Science, Technology and Innovation
ISSN 2544-9125 • 2021; 12 (1): 28-34
doi: 10.5604/01.3001.0015.5608

STI • 2021;12 (1): 28-34

Changes in the concentrations of selected toxic and essential elements in ewe milk from area with a potentially undisturbed environment

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Original article

Abstract

The aim of this study was to evaluate the effect of lactation on the concentration of selected essential and toxic elements in sheep milk forma area of Slovakia with potentially undisturbed environment and to find the actual contamination of selected area, in view of its environmental character. The research was conducted with 400 sheep (Tsigai breed), where the milk samples were taken during the lactation periods (early, middle and late lactation stage). Sheep were reared on the extensive pastures, reared indoors afterwards, fed with pasture ad libidum. Milk samples were collected after morning and afternoon milking. The samples of milk were analysed toxic and essential elements (Ca, Se, Mg, Zn, Fe, Cu, As, Cd, Hg, Ni, Pb) by the method of atomic absorption spectroscopy (AAS). The macro elements concentration in milk changes following the stages of lactation ($p < 0.05$). There was found that Ca milk concentration increased gradually in the following stages of lactation while Mg, Se, and Fe only in the last stage of lactation. With order hand, the milk concentration of Zn was the highest during summer ($p < 0.05$). Simultaneously the contents of essential elements (Cu) and toxic elements (As, Cd, Hg, Ni, Pb) in milk were very low, below the limit of quantification. In conclusion, ewe's milk from potentially undisturbed environmental areas of Slovakia is safe and poses no risk to consumer health, and is suitable for use directly or in dairy processing.

Keywords

- essential elements
- toxic elements
- sheep
- milk
- environmental burdes

Authors contributions

A – Conceptualization
B – Methodology
C – Validation
D – Data collection
E – Data analysis
F – Writing – original draft preperation
G – Writing, reviewing & editing
H – Project administration

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Article info

Article history

- Received: 2021-09-29
- Accepted: 2021-12-01
- Published: 2021-12-01

Publisher

University of Applied Sciences in Tarnow
ul. Mickiewicza 8, 33-100 Tarnow, Poland

Financing

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-18-0227.

Conflict of interest

None declared.

Introduction

Milk and dairy products are important components of the human diet. Milk has been described as a complete food because it contains vital nutrients including proteins, essential fatty acids, lactose, vitamins, and minerals in balanced proportions. However, milk and dairy products can also contain chemical hazards and contaminants which constitute a technological risk factor for dairy products, for related commercial image, and above all, for the health of the consumer [1]. The importance of milk in the human diet is widely established and its regular consumption is recommended, especially for young children. In recent decades sheep milk has assumed an increasingly important role in the human diet, not just for infants but also for adults and especially nursing mothers [2, 3].

Sheep milk may contain various elements of nutritional or toxicological importance, and their levels can vary according to intrinsic factors (season, feeding, and environment) [2]. Because of the nutritional habit of these small ruminants to graze plants and grass, they may be considered such environmental bio-indicators and their milk is a good matrix to monitor the pollution status. Heavy metals, such as arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg) whose toxicity is well known [4], are widely dispersed in the environment and their contamination sources are various: grazing animals are exposed to their accumulation by ingestion of water, grass, and feed. Studies about the presence of toxic trace elements in sheep milk have been frequently carried out in the area of the Middle East, where the livestock of these species is more common [5, 6, 7]. From the nutritional point of view, metals contents of milk and dairy products can be grouped into essential elements (calcium, zinc, magnesium, iron, copper, selenium) at low doses and non-essential or toxic ones (cadmium, lead, nickel, mercury, arsenic). The presence of the latter, even in low concentrations, is invaluable and leads to metabolic disorders with extremely serious consequences [8].

Dairy animals ingest metals while grazing on the pasture and when fed on contaminated concentrate feeds. Toxic metals such as lead and cadmium are common air pollutants and are emitted into the air as a result of various industrial activities [9, 10]. Various industrial environmental contamination of soil, waters, foods, and plants with these metals causes their incorporation into the food chain and imposes a great threat to human and animal health [11]. Lead and cadmium residues in milk and dairy products are of particular concern since they are largely consumed by infants and children. Food is the main route of lead and cadmium exposure in the general population (representing > 90% of the total Cd intake in non-smokers), although inhalation can play an important role in very contaminated areas [9, 12]. Lead and cadmium are considered potential carcinogens and are associated with the etiology of a number of diseases in the cardiovascular system, kidneys, nervous system, blood, and skeletal system [13].

Micronutrient elements such as Fe, Cu, Zn, Se and macronutrients Ca, Mg, are essential for many biological functions [14, 15]. Deficiencies of such elements contribute significantly to the global burden of disease; however, if present at higher levels, they can have a negative effect on human health. Both toxicity and necessity vary from element to element [14]. The trace element contents of milk and dairy products depends on the stage of lactation, nutritional status of the animal, environmental and genetic factors, characteristic of the manufacturing practices and possible contamination from the equipment during processing [16].

