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CONTENT OF SELECTED ESSENTIAL AND POTENTIALLY TOXIC TRACE ELEMENTS IN MILK OF COWS MAINTAINED IN EASTERN POLAND

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Abstract

Concentration of toxic metals in milk, especially in industrial regions, may serve as a direct bioindicator of the quality of milk and its products. But it can also function as an indirect indicator of contamination of the environment where milk is produced. The aim of the present study was to evaluate the content of some trace elements, i.e. Fe, Cu, Zn and Mn, as well as toxic elements (Cd and Pb) in milk from cows maintained on farms in three regions of Poland, including the production season as another aspect of the investigations. In total, 446 milk samples were analyzed. Significant influence of the season and production region on all the analyzed elements except Fe was noticed. Milk collected in the Biebrza and Bieszczady regions proved to be a richer source of Cu and Mn. Regardless of the production region, a higher content of Zn and Cu were observed in the summer, while more Mn was found in the winter milk. In none of the analyzed milk samples, Pb or Cd exceeded the permissible threshold.

Key words: bovine milk, trace elements, season, Eastern Poland.

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ZAWARTOŚĆ ISTOTNYCH I POTENCJALNIE TOKSYCZNYCH WYBRANYCH PIERWIASTKÓW ŚLADOWYCH W MLEKU KRÓW UTRZYMYWANYCH NA TERENIE WSCHODNIEJ POLSKI

Abstrakt

Stężenie metali ciężkich w mleku, przede wszystkim w rejonach uprzemysłowionych, jest bezpośrednim bioindykatorem jakości mleka i jego przetworów, a także wskaźnikiem pośrednim, wskazującym na stopień zanieczyszczenia środowiska, w którym produkuje się mleko. Celem pracy była ocena zawartości wybranych pierwiastków śladowych, tj. Fe, Cu, Zn i Mn, oraz toksycznych (Cd i Pb), w mleku krów utrzymywanych w gospodarstwach wschodniej Polski, z uwzględnieniem sezonu produkcji. Łącznie badaniami objęto 446 próbek. Stwierdzono istotny wpływ sezonu i regionu produkcji na zawartość analizowanych pierwiastków, z wyjątkiem Fe. Mleko pobierane w regionie Biebrzy i Bieszczad okazało się cennym źródłem Cu i Mn. Niezależnie od regionu produkcji, wyższą zawartość Zn i Cu stwierdzono w mleku pozyskiwanym w sezonie letnim, natomiast Mn w okresie zimowym. We wszystkich analizowanych próbkach mleka stężenie ołowiu i kadmu było stosunkowo niskie i mieściło się w dopuszczalnych granicach.

Słowa kluczowe: mleko krowie, pierwiastki śladowe, sezon, wschodnia Polska.

INTRODUCTION

Essential metals, beside basic nutrients, have a vital role in the functioning of a human organism. They participate in numerous metabolic processes and regulate biochemical reactions; they appear as activators or components of some specific enzymes. Fe, Cu, Zn and Mn play a crucial role in protection of a human body against the negative effect of toxic free radicals (Cashman 2006, Scherz, Kirchoff 2006). But bovine milk also contains heavy metals, which reduce its nutritional value and pose a hazard to the human health. Pb and Cd prove to be the most widespread environmental toxicants found in milk (Licata et al. 2004).

The content of essential micronutrients and trace elements in milk depends mainly on external factors, i.e. given content of elements element content in soil, feed and water as well as its assimilability. To a lesser extent, concentration of mineral components is conditioned by factors related to secretion of milk, i.e. a cow's breed, stage of lactation, animal health (Cashman 2006, Dobrzański et al. 2005, Gabryszuk et al. 2010, Malbe et al. 2010, Sola-Larrańaga, Navarro-Blasco 2009, Vidovic et al. 2005). Another important factor influencing the occurrence of trace elements, including toxic ones, is mainly of anthropogenic origin (Dobrzański et al. 2005, Licata et al. 2004). Some authors also point to the influence of a season and production region on the content of these elements (Dobrzański et al. 2009, Elsayed et al. 2011, Li-Qiang et al. 2009, Patra et al. 2008, Sola-Larrańaga, Navarro-Blasco 2009).

