Original Paper

Concentrations of Selected Chemical Elements in Sheep's Milk and Sheep's Milk Products with Estimated Intake of Selected Elements

Martina Pšenková*[1], Róbert Toman[1], Ivona Jančo[2], Mária Mičiaková[1], Martin Janíček[1]

[1]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Animal Husbandry, Nitra, Slovakia

[2]AgroBioTech Research Center, Slovak University of Agriculture in Nitra, Slovakia

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The aim of this study was to determine the concentrations of 10 elements – essential calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe) and toxic arsenic (As), nickel (Ni), antimony (Sb), strontium (Sr) and lithium (Li) in samples of milk and milk products from two farms located in regions with different environmental loads. The samples were analyzed using the inductively coupled plasma emission spectrometry method. In the case of sheep's milk, we recorded statistically significant differences in the concentration of Mg, Fe (P <0,01). In the case of the toxic elements As and Ni, we noted a slight exceedance of the highest permissible limits set by legislation. The estimation of the risk of consumption of monitored elements revealed that the share of intake of most elements through dairy products was below 1%. However, higher values from the preliminary tolerable daily intake (PTWI) were found in the case of As with values up to 15% and in the case of a share of the tolerable daily intake (TDI) for the monitored element Sr up to 35%. The results show that the monitored dairy products can be a good source of essential elements, but it is necessary to increase their consumption in the Slovak population. Despite the low values of toxic elements, further rigorous monitoring is needed, especially of As and Sr, which may pose a certain risk to the health of the Slovak population with long-term intake.

Keywords: sheep milk, environmental burden, essential elements, toxic elements, risk assessment

1 Introduction

Milk is a white colloidal suspension produced by the mammary gland of mammals. It is a natural animal product that can be consumed directly without any other additives (Minárik et al., 2023). The main components of milk are water, fat, lactose, whey protein and minerals. Their amounts vary according to the type of animal (Zamberlin et al., 2011). Sheep's milk has a better fatty acid profile, with higher levels of monounsaturated fatty acids and polyunsaturated fatty acids, with smaller fat droplets than cow's milk (Balthazar et al., 2017). Milk is consumed worldwide, especially during infancy, and is of economic importance to many countries. Cow, sheep, and goat milk account for 87% of global milk production (Chen et al., 2020). Currently, the consumption of sheep and goat milk, as well as cheese, is increasing, especially at the local level through purchasing from small farmers (Kováčová et al., 2021). The quality of sheep's milk is of paramount importance in controlling the quality of dairy products (cheese, butter) made from it. In the world, most sheep milk is transformed into cheese and yoghurt. In Asian and African countries, sheep's milk is processed into butter. Fresh sheep's milk is rarely consumed because of its high fat and total solids content. Total cheese production from sheep milk in the world is 680.30 million kg and butter and ghee is 63.25 million kilogram (FAO, 2016). Milk contains 20 elements that are considered nutritionally essential for humans and can be classified into macro and micro elements (Bilandžič et al., 2019). The most important elements in milk are calcium, sodium, potassium, magnesium and, to a lesser extent, iron (Chen et al., 2020). Children need calcium, magnesium, and potassium for normal metabolism, growth, and development (Oana et al., 2016; Zhou et al.,

***Corresponding Author:** Martina Pšenková, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Animal Husbandry, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovakia
martina.psenkova@uniag.sk https://orcid.org/0000-0002-4877-908X

2017) and milk and dairy products are excellent sources of these elements (Gaucheron, 2011). It is beneficial to human health to consume milk regularly (Srbely et al., 2019). The intake of milk and dairy products contributes to building bones mass (Fardelonne, 2019; Ratajczak et al., 2021), positive effects on bone mineral density have been found, as well as reduced fracture risk in some populations (Melse-Boonstra, 2020). According to WHO (2006), children younger than 11 years old should consume 2-3 portions of dairy products per day. It is important to note that the content of minerals is not a stable, but their amount depends on many factors such as the age of the animal, health status, lactation period, breeding method, season or feed quality, but also the method of milk processing, production process, fermentation or possible fortification (Bakircioglu et al., 2018; Garcia et al., 2006; Pietrzak-Fiecko and Kamelska-Sandowksa, 2020). Elemental concentrations also vary significantly between milk types (Chen et al., 2020). Concentrations of calcium, phosphorus, magnesium, zinc, manganese and copper are higher in sheep's milk compared to cow's milk, while the opposite trend is known for calcium and sodium (Park et al., 2007). Goat's milk also has a higher level of K, Ca, Cl, P, Se, Zn and Cu compared to cow milk (Zenebe et al., 2014).

