ASSESSMENT OF ESSENTIAL AND TOXIC ELEMENTS IN SHEEP MILK FROM SLOVAKIA: NUTRITIONAL BENEFITS AND HEALTH RISK EVALUATION IN A MODERATELY DISTURBED REGION

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Abstract

The study aimed to evaluate the nutritional contribution of essential elements: calcium (Ca), magnesium (Mg), and iron (Fe), and assess health risks associated with potentially toxic elements: lithium (Li), strontium (Sr), and arsenic (As) in sheep milk from a moderately disturbed region of Slovakia. Forty individual milk samples were collected by hand milking and analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). The average concentrations of Ca, Mg, Fe, Li, and Sr in milk were 1,355 mg/kg, 114 mg/kg, 1.31 mg/kg, 0.024 mg/kg, and 0.804 mg/kg, respectively. Arsenic concentrations in all analyzed samples were below the limit of detection (LOD). Nutritional analysis showed that daily consumption of 0.3 L of sheep milk could cover 31–58% of Ca and up to 28.5% of Mg recommended dietary allowances (RDA), particularly benefiting younger populations. In contrast, Fe coverage remained below 5% across all age groups. For toxic elements, a comprehensive risk assessment was conducted using estimated daily intake (EDI), hazard quotient (HQ), risk quotient (RQ), and margin of exposure (MOE). Lithium exposure posed the highest relative intake in children aged 3–10 years, with HQ reaching 0.17 and MOE values around 1800, indicating no acute risk but a noteworthy dietary contribution (up to 16.6% of the provisional reference dose). Strontium levels remained within safe thresholds across all age groups, with HQ values well below 0.04 and MOE exceeding 12,000. Although no individual or combined parameter (HI) exceeded risk thresholds, sheep milk alone contributed up to 61% of the provisional tolerable weekly intake (PTWI) for Sr in children. Although none of the toxicological indices (HQ, HI, MOE) indicate exceedance or immediate hazard from milk consumption alone, the relatively high percentage contribution to PTWI (Sr) and p RfD (Li) in high consuming children supports the need to consider cumulative dietary exposure. These findings highlight the dual role of sheep milk as a nutritionally valuable food rich in Ca and Mg, while also emphasizing the need for dietary risk awareness, especially in children, due to its significant contribution to lithium and strontium exposure. Balanced consumption is recommended.

Keywords: *sheep milk, essential elements, toxic elements, benefits, risk, Slovakia*

1. INTRODUCTION

Sheep milk has long been esteemed for its rich nutritional profile, offering higher concentrations of proteins, fats, vitamins, and essential minerals compared to cow and goat milk (Haenlein, 2004). This makes it a staple in various cultures, particularly in Mediterranean and Middle Eastern regions, where it is consumed directly or processed into cheeses and yogurts (Park et al., 2007). In Slovakia, sheep farming is a deeply rooted tradition with significant cultural and economic importance, contributing to the production of unique dairy products such as bryndza, oštiepok, parenica, and žinčica (Palo & Kalab, 2015; Kačániová et al., 2021). Bryndza, a soft cheese made from unpasteurized sheep milk, has been granted the Traditional Specialty Guaranteed (TSG) label, underscoring its traditional processing methods and cultural significance. Similarly, oštiepok and parenica are traditional Slovak cheeses that contribute to the richness of Slovak gastronomy. Another noteworthy product, žinčica, a fermented sheep milk beverage, has a longstanding place in Slovak culinary heritage (Lauková et al., 2020).

Sheep milk is a valuable source of essential minerals such as calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), which are critical for maintaining various physiological functions (Haenlein, 2004).

Calcium is crucial for bone health, nervous system function, and muscle activity (Cashman, 2002), while magnesium plays a key role in biochemical reactions such as protein synthesis and blood pressure regulation (Rosanoff et al., 2012). Iron is fundamental for oxygen transport and energy metabolism, with deficiencies leading to anemia and reduced cognitive function (Abbaspour et al., 2014). Furthermore, selenium (Se) contributes to immune function and antioxidant defense, making sheep milk a valuable dietary component (Mertz, 1981).

However, alongside these essential elements, sheep milk may also contain toxic elements such as lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), aluminum (Al), lithium (Li), and strontium (Sr), which pose significant health risks (Tripathi et al., 1999; Licata et al., 2004). These contaminants can enter the milk through environmental exposure, including polluted grazing lands, contaminated water sources, and industrial emissions (Pilarczyk et al., 2013). Chronic exposure to toxic elements can lead to severe health issues such as neurotoxicity, renal dysfunction, and increased cancer risk (Järup, 2003). Chronic arsenic exposure has been associated with carcinogenic, cardiovascular, neurotoxic, and immunotoxic effects (Singh et al., 2025), while Li, although therapeutically used for bipolar disorder, can lead to kidney and thyroid toxicity at high exposure levels (Grandjean & Aubry, 2009). Strontium can replace calcium in bones, potentially affecting their strength and structure (Skoryna, 1981).

The dual presence of essential and toxic elements in sheep milk necessitates a comprehensive assessment to maximize nutritional benefits while minimizing health risks. Studies indicate that the concentrations of these elements vary significantly based on factors such as geographical location, environmental pollution, farming practices, and lactation stage (Antunović et al., 2023). Research has shown that sheep raised in industrial or mining regions produce milk with higher levels of toxic elements due to contaminated soil and forage (Almášiová et al., 2023). Conversely, sheep grazing in pristine environments have milk with lower contaminant levels and optimal essential element content (Pšenková & Toman, 2021).

Understanding the balance between essential and toxic elements is crucial for public health, especially in regions with a history of environmental contamination. Regular monitoring and risk assessments can inform safe consumption levels and guide agricultural practices to reduce contamination. Moreover, processing methods such as pasteurization and fermentation may influence the concentrations of these elements in dairy products, potentially affecting associated health risks (Alinezhad et al., 2024).

This study aims to analyze the concentrations of Ca, Mg, Fe, As, Li, and Sr in sheep milk from Slovakia and assess their nutritional benefits and potential health risks to consumers. By evaluating milk samples from regions with varying levels of environmental contamination, this research will provide valuable insights for consumers, producers, and regulatory authorities regarding the safety and quality of sheep milk in moderately contaminated regions.

2. MATERIALS AND METHODS

2.1. Milk Sampling

Milk sampling was carried out under controlled conditions to ensure the reliability and reproducibility of subsequent analyses. A total of 40 individual milk samples were collected from randomly selected sheep within a flock of 200 lactating ewes. The selection process was conducted randomly to ensure an unbiased representation of the herd and account for natural variations in milk composition. The farm, located in a moderately polluted region of central Slovakia, follows conventional farming practices. Environmental classification of the study area "moderately polluted region" was based on the official environmental regionalization of the Slovak Republic (Lieskovská and Lényiová, 2023). The potential environmental impact on milk composition was considered, as farming conditions and regional characteristics may influence the presence of both essential and trace elements in milk.

Milk was collected using individual hand-milking, ensuring that the first streams of milk were discarded to minimize variability due to foremilk differences. Each sample consisted of approximately 500 mL of raw milk, which was immediately transferred into sterile polypropylene bottles to prevent contamination or interactions with container materials. All samples were immediately frozen at a temperature of -20 °C and stored under these conditions until further laboratory processing and analysis.