The presence of heavy metals and trace elements in milk has been reported in different countries and regions [7, 17, 18, 19]. Moreover, an additional insight into metal uptake and assessment of human risks associated with the consumption of milk are still needed.

Materials and methods

The monitoring of the area was realized during the lactation stages on the selected farm of Slovakia. According to the Ministry of Environment of Slovak Republic (SR) and Slovak Environmental Agency, regions of SR are divided into three types of environmental quality: 1st environmental quality – regions with an undisturbed environment and convenient environment; 2nd environmental quality – regions with a slightly disturbed environment, areas with disturbed environment and areas with the widely disturbed environment; 3rd environmental quality – regions with the heavily disturbed environment [20]. For monitoring in this study, the village Novoť (North Slovakia) which is characterized as an area with a potentially undisturbed environment was selected. For this area, the environmental burden from industrial production, form transport, and logistics facilities, and from waste management facilities is possible.

The research was conducted with 400 ewes (Tsigai breed), an average age of 6 years. The total number of samples of milk were 15 from each lactation stage and about 500 ml sample of milk were taken during the early (May), middle (July) late lactation stage (September). Sheep were reared on the extensive pastures, reared indoors afterward, fed with pasture ad libitum. Milk samples were collected after morning and afternoon milking. Despite the fact that there was a large number of animals on the farms, average milk samples were obtained from milk tanks at the end of milking.

To determine the environmental impact of the selected area, green pasture was also analyzed. At the same time, fifteen samples of green pasture from different places grazed by examined sheep (5 samples from each lactation stage) were collected and stored in plastic bags at −18°C for the following analysis.

Milk and grass samples were mostly mineralized by microwave decomposition with HNO_3 and H_2O_2 (microwave oven MARS 6 240/50). Moreover, the determination of Se was additionally required cooling, treating by HCl, and heating at 90°C for reduction from Se^{6+} to Se^{4+}. Only, As measurement needed the dry mineralization with oxidation mixture (oxygen, oxides of nitrogen, ozone) at 300–400°C. The ash was re-diluted in a solution of HCl. As and Se in milk were analyzed using the hydride generation atomic absorption spectroscopy (HG-AAS) method with Spectr AA-220 FS (The Netherlands). Ca, Fe and Mg in milk and feed were detected using the inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 720-ES, USA). Cd, Pb, and Ni in milk and feed were analyzed using the electrothermal atomization atomic-absorption spectrometry (ETA-AAS, Agilent DUO AA 240Z/240FS, USA). Zn and Cu in milk and feed were analyzed using the (F-AAS, DUO AA 240Z/240FS, USA). Hg in milk and feed was analyzed using the Advanced Mercury Analyzer and atomic-absorption spectrometry (AMA-AAS, Altec CR) without the need for chemical preparation of the sample. All analyses were conducted in certified testing laboratory Eurofins/Bel Novamann (Nové Zámky, Slovak Republic).

Data were analyzed with statistical software IBM SPSS Statistics 20. The results are presented as the arithmetic mean with standard deviation and maximum and minimum value of concentrations of selected metals. Means of concentrations of metals during the lactation stages were compared using One-way analysis ANOVA and Tukey HSD test and differences between lactation stage effect declared significant at $p < 0.05$ level.

Results and discussion

The average concentrations of elements in feed and sheep milk as well as other statistical measurements were determined (Table 1 and Table 2). During the spring season content of essential elements in feed (Ca, Zn, Fe, Mg, Cu) and toxic metals (As, Cd, Hg, Ni, Pb) was higher than during the autumns season. In the case of the autumn season, content Se and all analyzed toxic metals were below the LOQ (limit quantification). Bushra et al. [21] studied concentrations of toxic elements in the feed from rural and urban areas. They found that the content of Cd, Ni and Pb was 0.27, 1.68, 4.11 mg/kg in urban areas and in rural areas 0.037, 0.024, 4.52 mg/kg, respectively. Compared to the results of their study, on the farm of Novoť (potentially undisturbed environment), the content of Pb and Cd was lower, and the content of Ni was higher. Lower content of Ni, As, Cd, Pb in feed state Zhou et al. [22] compared to results of this study.

Animal studies demonstrate that nickel has negative effects on the structure and function of the testis, seminal vesicles, and prostate gland; there is a similar report on adverse effects on spermatozoa [23, 24]. Lukáč et al. [25] reported the negative effect of nickel on spermatogenesis. The decrease in the relative volume of germinal epithelium indicates alterations of spermatozoa production. Cadmium causes tissue damage in humans and animals and many toxicological studies have found functional and structural changes in the kidneys, liver, lungs, bones, ovaries, and fetal effects [26, 27].