However, milk and dairy products may also contain varying amounts of contaminants. Heavy metals are widely distributed in the environment. Toxic elements that enter the human body through the food chain can cause health problems. The presence of toxic elements even in low concentrations when consumed regularly can have adverse health effects, lead to metabolic disorders, adversely affect the development or function of organs and even cause the development of diseases (Girma et al., 2014; Khan et al., 2014). The accumulation of toxic elements can lead to growth disorders, mental retardation, neurological or cardiovascular diseases (Jaishankar et al., 2004). The presence of heavy metals in milk is controlled according to the maximum limit set by the EU (EC No. 1881/2006).

While the concentration of essential elements in milk is considered an indicator of the nutritional value of milk (Zhou et al., 2017), the absence of toxic elements is an indicator of its safety and an indirect indicator of the quality of the environment and the degree of contamination (González-Montana et al., 2012). Since higher content of toxic elements in environment and consequently in animal products such as milk and dairy product is expected in areas with higher level of contamination, the presented study aimed to determine the content of essential and toxic elements in samples of sheep milk collected from 2 farms with different environmental burden. Another purpose was to evaluate the share of the intake of these products on the intake of essential elements from food based on the detected concentrations of elements in milk and milk products and to determine the risk of intake of toxic elements from these sources by humans.

2 Material and Methods

Samples of sheep milk, cheese, and žinčica (drink made from sheep milk whey) from selected farms were collected. The farm 1 is in a region without environmental burden (region called Orava) and farm 2 is in a region with moderately disturbed environment (region called Zvolensko-Poiplie) according to the division made by The Ministry of the Slovak Republic and the Slovak Environmental Agency. The mentioned authorities have determined the environmental regionalization of Slovakia, and the country is divided into three types of environmental quality: regions with potentially undisturbed areas, regions with slightly disturbed areas, and heavily disturbed areas.

During the period of lactation, we took every two weeks samples of sheep milk, cheese, and milk drink – žinčica. From April to late September 12 samples of each product were collected, 36 samples in total. Milk samples were collected from sheep of the valachian breed × lacaune and each farm from which the samples were collected has approximately 450 sheeps and on both farms, sheep are fed semi-intensive system of nutrition.

Samples of sheep milk were taken from milk tanks as representative of all individuals and stored in freezers at -18 °C until analysis was carried out. To produce žinčica and cheese only milk originally from selected farms were used, not additional milk was mixed during preparation. Samples of žinčica were taken from the container and samples of cheese were cut from representative bigger cheese and consequently stored the same way as samples of milk.

Analysis of concentrations of the selected essential elements (Ca, Mg, Zn, Cu, Fe) and toxic elements (Ni, As, Li, Sr, Sb) were determined using an inductively coupled plasma-optical emission spectrometry (ICP OES) with axial plasma configuration and with auto-sampler SPS-3.

2.1 Statistical Analysis

All results of this study were processed using Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA). All obtained results are listed as mean values with standard deviation. Differences in concentrations of the analyzed elements in sheep milk, cheese, and žinčica between farms were compared by the Studentov *t*-test. A probability level of $P<0.05$, $P<0.01$ and $P<0.001$ were considered statistically significant.

2.2 *Assessment of the Intake of Essential Elements and Estimation of the Risk of Intake of Toxic Elements by Consumption of Dairy Products*

The concentrations of essential elements found in sheep products were compared with the recommended nutritional doses for the population of Slovakia (MZ SR, 2015), in the age categories with the highest need for the given element. For each essential element, the % share of dairy product consumption in their recommended intake was expressed.

For toxic elements, we calculated the value of the estimated daily intake (*EDI*) based on the average concentrations found in the dairy products samples.

The relationship was used for the calculation:

$$EDI = C_{metal} \times W_{milk} / BW$$

where: C_{metal} – the concentration of the element in the analyzed sample; W_{milk} – the daily milk consumption; *BW* – the average body weight (70 kg)

The average consumption of milk in Slovakia, according to the Statistical Office, is 0.12 kg, cheese 0.04 kg and žinčica 0.12kg per capita.