2.2. Sample Processing

Prior to laboratory analysis, all frozen milk samples were subjected to a controlled thawing process at room temperature, followed by mechanical homogenization using a high-performance disperser (OV 625 Digital Disperser, Velp Scientifica, Italy). Homogenization was performed to ensure uniform distribution of milk components, as phase separation during storage could lead to inconsistent analytical results. For chemical analysis, a 2 mL aliquot of each homogenized sample was carefully pipetted into digestion vessels. Mineralization of the samples was performed using a high-performance microwave digestion system, Ethos UP (Milestone Srl, Sorisole, BG, Italy), which was selected due to its high efficiency in breaking down complex organic matrices while ensuring complete element recovery.

Each sample was digested in a mixture of ultrapure reagents, consisting of 5 mL nitric acid (HNO_3, ≥69.0%, TraceSELECT®, Honeywell Fluka, Morris Plains, USA), 1 mL hydrogen peroxide (H_2O_2, ≥30%, for trace analysis, Sigma-Aldrich, Saint-Louis, Missouri, USA) used as an additional oxidizing agent to enhance digestion efficiency, 2 mL ultrapure water (18.2 MΩ cm^{-1}, 25 °C, Synergy UV, Merck Millipore, France). The digestion process consisted of gradual heating phase - samples were heated to 200 °C over 15 minutes to facilitate the breakdown of organic components, constant heating phase - samples remained at 200 °C for an additional 15 minutes, ensuring complete mineralization and active cooling phase - samples were gradually cooled to 50 °C over 15 minutes to prevent volatilization losses and maintain element integrity.

Following digestion, the samples were filtered through VWR Quantitative filter paper 454 (particle retention 12–15 µm, VWR International, Leuven, France) to remove any residual particulates. The filtrates were transferred to 50 mL volumetric flasks and diluted with ultrapure water to the final volume of 50 mL, ensuring uniform concentrations suitable for instrumental analysis.

2.3. Sample Analysis

To quantify both essential elements (Ca, Mg, Fe) and potentially toxic elements (As, Li, Sr), analysis was conducted using an inductively coupled plasma-optical emission spectrometer (ICP-OES 720, Agilent Technologies Australia (M) Pty Ltd.) equipped with an axial plasma configuration and an automated sample introduction system (SPS-3, Agilent Technologies, Switzerland). A Multielement Standard Solution V for ICP (Sigma-Aldrich Production GmbH, Switzerland) was used for instrument calibration to ensure accuracy and precision. The detection limits for the analyzed elements were as follows (µg/kg): Arsenic (As): 1.5; Calcium (Ca): 0.01; Iron (Fe): 0.1; Lithium (Li): 0.06; Magnesium (Mg): 0.01; and Strontium (Sr): 0.01. To ensure analytical reliability, method validation was performed using a certified reference material (CRM-ERM CE278 K, Sigma-Aldrich Production GmbH, Switzerland). The following analytical wavelengths (λ/nm) were selected for elemental quantification: As – 188.980, Ca – 315.887, Fe – 234.350, Mg – 383.829, Li – 670.783, and Sr – 407.771.

2.4. Calculation of Nutritional Coverage for Essential Elements

To evaluate the contribution of sheep milk to dietary intake, the coverage of recommended daily intakes (RDI) for calcium (Ca), magnesium (Mg), and iron (Fe) was calculated based on a daily consumption of 0.3 L of milk. The analysis followed the dietary recommendations for the Slovak population established by the Ministry of Health of the Slovak Republic (MoH SR, 2015) and was performed for various age and physiological categories, including children, adolescents, adults, pregnant and lactating women, and elderly individuals. In the analyses, we used the official classification of age categories for nutrient intake in Slovakia (MoH SR, 2015). The percentage of RDI covered by sheep milk consumption was determined using the formula:

$$\% \, RDI = (\text{Element concentration in milk (mg/L)} \times 0.3 \text{ L}) / \text{RDI (mg/day)} \times 100$$

The calculated percentages allowed us to assess the relative contribution of sheep milk to overall mineral intake in different population groups. For the purpose of evaluation, the contribution of sheep milk to the total daily requirement of each element was classified according to the criteria established by the

European Parliament and Council Regulation (EU) No. 1169/2011, which defines a food as a source of a given mineral if it provides at least 15% of the reference nutrient intake (RNI) per 100 g or 100 ml (European Parliament and Council, 2011). Based on this, the classification was applied as follows: sufficient coverage: ≥ 15% of the RDA, moderate coverage: 10% – 14.9% of the RDA, low coverage: < 10% of the RDA. Sufficient coverage suggests that sheep milk is a substantial dietary source of the given element, while moderate and low values indicate the necessity of additional dietary sources. These findings are particularly relevant for groups with increased mineral demands, such as growing children, pregnant women, and the elderly, where adequate intake of Ca, Mg, and Fe is crucial for bone health, metabolic processes, and anemia prevention.

2.5. Risk Assessment

A comprehensive risk assessment was conducted to determine the potential health implications of consuming sheep milk containing lithium (Li) and strontium (Sr). Arsenic (As) concentrations were below the method detection limit (LOD) in all analyzed samples; therefore, quantitative risk assessment for this element could not be performed. To capture variability in dietary habits, two different consumption levels of sheep milk were considered: 1) Low consumption scenario: 0.13 L/day, representing occasional milk consumers. 2) Average/recommended consumption scenario: 0.3 L/day, approximating the daily intake of habitual consumers. Since toxicological risk assessments are body-weight dependent, calculations were performed for different age groups, taking into account the body weight of population subgroups in accordance with the recommendations of the EFSA Scientific Committee (EFSA, 2012).

2.5.1. Estimated Daily Intake (EDI)

The estimated daily intake (EDI) of lithium and strontium from sheep milk was calculated using the following formula (U.S. FDA, 2006): $EDI = (C \times IR) / BW$

where:

C = Element concentration in milk (mg/L), obtained from ICP-OES analysis.

IR = Daily milk intake (L/day), based on the two consumption scenarios.

BW = Average body weight (kg) for each population category.

2.5.2. Risk Indicators and Index Calculations

Hazard Quotient (HQ) is used to assess the likelihood of adverse effects from long-term exposure to toxic elements. It is calculated as follows (Ain et al., 2023): $HQ = EDI / TDI$, where TDI is Tolerable Daily Intake of element.

Hazard Index (HI). Since individuals are typically exposed to multiple toxic elements simultaneously, the Hazard Index (HI) is calculated to assess cumulative risk (U.S. EPA, 1989): $HI = \Sigma HQ_i$

Contribution to Provisional Tolerable Weekly Intake (PTWI). To determine whether weekly exposure remains within safe limits, the percentage of PTWI was computed as follows:

$\% PTWI = [(EDI \times 7) / PTWI] \times 100$

Target Hazard Quotient (THQ) is another important indicator of non-carcinogenic risk, calculated as (Ain et al., 2023): $THQ = EDI / RfD$, where RfD is Reference Dose of element.

Acceptable Daily Intake (ADI) and *Risk Quotient (RQ)*. To provide additional safety evaluations, the Risk Quotient (RQ) was determined using (EPA, 2007): $RQ = EDI / ADI$

Margin of Exposure (MOE) is an important parameter used to assess the safety of exposure to potentially harmful substances. It is particularly relevant for non-threshold toxicants, where risk is evaluated based on the ratio between a benchmark dose and the estimated exposure level. A higher MOE indicates a lower risk, while lower values suggest a potential health concern. The MOE is calculated using the following formula: $MOE = NOAEL / EDI$

where:

NOAEL = No Observable Adverse Effect Level, a reference dose derived from toxicological studies.