Milk and dairy products are staple components of a daily diet of contemporary consumers, especially children.Thus, it is crucial to monitor regularly milk quality, paying special attention toxic metals. Theirs concentration in milk, especially in industrial regions, may serve as a direct bioindicator of the quality milk and its products, but cal also be an indirect indicator of contamination in the environment where milk is produced (LICATA et al. 2004, VIDOVIC et al. 2005).

The aim of the present study was to evaluate the concentration of some trace elements (essential and potentially toxic ones) in milk of cows maintained on farms located in three regions of Eastern Poland, i.e. the Biebrza River region (a buffer zone of Biebrza National Park), the Bieszczady Mountains and the Lublin region. Additionally, two production seasons were taken into consideration.

MATERIAL AND METHODS

Milk samples were collected individually from each cow (daily milk) during the summer (June-August) and winter (December-February). The research included a total of 446 milk samples (Biebrza – 134, Bieszczady – 163 and Lublin – 149) obtained from three areas in Poland (Figure 1). The Biebrza and Bieszczady regions are considered highly valuable nature areas and very attractive tourist destinations. They are unique in that the human interference with nature is almost negligible. Extensive milk production dominates in the farming practice in those regions, owing to large areas of available grasslands. In the Biebrza region the main cattle breeds are the Whiteback and Black-and-White cows, but in the Bieszczady Mountains the Simmental cows are reared. The nutrition of cows in spring relies mainly was on seasonal pasture forage comprising grasses and legumes supplemented with hay or straw. During the winter season, the animals were fed hay sillage, fodder beet with hay or straw additives. Milk samples from the Lublin region were obtained from intensive milk production farms maintaining the Simmental and Black and White cows. All year, the animals were given maize silage and hay silage, while in the summer their diet was supplemented with forage. On all the farms, dietary rations were enriched with nutritive fodder.

All analyses were performed in the Agroecological Central Laboratory of the University of Life Sciences in Lublin. All reagents were of analytical grade, with 65% HNO_3 and 70% $HClO_4$ of suprapure quality (POCh, Poland). The samples were wet-mineralized (Kjeldahl Flask Digestion) according to the official procedure AOAC 986.15 (AOAC 2000). The content of microelements (Cu, Fe, Zn, Mn) was determined with the flame atomic absorption spectrometry technique using a SOLAR 939 spectrometer (Uni-