Table 1. Concentrations of mineral and selected toxic elements in feed of sheep (mg/kg)

Green forage											
Elements	**Ca**	**Zn**	**Se**	**Mg**	**Fe**	**Cu**	**As**	**Cd**	**Hg**	**Ni**	**Pb**
Spring season	7260.0	35.2	0.055	3010.0	285.0	12.2	1.8	0.17	0.4	8.8	4.8
Summer season	2569.0	34.3	0.013	1256.2	135.8	5.63	< 0.03*	< 0.01*	< 0.3*	< 0.10*	< 0.30*
Autumn season	1125.0	33.0	< 0.03*	285.0	83.0	1.9	< 0.03*	< 0.01*	< 0.3*	< 0.10*	< 0.30*

* Concentrations with this index are below the LOQ (limit quantification).

Table 2. Descriptive statistics for concentrations of essential and toxic elements in sheep´s milk

Element, mg/kg	Mean±SD	Min	Max
Ca	1650.00 ± 750.61	1015.00	2430.00
Mg	132.67 ± 44.42	89.00	183.00
Zn	3.55 ± 2.22	3.20	6.00
Se	< 0.030	–	–
Fe	0.76 ± 0.45	0.65	1.45
Cu	< 0.50	–	–
As	< 0.030	–	–
Cd	< 0.0040	–	–
Pb	< 0.010	–	–
Ni	< 0.10	–	–
Hg	< 0.002	–	–

Abbreviations: SD – standard deviation; Min – minimum value; Max – maximum value.

A positive result of this study was that the content of toxic metals, which were detected in feed on the farm, did not affect their occurrence in sheep milk. Content of all toxic elements (As, Hg, Ni, Pb, Cd) in milk was below the LOQ (Table 2). The content of Pb in milk in this work was significantly lower than in milk samples from Iran [6, 7] and very low in comparison to the results of Anastasio et al. [28] and Licata et al. [29].

It is evident (Table 3) that milk of sheep from the farm of Novoť was found to significantly decrease concentrations of Ca and Mg during the early and middle lactation stage and significantly increase between the middle and late lactation stage. Concentrations of Zn were identical during the early and middle lactation stage and in the last stage of lactation significant decrease of Zn concentrations was recorded. Concentrations of Se were below the LOQ during the lactation stages and the highest concentration of Fe was found in the middle stage of lactation. Cu and toxic elements (As, Cd, Hg, Pb, Ni) during the lactation stages were below the LOQ.

Compared to the present study, Antunovič et al. [30] in sheep's milk in Croatia found increased concentrations Ca, Mg during the lactation and increased concentrations Fe and Se during the early and middle stages of lactation. Al-Wabel [31] recorded in sheep´s milk in Saudi Arabia lower concentrations of Zn (3.09 mg/kg), Ca (822.50 mg/kg), and higher concentration of Cu (0.62/mg) and Fe (5.01 mg/kg). In the milk of Bulgarian breeds of sheep (Strednostaroplaninska and Tetevenska sheep) Gerchev and Mihaylova [32] found similar concentrations of Ca (223 and 208 mg/100g) as well as a higher concentration of Fe (0.181 and 0.12 mg/100g).

The concentrations of elements in raw milk are also affected by animal forage, feed, and water [31, 33]. Animal feed with elevated levels of these elements also causes an increase of their level in milk [21]. Concentrations of health-beneficial elements, e.g. Fe, Zn in milk are dependent on the animal species, feed, milk sample collection time, environmental conditions, and manufacturing processes [34]. Changes in the composition of milk can also be affected by many genetic (breed, herd) and physiological factors (lactation, age, animal health), but also the environment (food, climate, season, method of milking) [35].

Table 2. Descriptive statistics for concentrations of essential and toxic elements in sheep´s milk

Element mg/kg	Stage of lactation (Mean±SD)		
	Early	Middle	Late
Ca	2430.00 ± 1.42a	1015.00 ± 1.58c	1675.00 ± 1.65b
Mg	183.00 ± 1.35a	89.00 ± 1.24c	171.00 ± 1.12b
Zn	6.00 ± 0.16a	6.00 ± 0.16a	3.20 ± 0.11b
Se	< 0.030	< 0.030	< 0.030
Fe	< 0.50	1.45 ± 0.16	0.65 ± 0.002
Cu	< 0.50*	< 0.50*	< 0.50*
As	< 0.030*	< 0.030*	< 0.030*
Cd	< 0.0040*	< 0.0040*	< 0.0040*
Pb	< 0.010*	< 0.010*	< 0.010*
Ni	< 0.10*	< 0.10*	< 0.10*
Hg	< 0.002*	< 0.002*	< 0.002*

SD – standard deviation; a, b, c – means within a row with different superscripts differ ($p < 0,05$); a means within a row with the same superscripts – non significant, * – values below LOQ (limit quantification).

Conclusions

The results indicate that the content of selected essential elements and toxic metals in feed and milk changes dependently on the season of year and lactation stage. The work showed a significant increase in the content of Ca, Mg, Se and Fe during the lactation stage. The concentration of essential element (Cu) and toxic elements (As, Cd, Hg, Ni, Pb) in milk was low, below the LOQ. It can be concluded that the use of ewe milk from this area for direct use or for dairy products processing is appropriate, safe and poses no health risk for the consumers.

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