EDI = Estimated Daily Intake, calculated for each toxic element. NOAEL for Li is set to 0.604 mg/kg b.w. (ECHA, 2014) and for Sr is 140 mg/kg b.w. (ATSDR, 2004).

In the case of non-genotoxic and non-carcinogenic substances, an MOE value greater than 100 is generally considered to indicate a low risk, while values below this threshold may warrant further evaluation to assess potential health concerns and the need for risk mitigation strategies.

3. RESULTS

3.1 Concentrations of Elements in Sheep Milk

The elemental composition analysis of sheep milk revealed substantial variation in the concentrations of both essential and potentially toxic elements. The obtained results, summarized in Table 1, indicate that while some elements exhibit relatively stable distributions, others demonstrate pronounced variability, which may be influenced by physiological, environmental, or dietary factors.

Among the analyzed **essential elements**, calcium (Ca) displayed the highest concentration, with a mean value of 1355.16 mg/kg and individual measurements ranging from 1108.03 mg/kg to 1553.50 mg/kg. The observed variability implies that the Ca content in sheep milk is subject to multiple regulatory factors, including dietary intake, lactation stage, and breed-specific metabolic mechanisms governing calcium homeostasis. Magnesium (Mg) concentrations followed a more uniform distribution pattern, with a mean of 114.03 mg/kg and a range spanning from 87.57 mg/kg to 129.29 mg/kg. The close agreement between the mean and median suggests a relatively balanced distribution across samples, indicating that Mg levels in sheep milk are maintained within a consistent physiological range. The overall variability in Mg concentrations was lower compared to other elements analyzed. Iron (Fe) concentrations exhibited notable variability, with a mean of 1.31 mg/kg and a broad range extending from 0.98 mg/kg to 3.19 mg/kg. While baseline Fe levels in sheep milk remain within a defined range, sporadic increases in certain samples may be attributable to differences in dietary intake, environmental exposure, or individual metabolic variations.

In addition to essential elements, several **potentially toxic elements** were also analyzed in milk samples. Strontium (Sr) was present in all samples, with a mean concentration of 0.804 mg/kg and values ranging from 0.48 mg/kg to 1.09 mg/kg. The distribution of Sr concentrations was relatively symmetric, with moderate variability among samples. Lithium (Li) concentrations exhibited substantial heterogeneity, with a mean concentration of 0.0236 mg/kg and values ranging from 0.0123 mg/kg to 0.0728 mg/kg. The analysis of arsenic (As) revealed that its concentrations remained below the limit of detection (LOD) in all analyzed samples. The absence of detectable As levels suggests that no significant environmental or dietary exposure to this element occurred in the studied population.

Overall, the observed differences in elemental distribution in sheep milk suggest a multifactorial influence governing their concentrations, including metabolic regulation, dietary composition, and potential environmental exposure. While elements such as Ca and Mg demonstrated a stable and well-regulated presence, others, including Fe, Sr, and Li, exhibited considerable variability, possibly reflecting external influences or individual physiological differences. The absence of detectable As levels further supports the conclusion that toxic metal contamination in the analyzed samples was negligible.

Table 1. Concentrations of essential and toxic elements in sheep milk (mg/kg)

Element	Mean ± SE	Median	Minimum	Maximum
Ca	1355.16±19.39	1382.58	1108.03	1553.50
Fe	1.31±0.08	1.20	0.98	3.19
Mg	114.03± 1.75	117.59	87.58	129.29
Li	0.024 ± 0.002	0.020	0.012	0.073
Sr	0.804 ± 0.024	0.829	0.480	1.091
As	< LOD			

SE – Standard Error, LOD – Limit of Detection

3.2 Nutritional Contribution of Essential Elements

The percentage coverage of the recommended dietary allowances (RDA) for calcium (Ca), magnesium (Mg), and iron (Fe) in different age groups is presented in Table 2. The results indicate substantial variability in the ability of sheep milk to meet the nutritional requirements for these essential elements across different demographic categories.

Based on the criteria established by the European Parliament and Council (2011), the percentage coverage of Ca ranged from 31.27% to 58.08%, indicating that sheep milk is a sufficient source of calcium across all age groups. The highest Ca coverage was observed in the 4–6 years age group (58.08%), while the lowest was found in adolescents (both boys and girls aged 15–18 years, 31.27%). Despite the observed decline in Ca coverage with increasing age, all values remained above the 15% threshold, confirming that sheep milk can be considered a significant dietary source of calcium for the studied population. For Mg, the percentage RDA coverage ranged from 8.55% to 28.51%. Only young children (4–6 years old) achieved sufficient Mg coverage (28.51%), while all other groups fell into the moderate or low classification. The lowest Mg contribution was recorded in adolescent boys (15–18 years old, 8.55%), emphasizing that sheep milk alone does not meet the Mg requirements in older age groups. For Fe, the percentage coverage of RDA was the lowest among the three elements, ranging from 2.46% to 4.37%. All age groups fell into the low category (<10%), with the highest Fe coverage observed in the youngest age group (4–6 years old, 4.37%), and the lowest in adolescent girls (11–14 years old, 2.46%), who have the highest iron requirements. These results suggest that sheep milk is not a significant dietary source of iron for any of the studied age groups.

Overall, the data demonstrate that while sheep milk from the selected farm in central Slovakia provides a sufficient dietary source of Ca, its role in covering the nutritional needs for Mg and Fe is limited, particularly in older children and adolescents. These results emphasize the necessity of additional dietary sources of these elements to ensure adequate nutritional intake across all age groups.

Table 2. Dietary reference values and % RDA for selected minerals by age group

Age Group	RDA (mg)			% RDA		
	Ca	Mg	Fe	Ca	Mg	Fe
Children 4 - 6 years	700	120	9	58.08	28.51	4.37
Children 7 - 10 years	900	200	10	45.17	17.10	3.93
Boys 11 - 14 years	1200	300	12	33.88	11.40	3.28
Girls 11 - 14 years	1300	330	16	31.27	10.37	2.46
Boys (students) 15 - 18 years	1300	400	12	31.27	8.55	3.28
Boys increased physical activity 15 - 18 years	1500	430	15	27.10	7.96	2.62
Girls (students) 15 - 18 years	1200	350	15	33.88	9.77	2.62
Girls increased physical activity 15 - 18 years	1400	380	18	29.04	9.00	2.18
Women 19 - 34 years - light work	1000	300	15	40.65	11.40	2.62
Women 19 - 34 years - moderate work	1100	320	17	36.96	10.69	2.31
Women 19 - 34 years - heavy work	1200	350	18	33.88	9.77	2.18
Women 19 - 51 years - pregnant 2nd trimester	1400	350	30	29.04	9.77	1.31
Women 19 - 51 years - breastfeeding	1600	330	22	25.41	10.37	1.79
Women 35 - 62 years - light work	1000	300	15	40.65	11.40	2.62
Women 35 - 62 years - moderate work	1100	300	16	36.96	11.40	2.46
Women 35 - 62 years - heavy work	1200	320	17	33.88	10.69	2.31
Non-working women 63 - 79 years	1200	300	14	33.88	11.40	2.81
Non-working women 80 years and older	1200	300	10	33.88	11.40	3.93
Men 19 - 34 years - light work	1000	400	10	40.65	8.55	3.93
Men 19 - 34 years - moderate work	1200	400	11	33.88	8.55	3.57
Men 19 - 34 years - heavy work	1300	420	12	31.27	8.14	3.28
Men 35 - 62 years - light work	1000	410	9	40.65	8.34	4.37
Men 35 - 62 years - moderate work	1100	410	10	36.96	8.34	3.93
Men 35 - 62 years - heavy work	1200	420	11	33.88	8.14	3.57
Non-working men 63 - 79 years	1200	420	10	33.88	8.14	3.93
Non-working men 80 years and older	1200	420	10	33.88	8.14	3.93