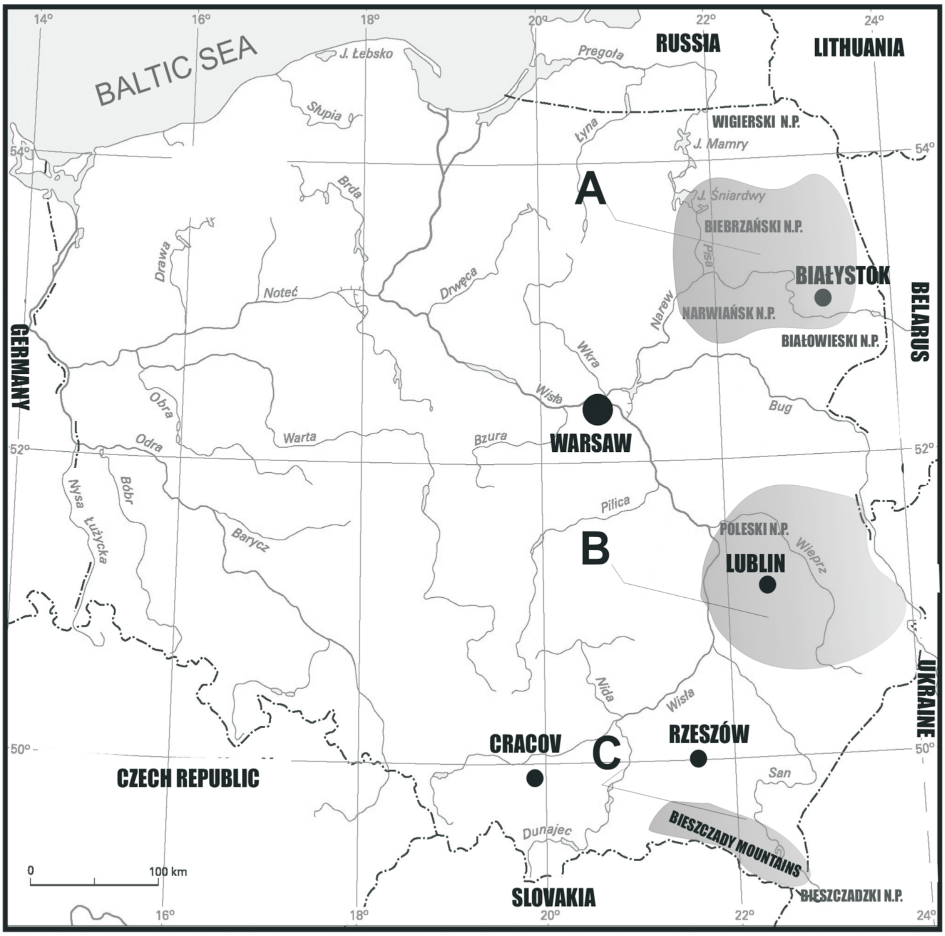

Fig. 1. Location of the research regions in Poland:
A – Biebrza River region, B – Lublin region, C – Bieszczady Mountains

cam). The content of Pb and Cd was established using the flameless atomic absorption spectrometry method in a SpectrAA 88OZ (Varian) spectrometer.

The results were analyzed statistically with the StatSoft Inc. Statistica ver.6 software (Statsoft INC. 2003) based on a two-factor analysis of variance, achieving mean values and standard deviation. Significance of differences between mean values was determined with Tukey's HSD test.

RESULTS AND DISCUSSION

The results indicate significant differences between the seasons in the content of Zn, Cu and Mn in milk collected in the three regions. Higher concentrations of Zn and Cu, regardless of the production region, were noticed in the summer, and the content of Mn was higher in the winter season (Table 1). Differences between the seasons (in favor of the summer) for the Zn content ranged from 0.426 mg L^{-1} to 0.753 mg L^{-1}, and for Cu – from 0.008 mg L^{-1} to 0.030 mg L^{-1}. Milk produced in the Bieszczady Mountains in the winter was characterized by twice as much Mn (0.132 mg L^{-1}) as in the summer season (0.066 mg L^{-1}). Seasonal differences in the Mn content did not occur in the other regions. Other authors also report seasonal variation in concentrations of the analyzed elements (ELSAYED et al. 2011, GUSTAFSON et al. 2007, RODRIGUEZ et al. 2001, SOLA-LARRAÑAGA, NAVARRO-BLASCO 2009). Like in our study, higher levels of the elements, especially Cu and Zn, were found in milk obtained in the summer, i.e. from cows fed mainly pasture forage. A contrary result, i.e. higher concentrations of Cu and Zn in milk in winter, was obtained GÓRSKA and OPRZĄDEK (2006). The level of Zn ranged from 3.2 (in summer) to 3.7 mg L^{-1} (in winter), and Cu – from 0.048 to 0.050 mg L^{-1}, respectively. In another study conducted in Silesia by DOBRZAŃSKI et al. (2005), milk of cows kept on pasture was characterized by more Cu (0.065-0.089 mg L^{-1}) and less Zn (3.085-3.164 mg L^{-1}) than in our investigations. The concentration of Mn, however, ranged from 0.051 mg L^{-1} in Upper Silesia to 0.102 mg L^{-1} in Lower Silesia.

