléka za normovanou laktaci. Do sledování bylo zařazeno 35 dojnic ustájených v jedné skupině, u kterých byl proveden odběr krve a mléka při dvou po sobě jdoucích kontrolách užítkovosti s odstupem 4 týdny. Aktuální denní dojivost se u dojnic zařazených do sledování pohybovala od 19,6 až do 62,6 l. Z hlediska fáze laktace byly do hodnocení zařazeny výsledky vyšetření od 7 do 188 dní po porodu. Koncentrace jednotlivých prvků v krvi svědčila o dostatečném zásobení dojnic jednotlivými stopovými prvky. V krevním séru byla zjištěna koncentrace Zn 1063,9 \pm 181,1 $\mu\text{g/l}$ a koncentrace Cu 826,8 \pm 138,1 $\mu\text{g/l}$. V plné krvi byla stanovena koncentrace Mn 14,0 \pm 3,2 $\mu\text{g/l}$ a Se 183,8 \pm 29,6 $\mu\text{g/l}$. Koncentrace jednotlivých stopových prvků v mléce byla následující: Zn 3855,2 \pm 814,7 $\mu\text{g/l}$; Cu 36,3 \pm 14,4 $\mu\text{g/l}$; Mn 20,1 \pm 8,3 $\mu\text{g/l}$; Se 28,6 \pm 7,1 $\mu\text{g/l}$.

V chovu s dostatečnou suplementací jednotlivých stopových prvků jsme nenalezli signifikantní korelační vztahy mezi koncentrací stopových prvků v mléce a v krvi. Korelační vztahy mezi aktuálním nádojem a koncentrací sledovaných mikroprvků v mléce nebyly jednoznačně potvrzeny. Pouze mezi koncentrací mědi v mléce a denní dojivostí byl významný korelační vztah Cu: $r = -0,302$, $p \leq 0,05$). Mezi dnem laktace a koncentrací stopových prvků v mléce byl zjištěn pozitivní korelační vztah u Mn ($r = 0,419$, $p \leq 0,01$) a naopak negativní korelační vztahy byly zjištěny u Cu ($r = -0,258$, $p \leq 0,05$; Se: $r = -0,277$, $p \leq 0,05$). Výše denního nádoje ovlivnila negativně pouze koncentraci Cu v mléce, koncentrace Se, Zn a Mn nebyla výši nádoje ovlivněna. S postupující laktací se koncentrace Mn v mléce zvyšovala, koncentrace Cu a Se naopak mírně klesala a koncentrace Zn v mléce se neměnila.

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