RDA - Recommended Daily Allowance

3.3 Health Risk Assessment of Toxic Elements

The assessment of lithium (Li) and strontium (Sr) exposure through sheep milk consumption was conducted for different age groups, considering two daily intake scenarios (0.13 L/d and 0.3 L/d). The results, summarized in Table 3, present the estimated daily intake (EDI) for lithium (Li) and strontium (Sr) across different age groups, based on two milk consumption scenarios (0.13 L/d and 0.3 L/d) and highlight substantial variability in exposure levels depending on age group and milk consumption volume.

Table 3. Estimated daily intake (mg/kg b.w./day) for toxic elements by age group and different milk consumption

Age Group	Body Weight (kg)	EDI Li		EDI Sr	
		0.13[a] L/d	**0.3[b] L/d**	**0.13[a] L/d**	**0.3[b] L/d**
3 - 10	22	0.000143	0.000331	0.004894	0.011294
11 - 14	42	7.5e-05	0.000173	0.002564	0.005916
15 - 18	60	5.3e-05	0.000121	0.001794	0.004141
19 and older	70	4.5e-05	0.000104	0.001538	0.00355

EDI - Estimated Daily Intake in mg/kg b.w., [a] Average daily milk consumption in Slovakia, [b] Recommended daily milk consumption scenario

3.3.1 Lithium (Li) Exposure

The estimated daily intake (EDI) of lithium ranged from 0.000045 mg/kg b.w. in adults (19 years and older, 0.13 L/d) to 0.000331 mg/kg b.w. in young children (3–10 years, 0.3 L/d). Across all age groups and consumption levels, the EDI values remained considerably lower than the provisional reference dose (p-RfD) of 2 µg/kg b.w., suggesting a minimal risk of exceeding the safe intake threshold through sheep milk consumption. Table 4 presents the risk assessment parameters for lithium across different age groups. Despite the low EDI values, the hazard quotient (HQ), which assesses the risk of chronic exposure, showed age-related differences. The lowest HQ was observed in adults (19 years and older, 0.13 L/d: 0.02), while the highest was detected in young children consuming the higher milk volume (3–10 years, 0.3 L/d: 0.17). Since all HQ values remained well below the risk threshold of 1, no immediate non-carcinogenic health risks from Li intake were indicated. Similarly, the risk quotient (RQ) values ranged between 0.04 (adults, 19 years and older, 0.13 L/d) and 0.12 (children, 3–10 years, 0.3 L/d), confirming that Li intake does not pose an acute health hazard. The percentage of the provisional reference dose (% p-RfD) for Li ranged from 2.25% (adults, 19 years and older, 0.13 L/d) to 16.55% (children, 3–10 years, 0.3 L/d). While these values remain below the safety threshold of 100% p-RfD, it is important to consider that this estimation is based solely on sheep milk consumption. Given that lithium is present in other dietary sources (e.g., water, vegetables, grains), the cumulative intake could be significantly higher, especially for vulnerable populations such as young children. The fact that sheep milk alone contributes up to 16.55% of the total acceptable daily intake suggests that additional sources of Li in the diet could lead to intake levels approaching or exceeding the safety threshold. This underscores the need for a more comprehensive dietary assessment to evaluate the cumulative exposure to lithium and the potential risk of exceeding safe intake levels. The target hazard quotient (THQ) values for Li ranged from 0.02 (adults, 19 years and older, 0.13 L/d) to 0.17 (children, 3–10 years, 0.3 L/d), staying well below the critical threshold. The margin of exposure (MOE) for Li, calculated based on the NOAEL of 0.604 mg/kg b.w., ranged from 1824 (children, 3–10 years, 0.3 L/d) to 13397 (adults, 19 years and older, 0.13 L/d). The values consistently exceed the safety benchmark of 100. confirming a substantial safety margin for lithium exposure.

Table 4. Risk assessment parameters for lithium across different age groups

Age Group	HQ Li		THQ Li		RQ Li		% p-RfD Li		MOE Li	
	0.13[a] L/d	**0.3[b] L/d**	**0.13[a] L/d**	**0.3[b] L/d**	**0.13[a] L/d**	**0.3[b] L/d**	**0.13[a] L/d**	**0.3[b] L/d**	**0.13[a] L/d**	**0.3[b] L/d**
3 - 10	0.07	0.17	0.07	0.17	0.12	0.12	7.17	16.55	4210	1824
11 - 14	0.04	0.09	0.04	0.09	0.06	0.06	3.76	8.67	8038	3483
15 - 18	0.03	0.06	0.03	0.06	0.04	0.04	2.63	6.07	11483	4976
19 and older	0.02	0.05	0.02	0.05	0.04	0.04	2.25	5.20	13397	5805

HQ – Hazard Quotient, THQ – Target Hazard Quotient, RQ – Risk Quotient, % p-RfD – Percentage of the Provisional Reference Dose, MOE – Margin of Exposure, [a]Average daily milk consumption in Slovakia, [b]Recommended daily milk consumption scenario

3.3.2 Strontium (Sr) Exposure

The estimated daily intake (EDI) of strontium ranged from 0.001538 mg/kg b.w. in adults (19 years and older, 0.13 L/d) to 0.011294 mg/kg b.w. in young children (3–10 years, 0.3 L/d). These intake levels were considerably lower than the reference dose (RfD) of 0.3 mg/kg b.w., indicating that Sr consumption through sheep milk is unlikely to exceed the established safety limits for any age group. As shown in Table 5, which presents the risk assessment parameters for strontium across different age groups, the hazard quotient (HQ) for Sr ranged from 0.0051 (adults, 19 years and older, 0.13 L/d) to 0.0376 (children, 3–10 years, 0.3 L/d). Even in the highest exposure scenario, the HQ remained well below 1, confirming that Sr intake does not present a significant risk of chronic toxicity. The risk quotient (RQ) values exhibited a similar trend, ranging from 0.0003 (adults, 19 years and older, 0.13 L/d) to 0.0024 (children, 3–10 years, 0.3 L/d). All values were orders of magnitude below the risk threshold, reinforcing the conclusion that Sr intake through sheep milk is not a significant concern. The percentage of provisional tolerable weekly intake (% PTWI) for Sr ranged from 8.28% (adults, 19 years and older, 0.13 L/d) to 26.35% (children, 3–10 years, 0.13 L/d). In the higher consumption scenario (0.3 L/d), the values increased significantly, ranging from 19.11% (adults, 19 years and older) to 60.81% (children, 3–10 years). While these values remain within the safety threshold of 100% PTWI, they highlight a potential concern: sheep milk alone contributes a disproportionately high percentage of the total allowable Sr intake. This means that even moderate consumption of other Sr-containing foods (such as leafy vegetables, grains, and drinking water) could lead to intake levels exceeding the recommended safety limits. The fact that milk alone accounts for over 60% of the PTWI in young children suggests that cumulative dietary exposure to Sr could be a health concern, particularly in regions where drinking water and staple foods also contribute significantly to Sr intake. This warrants further dietary assessments to ensure that total Sr intake remains within safe levels, particularly for populations with high milk consumption. The target hazard quotient (THQ) for Sr ranged from 0.01 (adults, 19 years and older, 0.13 L/d) to 0.04 (children, 3–10 years, 0.3 L/d), remaining below critical risk levels. The margin of exposure (MOE) for Sr, calculated based on the NOAEL of 140 mg/kg b.w., demonstrated exceptionally high safety margins across all groups. The lowest MOE value was recorded in children (3–10 years, 0.3 L/d: 12396), while the highest was observed in adults (19 years and older, 0.13 L/d: 91020), confirming safe exposure levels.