In both analyzed seasons, a region influence on the content of mentioned microelements was also stated. In the winter season milk from Lublin region was characterized by the highest Zn content (4.764 mg L^{-1}) at the lowest Cu content (0.032 mg L^{-1}) and Mn (0.039 mg L^{-1}). However, milk collected in the Biebrza region contained over twice, and in the Bieszczady over three times more of Mn, respectively: 0.084 mg L^{-1} and 0.132 mg L^{-1}. In the case of Cu level, the differences between regions were much lower. In all regions in the summer season Zn and Cu content changes were similar to the changes in the winter. Concentration of Zn ranged from 4.312 mg L^{-1} (Biebrza region) to 5.341 mg L^{-1} (Lublin region), and Cu from 0.040 mg L^{-1} (Lublin region) to 0.078 mg L^{-1} (Biebrza region). The differences in concentration of these elements in milk mainly may be due to their diverse content in a feed (DOBRZAŃSKI et al. 2005, GABRYSZUK et al. 2010, MALBE et al. 2010, SCHERZ, KIRCHOFF 2006). MALBE et al. (2010) shows the effect of balancing a ration for animals kept in conventional and organic farms on the content of elements in milk. Low Cu concentration in the milk of cows kept in the Lublin region may indicate a high level of Zn in the ration. According to many authors (ARANCIBIA et al. 2006, LICATA et al. 2004), Zn taken from a feed, interacting with the copper absorption system, reduces the Cu bioavailability. HOSNEDLOVA et al. (2005) point at a gradual increase

Table 1

Content of trace elements ($\overline{x}$±SD) in milk of cows maintained in three regions of Eastern Poland

Microelement content (mg L^{-1})	Biebrza region		Bieszczady region		Lublin region		Factor		
	winter season	summer season	winter season	summer season	winter season	summer season	region	season	season x region interaction
No.	66	68	76	87	77	72			
Zn	3.559±1.785^a	4.312±1.896^b	4.416±1.612	4.842±1.822	4.764±1.864^a	5.341±2.413^b	*x*	*x*	*x*
Cu	0.048±0.013^a	0.078±0.028^b	0.039±0.007	0.048±0.019	0.032±0.011	0.040±0.017	*x*	*x*	*xx*
Fe	0.264±0.087	0.337±0.101	0.306±0.070	0.377±0.059	0.188±0.051	0.252±0.062	ns	ns	ns
Mn	0.084±0.029	0.085±0.052	0.132±0.056^B	0.066±0.028^A	0.039±0.013	0.030±0.015	*xx*	*xx*	*xx*

No. – number of cows
a, b, A, B – differences between seasons, *a, b* – differences significant at $P \le 0.05$
A, B – differences significant at $P \le 0.01$, *x* – differences significant at $P \le 0.05$, *xx* – differences significant at $P \le 0.01$, ns – not stated significant

of Zn concentration in milk, at a simultaneously Cu level decrease and an increase of cows' milk yield. In SIMSEK et al. (2000) opinion, a content of Cu is largely conditioned by an environment influence. This element high concentration (0.77-1.20 mg kg^{-1}), the authors noted in the milk from industrialized areas of Turkey. This is contrary to the results of own studies, in which showed higher levels of this element in the regions subjected to a small degree of human pressure, such as the Bieszczady and Biebrza regions.

The Fe content in milk was not found to have been affected by a region or production season, although the highest concentrations of this metal in milk were observed in the Bieszczady Mountains in summer (0.377 mg L^{-1}) and the lowest – in the Lublin region in winter (0.188 mg L^{-1}). Contradictory conclusions were presented by SOLA-LARRAÑAGA and NAVARRO-BLASCO (2009), who demonstrated the impact of a season on the Fe content in milk at a high value of Varimax factor (0.845). In another study conducted in seven regions of Egypt, with varying degrees of industrialization, the highest levels of Cu, Fe and Zn were recorded in the most heavily urbanized areas, although higher concentrations of Cu and Fe were noticed in summer months (May-June), and Zn – in winter (January-February) (ELSAYED et al. 2011). LI-QIANG et al. (2009), who evaluated milk from areas of China and Japan, stated that Zn and Fe can be transferred to milk from machinery and equipment used during collection and processing of raw milk.