From the determined values, we calculated the Estimated Weekly Intake (*EWI*) for As:

$$EWI = EDI \times 7 \text{ (days)}$$

Subsequently, we expressed the proportion (%) of intake of the toxic element on the preliminary tolerable weekly PTWI intake for As established by JEFCA (2012):

$$\%PTWI = EWI / PTWI \times 100$$

For Ni, Sr and Sb, for which the PTWI value is not determined, we will express the share (%) of element intake on the Tolerable Daily Intake (TDI) determined by WHO (2003, 2010) and EFSA (2020):

$$\%TDI = EDI / TDI \times 100$$

A Provisional Subchronic and Chronic Reference Dose (*p-RfD*) has been established for Li (EPA, 2008), so we expressed the proportion (%) of daily Li intake to this value:

$$\%p\text{-}RfD = EDI / p\text{-}RfD \times 100$$

3 Results and Discussion

3.1 *Concentrations of Elements of Milk and Dairy Products Between 2 Farms in Slovakia*

Table 1 shows the results of analyzes of selected types of sheep milk products for the presence of essential elements. In the case of essential elements, we found statistically significant differences between farms in sheep milk for the elements Mg, where the concentration of this elements was higher on farm 2 (P <0.01) and in case Zn (P <0.001), Cu (P <0.001) and Fe (P <0.01) was found statistically significant higher concentrations of farm 1 (potentially undisturbed environment). We recorded statistically significant differences with sheep's cheese in case Ca (P <0.05), Mg (P <0.01), where the concentration of these elements was higher of farm 2 (moderately disturbed environment) and in case of Cu the concentrations was higher on Farm 1 and this difference was statistically significant (P <0.001). We recorded in traditional Slovak milk drink – žinčica, the highest concentration of Ca (P <0.001) on Farm 2, where the difference between farms was statistically significant. In case of Fe (P <0.01) and Zn (P <0.001) we found higher concentrations of these elements on Farm 1 and differences were statistically significant. Concentrations of Zn we found higher on Farm 2.

In recent years, sheep's and goat's milk has played an increasingly important role in the human diet, not only for children and the elderly, but especially for nursing mothers (Haenlein, 2004; Kapila, et al., 2003). Suttle (2010) states that milk is a rich source of Ca, Mg, Zn, but on the other hand, it is not a sufficient source of Fe, Cu. If we look at the comparison with our results, it is confirmed that sheep's milk is not really a good source of Fe and Cu, as the concentration of Fe in our milk samples was around 1.5 mg.kg^{-1} and less, and the concentration of Cu in the milk samples was 0.11 mg.kg^{-1} and less. We recorded similar results with these elements in the traditional Slovak milk drink žinčica, but this drink made from sheep's milk has a significantly lower concentration of Ca compared to sheep's milk as a product. Compared to our results, Bilandžić et al. (2011, 2019) reported higher concentrations of Cu in milk from Croatia and (Sola-Larrañaga and Navarro-Blasco, 2009) in milk from Spain. Coni et al. (1996) reported higher concentrations of Cu, Fe, Mg, Zn in the milk of sheep from Italy compared to the concentrations of these elements on both monitored farms in this study. Al-Wabel (2008) found lower average concentrations of Ca (822.5 mg.kg^{-1}) and Zn (3.09 mg.kg^{-1}) and a higher concentration of Fe (5.01 mg.kg^{-1}) in the milk of sheep in Saudi Arabia and Cu (0.62 mg.kg^{-1}).

Table 1 Concentrations of essential elements in milk and dairy products between farms (mg.kg^{-1})

Elements	Farm	Mean ±SD		
		sheep milk	sheep cheese	milk drink – žinčica[1]
Ca	Farm 1	1,424.29 ±133.42	3,921.88 ±1,585.46	347.18 ±90.59
	Farm 2	1,373.19 ±180.92	5,036.77 ±743.77*	734.45 ±271.47***
Mg	Farm 1	135.74 ±11.45	245.42 ±85.19	79.19 ±17.54
	Farm 2	151.81 ±18.08**	333.03 ±58.33**	92.363 ±22.68
Zn	Farm 1	4.22 ±0.46***	13.98 ±5.91	0.120 ±0.09
	Farm 2	2.64 ±0.61	13.61 ±3.07	0.49 ±0.13***
Cu	Farm 1	0.11 ±0.02***	0.32 ±0.05***	0.19 ±0.05***
	Farm 2	0.05 ±0.02	0.044 ±0.01	0.04 ±0.01
Fe	Farm 1	1.57 ±0.27*	2.94 ±1.67	1.52 ±0.28**
	Farm 2	1.17 ±0.49	2.43 ±0.57	1.12 ±0.47