Table 5. Risk assessment parameters for strontium across different age groups

Age Group	HQ Sr		THQ Sr		RQ Sr		% PTWI Sr		MOE Sr	
	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d
3 - 10	0.02	0.04	0.02	0.04	0.0010	0.0024	26.35	60.81	28606	12396
11 - 14	0.01	0.02	0.01	0.02	0.0005	0.0013	13.80	31.85	54612	23665
15 - 18	0.01	0.01	0.01	0.01	0.0004	0.0009	9.66	22.30	78017	33807
19 and older	0.01	0.01	0.01	0.01	0.0003	0.0008	8.28	19.11	91020	39442

HQ – Hazard Quotient, THQ – Target Hazard Quotient, RQ – Risk Quotient, % PTWI – Percentage of the Provisional Tolerable Weekly Intake, MOE – Margin of Exposure, [a]Average daily milk consumption in Slovakia, [b]Recommended daily milk consumption scenario

3.3.3 Combined Risk Evaluation

The combined risk evaluation for Sr and Li across different age groups is presented in Table 6. When assessing total risk (Li + Sr), the total risk quotient (Total RQ) values ranged from 0.04 (adults, 19 years and older, 0.13 L/d) to 0.28 (children, 3–10 years, 0.3 L/d). Despite higher exposure in children, the total RQ remained below the 1.0 threshold, confirming an absence of immediate health risks. The total hazard quotient (Total THQ) values showed a similar trend, with the highest observed in young children consuming 0.3 L/d (3–10 years: 0.20), but still below critical risk levels. The hazard index (HI), representing the combined risk from lithium and strontium, ranged from 0.03 (adults, 19 years and older, 0.13 L/d) to 0.20 (children, 3–10 years, 0.3 L/d). Since all values remained below 1, no cumulative toxicological risk was identified. The total margin of exposure (Total MOE) remained consistently high, supporting the low-risk assessment. The lowest recorded total MOE was 1590 (children, 3–10 years, 0.3 L/d), which, while lower than other values, still indicates a substantial safety buffer. In contrast, adults consuming 0.13 L/d exhibited the highest total MOE of 11678, reinforcing the low-risk characterization.

The results indicate that while sheep milk alone does not immediately exceed toxicological safety limits for Li and Sr, its contribution to total dietary exposure is significant, particularly in young children. The EDI, HQ, RQ, THQ, HI, Total RQ, MOE, and Total MOE values remained below critical safety thresholds, suggesting no acute toxicological risk from milk consumption alone. However, the high percentage contribution of sheep milk to the provisional reference dose (% p-RfD) for Li and the provisional tolerable weekly intake (% PTWI) for Sr raises concerns regarding total dietary exposure. In young children consuming 0.3 L of sheep milk per day, milk alone accounts for up to 16.55% of the daily Li reference dose and 60.81% of the weekly Sr tolerable intake. This indicates that even moderate intake of other dietary sources (e.g., drinking water, vegetables, cereals) could lead to cumulative exposure levels approaching or exceeding the recommended limits. Therefore, long-term exposure and total dietary intake assessments are essential to ensure that cumulative exposure remains within safe limits, particularly for vulnerable populations such as young children.

These findings suggest that while sheep milk consumption alone does not pose an immediate toxicological hazard, its high relative contribution to Li and Sr intake warrants further investigation. A more comprehensive dietary risk assessment, considering all potential sources of Li and Sr in the diet, is needed to fully evaluate long-term health implications.

Table 6. Combined risk evaluation for Sr and Li across different age groups

Age Group	HI		Total THQ		Total RQ		Total MOE	
	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d	0.13[a] L/d	0.3[b] L/d
3 - 10	0.09	0.20	0.09	0.20	0.12	0.28	3670	1590
11 - 14	0.05	0.11	0.05	0.11	0.06	0.14	7007	3036
15 - 18	0.03	0.07	0.03	0.07	0.04	0.10	10010	4337
19 and older	0.03	0.06	0.03	0.06	0.04	0.09	11678	5060

HI – Hazard Index, THQ –Target Hazard Quotient, RQ –Risk Quotient, MOE – Margin of Exposure

4. DISCUSSION

4.1 Concentrations of Elements in Sheep Milk

The concentrations of essential and toxic elements in sheep milk observed in this study align with previously reported values in the literature but also reveal some notable variations. Calcium was found to be the most abundant essential element, with a mean concentration of 1355.16 mg/kg, ranging from 1108.03 mg/kg to 1553.50 mg/kg. This is consistent with findings by Antunović et al. (2023), who reported average Ca levels of 1400 mg/kg in Lacaune sheep milk from Croatia. Similarly, Pšenková & Toman (2021) recorded values between 1200 mg/kg and 1500 mg/kg in Slovakia, suggesting that our results are within the expected range for sheep milk produced under comparable conditions. The observed variability in Ca concentration may be attributed to differences in diet, soil mineral composition, and lactation stage, as previously suggested by Park et al. (2007). Additionally, Kulzhanova et al. (2024) found that calcium levels in sheep milk vary significantly depending on breed, with Kazakh fine-fleeced sheep exhibiting the highest Ca concentrations (231.23 mg/100g), while South Kazakh Merino sheep had the lowest (176.45 mg/100g). This suggests that genetic factors may also play a role in the mineral content of sheep milk. Raynal-Ljutovac et al. (2008) reported that sheep milk contains significantly higher levels of calcium compared to cow and goat milk, with values ranging from 1950 mg/kg to 2000 mg/kg. This suggests that our samples contain lower Ca concentrations than those reported in the literature, potentially due to differences in breed, feeding, or environmental factors.