The content of microelements recorded in Sweden (LINDMARK-MANSSON et al. 2003) was similar to our results obtained in the Biebrza and Bieszczady regions. The average concentrations of individual elements in cows' milk were as follows: Zn – 4.4 mg L^{-1}, Mn – 0.10 mg L^{-1}, and a higher content of Cu – 0.10 mg L^{-1}. In another study, conducted in Northern Sweden, milk was characterized by lower levels of these elements: Zn – 4.1 mg L^{-1}, Cu – 0.053 mg L^{-1} as well Mn – 0.024 mg L^{-1} (14). Very low concentrations of Zn (2.016 mg kg^{-1}) and Cu (0.002 mg kg^{-1}) were found by LICATA et al. (2004) in milk from various farms in Calabria, Italy. High levels of these components in milk were shown by RODRIGUEZ et al. (2001), who conducted their research on 8 farms located in the Canary Islands (Zn – 4.41 mg L^{-1} and Cu – 0.076 mg L^{-1}).

Nutritional evaluation of milk includes determination of the content of heavy metals such as Pb and Cd. It is important to emphasize that the mean Pb concentrations in the analyzed milk produced in three regions of Poland did not exceed the permissible limit, i.e. 0.020 mg L^{-1} (*Commission Regulation* 2006). In just two milk samples collected in the Lublin region (Table 2) the permissible level of heavy metals was surpassed. It was shown that regardless of the production season, milk collected in the Lublin region contained considerably more Pb than milk from the Bieszczady Mountains (winter – 4.18 μg L^{-1}, summer – 4.34 μg L^{-1}) and the Biebrza region (winter – 5.99 μg L^{-1}, summer – 6.93 μg L^{-1}). Presence of Cd was noticed in all

Table 2

Content of toxic elements in milk of cows maintained in three regions of Eastern Poland

Toxic elements content (µg L^{-1})		Biebrza region		Bieszczady region		Lublin region		Factor		
		winter season	summer season	winter season	summer season	winter season	summer season	region	season	season x region interaction
No.		66	68	76	87	77	72			
Cd	$\overline{x}$±SD	4.42±3.61	4.13±3.62	4.74±5.45[b]	2.22±2.13[a]	7.10±2.91	7.34±4.75	*xx*	*x*	*xx*
	min.	0.24	<0.02	0.12	0.14	0.83	0.32			
	max.	5.96	6.51	6.80	6.32	8.17	8.94			
Pb	$\overline{x}$±SD	6.44±5.42	6.21±4.02	8.25±6.22	8.80±3.81	12.43±5.60	13.14±4.52	*x*	ns	*xx*
	min.	0.15	0.50	0.53	3.93	3.40	3.84			
	max.	13.90	14.52	10.51	13.43	15.88	20.20			

No. – number of cows

a, b – differences between seasons within the region, significant at $P \leq 0.05$

x – differences significant at $P \leq 0.05$, *xx* – differences significant at $P \leq 0.01$, ns – not stated significant the analyzed milk samples, but its content did not exceed the permissible limit, i.e. 0.010 mg L^{-1} (*Commission Regulation* 2006). Milk from the Lublin region was characterized by the highest content of this element. Significant seasonal differences in the content of the analyzed heavy metals were observed only for Cd in milk of cows kept in the Bieszczady Mountains (summer – 2.22 μg L^{-1}, winter – 4.74 μg L^{-1}). Taking into consideration that animal feed, and consequently milk, is contaminated by dusts, fumes and gases emitted by industry and road traffic, the present results may indicate less heavy pollution in the Biebrza region and the Bieszczady Mountains than near Lublin. It should be added that the Lublin region is an area put to the strongest anthropopressure. The urban sprawl and increasing traffic lead to elevated emission of harmful substances. Intensive agricultural production in this region also contributes to pollution of the natural environment.