Farm 1 – potentially undisturbed environment; Farm 2 – moderately disturbed environment; SD – standard deviation; *P <0.05, **P <0.01, ***P <0.001; [1] – a typical Slovak milk drink that is produced during the production of sheep's cheese

Table 2 Concentrations of toxic elements in milk and dairy products between farms (mg.kg^{-1})

Elements	Farm	Mean ±SD		
		sheep milk	sheep cheese	milk drink – žinčica[1]
Ni	farm 1	0.072 ±0.07	0.186 ±0.13	0.159 ±0.04
	farm 2	0.187 ±0.09***	0.249 ±0.07	0.191 ±0.08
As	farm 1	0.097 ±0.08	0.332 ±0.21	0.141 ±0,15
	farm 2	0.198 ±0.16*	0.267 ±0.23	0.197 ±0.23
Li	farm 1	0.007 ±0.001	0.006 ±0.01	0.003 ±0.002
	farm 2	0.014 ±0.001***	0.014*** ±0.001	0.007 ±0.004***
Sr	farm 1	0.901 ±0.11	0.32 ±0.05***	0.084 ±0.06
	farm 2	1.047 ±0.22*	4.026*** ±0.69	0.5484 ±0.25***
Sb	farm 1	0.047 ±0.03	0.214 ±0.15	0.117 ±0.08
	farm 2	0.133 ±0.06***	0.259 ±0.15	0.221 ±0.13**

Farm 1 – potentially undisturbed environment; Farm 2 – moderately disturbed environment; SD – standard deviation; *P <0.05, **P <0.01, ***P <0.001; [1] – a typical Slovak milk drink that is produced during the production of sheep's cheese

Table 2 shows the concentrations of essential elements in dairy products between farms. Milk and milk products, in addition to their benefits for human health, can also be a potential source of toxic elements or various other contaminants that can cause health problems (Licata et al., 2004; Beikzadeh et al., 2019). Breeding sheep in areas that have a contaminated environment can cause higher concentrations of toxic elements in milk compared to milk that comes from an undisturbed environment, and therefore it is important to monitor such elements in disturbed areas (Norouzirad et al., 2018). In the case of milk, we noted statistically significant differences between farms in the elements Ni (P <0.001), As (P <0.05), Li (P <0.001), Sr (P <0.05) and Sb (P <0.001). For all toxic elements of milk, the concentrations were higher on farm 2, i.e. with a moderately disturbed environment. When analyzing the sheep's cheese, we found two statistically significant differences between the farms, in the case of Li (P <0.001) and Sr (P <0.001). In both cases, it was again a higher concentration on farm 2. We recorded the same statistically significant result at Li and Sr in milk drink – žinčica and in case of Sb (P <0.001). All of these results were higher on farm 2 with moderately disturbed environment.

3.2 Percentage Expression of Intake of Essential Elements and Estimation of Risk of Intake of Toxic Elements

Part of the assessment of the concentrations of essential elements was the expression of the proportion (%) of the element intake through the consumption of dairy products in the recommended daily intake (Table 3). Based on the consumption of selected dairy products in 2022, we found that the highest coverage of the daily requirement of essential elements would be ensured by the consumption of sheep's milk on farm 1. However, this fact must be taken on a theoretical level, as sheep's milk is not used for direct consumption – drinking – in Slovakia. In the case of farm 2, we found that the coverage of the daily requirement of essential elements would be ensured through cheese, and in the case of Ca at the level of 12.59%, in the case of Zn at the level of 3.4%. In the case of magnesium as another important element in dairy products, we found the highest coverage of the daily requirement through milk at the level of 4.34%.

The regulation of chemical elements is not balanced for some and for some known toxic elements, limits are not set, such as PTWI. For example, in the case of the toxic element Cd, the original PTWI value was abolished in 2010 because it did not meet the consumer protection requirement and was replaced in the long term by the PTMI value (Provisional Tolerable Monthly Intake (JECFA, 2012). For other elements, various regulatory values are set, such as TDI, p-RfD, PTMDI (Provisional Maximum Tolerable Daily Intake), etc. In our work, we used the available information and, when estimating the risk, we arrived at the results shown in Table 4.

The results of the risk analyses of human consumption of the analyzed products show that the share of most elements in PTWI, TDI or p-RfD in the case of Li is very low and is at the level of 1% and below in the case of all monitored products on both farms. A similar result was also recorded in the case of Ni, except for sheep cheese on farm 2, where the Ni intake in the TDI was at the level of less than 2%. These values are low and do not pose a significant risk to the consumer.