Magnesium concentrations in our samples averaged 114.03 mg/kg, with individual values ranging from 87.57 mg/kg to 129.29 mg/kg. These findings are consistent with the work of Pilarczyk et al. (2013), who reported Mg levels of 110–130 mg/kg in organic sheep farms. The relatively stable distribution of Mg in our samples suggests effective physiological regulation of this element in sheep, as has been previously reported by Rosanoff et al. (2012). Kulzhanova et al. (2024) also observed significant breed-dependent variations in Mg levels, with Ordabasy sheep milk containing the highest Mg concentrations (126.17 mg/100g), whereas South Kazakh Merino milk had the lowest (26.75 mg/100g). Such variations highlight the importance of breed selection in optimizing milk mineral content for nutritional purposes. Comparing our Mg values with other milk sources, Singh et al. (2019) found that Mg concentrations in cow, goat, and buffalo milk ranged between 113.5 mg/kg and 152.8 mg/kg, with goat milk showing the highest values. These results suggest that our sheep milk samples exhibit Mg concentrations similar to those of other ruminant species, indicating that Mg homeostasis is well-maintained across different dairy animals. Raynal-Ljutovac et al. (2008) highlighted that Mg levels in sheep milk typically range from 180 to 210 mg/kg, which is higher than the values detected in our study. Given the role of Mg in enzymatic functions and bone health, the observed lower values may suggest differences in dietary Mg intake or bioavailability in our study area.

Iron exhibited considerable variability, with concentrations ranging from 0.98 to 3.19 mg/kg, with a mean of 1.31 mg/kg. This is lower than the 2.05 mg/kg reported by Almášiová et al. (2023) in sheep milk from industrially exposed areas, suggesting that the environmental conditions of our study region may be less influenced by anthropogenic pollution sources. However, our results are comparable to the

1.1–1.5 mg/kg range observed by Licata et al. (2004) in Mediterranean sheep breeds. The variability in Fe levels may be due to individual metabolic differences among animals, dietary iron content, and the extent of environmental exposure to Fe-rich soil and water sources. One possible explanation for the Fe concentrations observed in our samples is the presence of a natural spring located near the farm where the milk was collected. This spring is known to contain elevated levels of iron and manganese, which could influence Fe concentrations in the local environment. This could explain why Fe concentrations in some of our milk samples were on the upper end of the observed range. However, further analysis of environmental Fe sources would be needed to confirm this hypothesis. Despite this, our mean Fe concentration suggests that sheep milk remains a low dietary source of Fe compared to other iron-rich foods. Interestingly, Kulzhanova et al. (2024) reported higher Fe concentrations in Kazakh fine-fleeced and meat Merino sheep milk (5.24–6.73 mg/100g), indicating that certain breeds may have a greater ability to accumulate Fe from their diet and environment. This supports the notion that Fe variability in milk is strongly influenced by breed, diet, and environmental conditions. According to Raynal-Ljutovac et al. (2008), sheep milk contains Fe levels ranging from 720 to 1222 µg/kg, which is notably higher than that found in cow and goat milk. This suggests that sheep milk may indeed be an important source of dietary iron, contributing to the prevention of iron deficiency. The observed variability in our study highlights the need for further investigation into the factors influencing Fe uptake and metabolism in sheep milk production. Bilandžić et al. (2021) reported Fe concentrations in raw cow milk from Croatia ranging from 0.41 to 3.29 mg/kg, with the highest values observed in regions with increased industrial activity. When compared to our sheep milk samples, which showed Fe concentrations between 0.98 mg/kg and 3.19 mg/kg, it is evident that our values overlap with the upper range observed in Croatian cow milk. This supports the notion that environmental factors, such as soil composition and potential contamination, play a crucial role in Fe accumulation in milk. Additionally, the similarity in Fe variability between Croatian cow milk and our sheep milk suggests that the animals in both studies were likely exposed to comparable dietary and environmental Fe sources.

Strontium was detected in all analyzed samples, with a mean concentration of 0.804 mg/kg and a range of 0.48 to 1.09 mg/kg. These values are within the range reported for sheep milk from Slovakia, for example 0.90 - 1.11 mg/kg across farms located in regions with different environmental burden (Almášiová et al., 2023) and 0.901 - 1.047 mg/kg in milk from farms located in regions without and with moderate environmental load (Pšenková et al., 2025). These differences may be attributed to variations in soil composition and regional environmental contamination, as their study focused on areas with a potential environmental burden. The presence of Sr in milk has been associated with environmental factors, particularly soil composition and groundwater contamination, as strontium is known to bioaccumulate in plants and, consequently, in animal tissues (Skoryna, 1981). While Sr is not classified as a toxic element at low concentrations, excessive intake can lead to its substitution for calcium in bones, potentially affecting bone strength and mineralization (Blashko et al., 2013). The results of our study are consistent with findings from other investigations on Sr levels in milk from different animal species. Licata et al. (2004) reported Sr concentrations in bovine milk from dairy farms in Calabria, Italy, with values ranging from 0.6 to 1.1 mg/kg, which overlap with the range observed in our sheep milk samples. This suggests that strontium uptake in dairy animals is largely dependent on regional environmental factors, particularly soil mineral composition and water sources. Similarly, Bilandžić et al. (2015) analyzed Sr concentrations in cow and goat milk using inductively coupled plasma optical emission spectrometry (ICP-OES) and found Sr levels between 0.4 and 1.3 mg/kg. Our values fall within this range, further supporting the conclusion that Sr levels in milk vary primarily due to differences in geological and agricultural conditions rather than species-specific metabolism. Furthermore, Pšenková et al. (2025) emphasized that Sr levels in sheep milk can be influenced by industrial pollution and historical contamination in certain regions of Slovakia. Their study found that sheep grazing in areas with potential environmental burdens exhibited slightly elevated Sr concentrations compared to those from cleaner regions. Given that our study area is not classified as highly industrialized but falls into the category of a moderately burdened environment (Lieskovská and Lényiová, 2023), where contamination levels are elevated, the slightly lower average Sr concentration in our samples compared to their study (0.95 mg/kg) is still consistent with expected trends. These findings support the value of periodic surveillance studies of Sr in dairy products to better characterize long-term dietary exposure, particularly in high intake subgroups. Although sheep milk is not a significant dietary source of Sr, its presence is relevant due to potential human exposure through dairy consumption. Given the bioaccumulative properties of Sr, ongoing monitoring is necessary to assess its long-term impact on consumer health, particularly in regions with naturally elevated Sr concentrations in the environment (Bilandžić et al., 2015).

Lithium (Li) concentrations varied significantly among samples, averaging 0.024 mg/kg, with a range from 0.012 to 0.073 mg/kg. These values fall within the expected range based on findings by Grandjean & Aubry (2009), who reported Li concentrations between 0.015–0.030 mg/kg in dairy products. Lithium is naturally present in the environment and can enter the food chain through soil and water. The study by Astolfi et al. (2020) analyzed Li concentrations in various mammalian milk samples, including cow, goat, and donkey milk, and found that cow milk contained an average of 0.0016 mg/kg, goat milk contained 0.020 mg/kg, and donkey milk contained 0.0058 mg/kg. These results suggest that our sheep milk samples exhibit higher Li concentrations than those reported in cow and donkey milk but are comparable to values observed in goat milk. Compared to plant-based milk alternatives, Astolfi et al. (2020) reported that the highest Li concentrations were found in oat milk (0.018 mg/kg), followed by almond milk (0.014 mg/kg), and rice milk (0.0046 mg/kg). The relatively low Li levels in these plant-based beverages suggest that animal-derived milk, including sheep milk, may be a more consistent dietary source of lithium. This finding is relevant considering the growing interest in lithium as a potential micronutrient involved in neurological functions and mood regulation. Although Li is therapeutically used in psychiatry, its presence in food sources is not commonly associated with adverse effects at the levels detected in our study (ECHA, 2014). Nonetheless, continued monitoring is necessary to prevent excessive dietary exposure.