Significant differences in concentrations of trace elements between regions were also found by DOBRZAŃSKI et al. (2009). In milk produced on farms in Upper Silesia (contaminated region) significantly higher concentrations of Cu (0.659 mg L^{-1}), Cd (7.39 μg L^{-1}) and Pb (0.042 mg L^{-1}) were observed compared to from Bystrzyca (ecologically clean area), i.e. 0.228 mg L^{-1}, 3.88 μg L^{-1} and 0.021 mg L^{-1}, respectively. It should be noted that the limit for lead content in milk was exceeded in both regions. According to the authors, such a high concentration of this element in milk from Lower Silesia, a region considered as environmentally clean, may be due to inadequate milking hygiene and the impact of airborne pollution from industrial areas.

Studies of other authors (ASLAM et al. 2011, ELSAYED et al. 2011, LICATA et al. 2004, SOLA-LARRAŃAGA, NAVARRO-BLASCO 2009, VIDOVIC et al. 2005) also indicate that the location of farms has a significant impact on the heavy metal content in milk. LICATA et al. (2004), who evaluated bovine milk produced in various farms in Calabria (Italy), confirmed the Cd presence in only 3 out of 43 milk samples, where it equalled 1.14, 3.42 and 22.8 mg kg^{-1}. The Pb presence, however, was observed in all the samples but the recorded values, ranging from 0.1 to 9.92 mg kg^{-1}, did not surpass the permissible limit for this microelement. Low Cd concentration was also noticed in milk of cows maintained in the north of Serbia, where it oscillated from 0.001 up to 0.016 mg kg^{-1} (VIDOVIC et al. 2005). In Pakistan (Faisalabad), the heavy metal residues in milk exceeded several-fold the permissible limits according to the European standards. The average content of Cd was 0.147 mg L^{-1} and Pb – 19.972 mg L^{-1} (ASLAM et al. 2011). Such high values, considered to be potentially dangerous to the health of animals and humans, were probably caused by contamination of soil and water.

Higher concentrations of Pb and Cd in milk are mainly due to the location of farms along roads or near factories and power plants (BILAND•IĆ et al. 2011, ELSAYED et al. 2011, LI-QIANG et al. 2009, PATRA et al. 2008, SIMSEK et al.

2000). Several-fold higher Pb content was found PATRA et al. (2008) in milk of cows kept near zinc smelter and aluminum processing plants (0.85 mg L^{-1}), compared to milk of cows kept in unpolluted areas (0.25 mg L^{-1}). SIMSEK et al. (2000) observed similar concentrations of Pb in an industrial area (0.049 mg kg^{-1}), and in areas with heavy traffic (0.032 mg kg^{-1}). In studies conducted in Lithuania, permissible limits for both analyzed elements were not exceeded, although, similarly to our study, a higher content was reported in the winter season (VALIUKENAITE et al. 2006). In the previous research of KRÓL et al. (2006), similar values of the concentration of both microelements were determined. The average Cd level varied from 0.002 mg L^{-1} in milk from Simmental cows to 0.006 mg L^{-1} in milk from Polish Holstein-Friesian Black and White cows, while the Pb content varied from 0.011 to 0.016 mg L^{-1}, respectively. Higher concentration of both elements was observed in milk obtained in the summer. The results obtained by ELSAYED et al. (2011) confirmed the significant influence of a season ($P \leq 0.05$) on the Pb content in milk. In the summer months, higher amounts of this element were found. There was no effect of a production season on the Cd content in milk.

CONCLUSIONS

In conclusion, it should be stated that the significant effect of a season and production region on the content of all the analyzed elements except Fe was noticed. Milk collected in the Biebrza and Bieszczady regions proved to be a richer source of Cu and Mn. Regardless of the production region, higher levels of Zn and Cu were observed in the summer season, and Mn was more abundant in the winter. The average concentration of Pb and Cd did not exceed the permissible limits. It should be underlined, however, that in all the analyzed milk samples the presence of both elements was observed, even in milk from the Biebrza and Bieszczady regions, which are commonly recognized as ecologically clean. Taking into consideration the fact that Pb and Cd accumulate in living organism, constant monitoring of these elements in milk and other food products seems advisable.

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