However, in the case of As, we recorded relatively higher values of the share of PTWI, which ranged from 3.76–15% on both monitored farms. The highest value was recorded for sheep's milk on farm 1. In the risk estimation, we recorded the highest values in the case of Sr, where we recorded the two highest exposures, namely in the case

Table 3 The intake of an essential element of dairy products compared to RND*

Farm 1					Farm 2				
Element	RND*	% z RND*			Element	RND*	% z RND*		
		milk	cheese	žinčica			milk	cheese	žinčica
Ca	1,600 mg[1]	10.6	9.8	0,87	Ca	1,600 mg[1]	10.29	12.59	1.83
Mg	420 mg[2]	3.9	2.34	0.75	Mg	420 mg[2]	4.34	3.17	0.88
Zn	16 mg[3]	3.17	3.49	0.03	Zn	16 mg[3]	1.96	3.4	0.12
Cu	1,800 µg[4]	0.73	0.71	0.42	Cu	1,800 µg[4]	0.31	0.11	0,09
Fe	30 mg[5]	0.63	0.39	0.2	Fe	30 mg[5]	0.47	0.29	0.15

*RND – recommended nutritional dose (Ministry of Health of Slovakia, 2015), [1] – RND for category of breastfeeding women 19 – 51 years; [2] – RND for hard working men 35–62 years and non-working men 65–80 years; [3] – RND for hard working men 19–34 years; [4] – RND for hard working men 19–34 years; 5RND for pregnant women from the second trimester

Table 4 Estimation of the risk of intake of toxic elements according to toxicological limits

	Element		Ni[1]	As[2]	Li[3]	Sb[4]	Sr[5]
Milk	farm 1	EDI	1.22 E-04	1.66 E-04	1.2 E-05	8.05 E-05	1.5 E-03
		%	0.9[a]	7.76[b]	0.6[c]	1.34[a]	1.19[b]
	farm 2	EDI	7.37 E-05	3.39 E-04	2.4 E-05	2.28 E-04	1.7 E-03
		%	0.56[a]	15[b]	1.2[c]	3.8[a]	1.38[b]
Cheese	farm 1	EDI	1.06 E-04	1.89 E-04	3.42 E-06	1.22 E-04	1.5 E-03
		%	0.82[a]	8.85[b]	0.17[c]	0.09[a]	25[b]
	farm 2	EDI	1.42 E-04	1.52 E-04	8 E-06	1.4 E-04	2.3 E-03
		%	1.89[a]	7.12[b]	0.4[c]	0.1[a]	38,34[b]
Žinčica	farm 1	EDI	2.29 E-05	8.06 E-05	1.71 E-06	6.68 E-05	4.8 E-05
		%	0.16[a]	3.76[b]	0.08[c]	1.11[a]	0.04[b]
	farm 2	EDI	1.09 E-04	1.12 E-04	0.4 E-05	1.26 E-04	3.13 E-04
		%	0.84[a]	5.25[b]	0.2[c]	2.10[a]	0.24[b]

EDI – estimated daily intake; [1] – TDI 13 µg.kg^{-1} (EFSA, 2020); [2] – PTWI 15 µg.kg^{-1} (JECFA, 2011); [3] – *p-RfD* 2 µg.kg^{-1} (U.S.EPA, 2008); [4] – TDI 6 µg.kg^{-1} (WHO, 2003); [5] – TDI 0.13 mg.kg^{-1} (WHO, 2010); [a] – expressed as % from TDI; [b] – expressed as % from PTWI; [c] – expressed as from *p-RfD* of sheep cheese on both farms, where the value of this element ranged on farm 1 at 25% of the TDI and on farm 2 at 38.34% of the TDI. In the case of other products, the exposure ranged at 0.04–1.38%.

The last toxic element monitored was Sb, for which we found an exposure level in the monitored sheep dairy products at 0.09–3.8% of the TDI with the highest exposure value in the case of milk from farm 2. Intake of Sb through the monitored products can be considered low.

4 Conclusions

The results of this study show that in the monitored products we found a slight excess of the established limits for the occurrence of toxic elements, especially in the case of As and Ni. Based on the assessment of the basic elements, sheep products can be considered as a good source of Ca and Mg, but it is necessary to increase the consumption of these products. The risk of health damage could be mainly affected by the occurrence of Sr, the intake of which from the monitored products could constitute a significant proportion, and to a lesser extent also the occurrence of As. Therefore, despite the lower concentrations of other elements that pose a risk to the health of the Slovak population, further consistent monitoring of these elements is necessary.

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