The absence of detectable arsenic (As) in all analyzed samples is a positive finding, suggesting no significant environmental contamination of grazing pastures. This aligns with the results of Licata et al. (2004), who also reported non-detectable As levels in sheep milk from uncontaminated areas. European Union regulations (Commission Regulation EC No. 1881/2006) set a maximum limit of 10 µg/kg for As in dairy products, and our findings confirm that the analyzed samples are well below this threshold.

The variability in elemental concentrations observed in our study highlights the complex interplay between environmental, physiological, and dietary factors that influence milk composition. Similar trends have been reported by Haenlein (2004) and Guiso et al. (2022), who emphasized that mineral content in sheep milk is subject to seasonal variations, soil composition, and farming practices. The relatively stable levels of Ca and Mg, coupled with low concentrations of toxic elements, support the notion that sheep milk remains a nutritionally valuable and safe food product. However, continued monitoring and risk assessments are essential, particularly in regions affected by industrial pollution or intensive agriculture.

4.2 Nutritional Contribution of Essential Elements

4.2.1 Calcium

Our results indicate that daily consumption of 0.3 L of sheep milk covers 58.1 % of the RDA for calcium in children aged 4–6 years, 45.2 % in those aged 7–10 years, decreases to 27.1 % in adolescents (15–18 years), and equates to 40.7 % in young adults (19–34 years) and 36.9 % in older adults (35–62 years). These results are in accordance with findings from Antunović et al. (2023), who reported 50–60 % RDA Ca in Lacaune sheep during lactation, and from Pšenková & Toman (2021), who observed 30–55 % RDA Ca in Slovak sheep milk from regions with moderate environmental burden. Additionally, Park et al. (2007) found that sheep milk provides 35–50 % RDA Ca across various breeds in Mediterranean climates.

Calcium bioavailability in sheep milk is enhanced by its casein–calcium complexes and favourable casein-to-whey protein ratio, which promote solubility and absorption in the gastrointestinal tract (Cashman, 2002; Raynal-Ljutovac et al., 2008). Seasonal fluctuations further influence calcium, magnesium, citrate and phosphate content in milk, as reported Dunshea et al. (2019). Breed-specific variations have been documented by Kulzhanova et al. (2024), indicating genetic and dietary drivers of mineral deposition.

Under Regulation (EU) No 1169/2011, foods providing ≥ 15 % of the reference nutrient intake per 100 mL may claim "source of calcium" (European Parliament & Council, 2011). Sheep milk qualifies across all age cohorts, most notably benefiting young children and the elderly. However, as RDA thresholds rise to 1200–1500 mg/day during adolescence and adulthood, complementary sources such as aged cheeses, fortified plant milks, and leafy greens are necessary to maintain adequate intake (Rizzoli et al., 2014; Weaver, 2015).

4.2.2 Magnesium

Our results show that a daily intake of 0.3 L of sheep milk covers 28.5 % of the RDA for magnesium in children aged 4–6 years, 17.1 % in those aged 7–10 years, 10.4-11.4 % in early adolescents (11–14 years), and only 8.6 % in older adolescents (15–18 years). In adults (19–34 years) and middle-aged cohorts (35–62 years), coverage remains at approximately 8.3 %, with seniors (63–79 years) likewise at 8.3 % (Pšenková & Toman, 2021; Pilarczyk et al., 2013). Magnesium solubility is enhanced by lactose (Rosanoff et al., 2012), yet high Ca:Mg ratios (> 10:1) can impede uptake (Rude et al., 2009). Sheep milk's Ca:Mg ratio (~12:1) may reduce Mg bioavailability, as demonstrated in Caco-2 cell assays (Kowalczyk et al., 2023). Seasonality can influence magnesium concentrations in sheep milk, with late-season milk showing higher Mg levels than early and mid-season samples (Li et al., 2022). Breed-dependent differences of up to 25 % further underline genetic influences (Kulzhanova et al., 2024).

Under Regulation (EU) No 1169/2011, foods providing 15–30 % RNI per 100 mL can be labeled as "moderate source" of a mineral (European Parliament & Council, 2011). Sheep milk fits this for children but falls short in other groups, necessitating dietary diversification with nuts, seeds, whole grains, and leafy greens. Adequate Mg intake supports over 300 enzymatic processes critical to bone, metabolic, and neuromuscular health (Mertz, 1981; Rizzoli et al., 2014; Weaver, 2015). Emerging clinical evidence links marginal Mg deficiency to cardiovascular disease, insulin resistance, and migraine risk (Barbagallo et al., 2021; DiNicolantonio et al., 2018). Meta-analyses reveal a 22 % reduction in hip fracture risk with higher Mg intake among older adults (Farsinejad-Marj et al., 2016).

4.2.3 Iron

Our results indicate that a daily intake of 0.3 L of sheep milk covers 4.4 % of the RDA for iron in young children (4–6 years), declines to 3.9 % in children (7–10 years), further decreases to 2.5-3.3 % in early adolescents (11–14 years), and reaches as low as 2.2 % in adolescent girls (15–18 years). In adults (19–34 years) and older adults (35–62 years), iron coverage remains between 2.6 % and 2.3 %, with minimal variation among seniors (63–79 years) at approximately 2.8 %. These values correspond to an average iron concentration of 1.31 mg/kg in our sheep milk samples, similar to values reported by Bilandžić et al. (2021) for cow milk (0.41–3.29 mg/kg) and Almášiová et al. (2023) for Slovak sheep milk (2.05 mg/kg). However, they are lower than concentrations observed in Kazakh fine-fleeced sheep (5.24–6.73 mg/100 g) by Kulzhanova et al. (2024) and align with Licata et al. (2004), who measured 1.1–1.5 mg/kg in Mediterranean goat milk. Recent research has explored dairy-based iron fortification, showing that microencapsulated iron salts added to milk maintain stability and sensory properties, achieving an additional 5–7 % RDA coverage without adverse organoleptic effects (Hallberg & Hulthén, 2000).

Iron bioavailability in milk is limited by the predominance of non-heme forms and competitive inhibition by calcium; Perfecto et al. (2017) demonstrated up to a 50 % reduction in iron transport in Caco-2 cells when iron was presented in dairy matrices. While lactoferrin-bound iron may enhance uptake, it represents only 10 % of total milk iron (Lönnerdal, 2009), limiting its overall impact on iron status. Dietary inhibitors such as phytic acid in grains and polyphenols in plant foods can reduce non-heme iron absorption by up to 50 %, while enhancers like ascorbic acid and fermentation processes can increase bioavailability by over 30 % (Hurrell & Egli, 2010; Rodríguez-Ramiro et al., 2019).

Under Regulation (EU) No 1169/2011, foods supplying less than 15 % of the RNI per 100 mL cannot be marketed as a "source of iron" (European Parliament & Council, 2011). Therefore, sheep milk should be consumed alongside iron-rich foods such as red meat, legumes, or fortified cereals, and paired with vitamin C-rich fruits to improve non-heme iron absorption (Zimmermann & Hurrell, 2007; EFSA, 2015). Iron is crucial for cognitive development, oxygen transport, and energy metabolism across all life stages, with deficiencies linked to anemia, impaired neurological function in children, and reduced work capacity in adults (Abbaspour et al., 2014). Public health modelling indicates that incorporating iron-fortified sheep milk into the diet of at-risk populations could reduce anemia prevalence by 8–12 % over five years, particularly when combined with nutrition education to improve dietary diversity (WHO, 2016). Also, agronomic biofortification studies indicate that supplementing sheep diets with iron-enriched feed can significantly increase iron content in their milk, thus enhancing its nutritional value and potentially benefiting consumer health (Witkowska et al., 2015).

4.3 Health Risk Assessment of Toxic Elements

4.3.1 Lithium

Our estimated daily intake (EDI) of lithium from sheep milk ranged from 0.000045 mg/kg b.w./day in adults (19 years and older, 0.13 L/day) to 0.000331 mg/kg b.w./day in young children (3–10 years, 0.3 L/day), corresponding to only 2.25–16.55 % of the provisional reference dose (p-RfD = 2 μg/kg b.w./day) (U.S. EPA, 2008; ECHA, 2014). The margin of exposure (MOE = NOAEL/EDI), using a NOAEL of 0.604 mg/kg b.w., ranged from 1824 to 13397, orders of magnitude above the commonly accepted benchmark of 100, indicating a large safety buffer and negligible non-carcinogenic risk. Lithium is almost completely absorbed (80–100 %) from the gastrointestinal tract, achieving peak serum levels within 2–4 hours post-dose (Gitlin, 2016). In plasma, it exists nearly entirely as the free ion, facilitating rapid distribution across cell membranes and penetration of the blood–brain barrier. Its apparent volume of distribution (0.6–0.9 L/kg) reflects extensive partitioning into total body water, including the brain and bone (MoHM, 2021). Lithium undergoes minimal metabolism and is eliminated almost exclusively via renal glomerular filtration with partial tubular reabsorption; elimination half-life varies between 18 and 36 hours depending on hydration status and renal function (StatPearls Publishing 2023). This predominance of renal clearance makes lithium levels highly sensitive to changes in kidney perfusion and requires that any risk assessment account for population-specific renal variability.

Toxicologically, HQ < 1 signifies that adverse effects from chronic lithium exposure through milk alone are highly unlikely, and MOE ≫ 100 further corroborates low concern for all age groups. However, our paediatric HQ of 0.17 (3–10 years) represents the highest relative exposure, highlighting the importance of monitoring cumulative intake in diets that include other Li-rich items such as drinking water and certain vegetables (Schrauzer, 2002). Typical total dietary lithium intake in adults (from water, grains, vegetables) ranges from 0.24 to 1.5 μg/kg b.w./day, so sheep milk contributes less than 1 % of overall exposure. Although low-dose lithium has been associated with neuroprotective and mood-stabilizing benefits (Grandjean & Aubry, 2009; WHO, 2006), cumulative exposure in regions with high environmental lithium (e.g., drinking water) warrants integrated dietary assessments (Memon et al., 2020). In summary, while lithium from sheep milk alone poses no acute or chronic toxicity risk, integrated exposure assessments, including water, diet, and environmental sources, are essential for vulnerable populations, particularly young children and individuals in high-Li regions.

4.3.2 Strontium

Our estimated daily intake (EDI) of strontium from sheep milk ranges from 0.001538 mg/kg b.w./day in adults (19 years and older, 0.13 L/day) to 0.011294 mg/kg b.w./day in young children (3–10 years, 0.3 L/day). When benchmarked against the provisional tolerable weekly intake, these EDIs represent 8.3–60.8 % of the PTWI. Calculated hazard quotients are 0.01–0.04, and margins of exposure using a NOAEL of 140 mg/kg b.w. (ATSDR, 2004) exceed 12000 even in the highest-exposure scenario, indicating minimal likelihood of non-carcinogenic risk across all age groups. Strontium is absorbed by passive diffusion and active Ca-channels in the small intestine (fractional absorption approximately 20–30 %), shares transporters with calcium, and is incorporated preferentially into growing bone where it can replace Ca^{2+} in hydroxyapatite lattices (Skoryna, 1981). Systemic distribution mirrors calcium kinetics; 98 % of body burden resides in the skeleton with a biological half-life of 15–30 years. Renal clearance eliminates the plasma fraction within hours, but skeletal Sr is released slowly via bone remodelling, explaining its long-term residence and potential cumulative effects (ATSDR, 2004).

While HQ and MOE suggest negligible risk from milk-borne Sr alone, the PTWI data reveal that sheep milk can supply up to 61 % of the weekly tolerable intake in 3–10-year-olds at 0.3 L/day. This is noteworthy because high skeletal Sr accumulation has been linked to Ca-deficient rickets in children (Özgür et al., 1996). Regulatory limits for Sr in dairy products are not harmonised: National Health Commission of the People's Republic of China (2025) sets 7 mg/kg (fresh milk), whereas the EU currently lacks a specific maximum level, relying instead on PTWI guidance. Our mean milk concentration (0.804 mg/kg) is an order of magnitude below Chinese limits and consistent with previous reports for Slovak sheep (0.6–1.25 mg/kg; Pšenková & Toman, 2021). Given that milk contributes disproportionately to total Sr intake in young children, periodic screening of Sr in milk may be useful for refining cumulative dietary exposure estimates, particularly in regions with Sr rich geology. Dietary diversification (e.g., alternating sheep and cow milk) and ensuring adequate calcium intake can mitigate skeletal Sr uptake. Future studies should couple dietary exposure with serum and bone Sr measurements to elucidate long-term health implications.

4.3.3 Combined Risk Evaluation

The combined hazard index (HI), integrating lithium and strontium exposures, ranged from 0.03 in adults (19 years and older, 0.13 L/day) to 0.2 in young children (3–10 years, 0.3 L/day). All HI values remained well below the critical threshold of 1.0 defined by the U.S. EPA for non-carcinogenic risk (U.S. EPA, 1989), indicating no appreciable aggregate hazard from concurrent ingestion of these elements via sheep milk. Total target-hazard quotients for Li and Sr summed to a maximum of 0.2 in the highest child-exposure scenario, again well under unity, while the total risk quotient peaked at 0.28, which is far below the level of concern identified by international food-safety agencies (U.S. EPA, 2007). These conservative indices incorporate surrogate reference doses that already include uncertainty factors of 100–1 000, underscoring the generous safety margin. The total margin of exposure remained ≥ 1590 for the worst-case scenario, comfortably exceeding the customary benchmark of 100 for non-genotoxic substances (EFSA, 2020). Although lithium and strontium share renal excretion pathways with calcium and sodium, current toxicokinetic models do not predict synergistic toxicity at concentrations < 1 % of their respective therapeutic or toxicological thresholds (Grandjean & Aubry, 2009; ATSDR, 2004).

From a nutritional-toxicological standpoint, these data confirm that simultaneous intake of Li and Sr through sheep milk poses a negligible combined risk for any Slovak age cohort, even under the high-consumption scenario of 0.3 L/day. Nevertheless, because Li burden is dominated by drinking water and Sr burden by leafy vegetables and cereal grains, a whole-diet approach remains essential. In vulnerable groups (young children, individuals with impaired renal function), periodic biomonitoring of blood Li/Sr and dietary counselling to ensure adequate calcium intake (which competitively inhibits skeletal Sr deposition) are prudent risk-management measures. Future research should couple longitudinal dietary surveys with biomarker data (serum, urine, bone biopsies) to validate these risk estimates and refine reference doses. Integration of physiologically based pharmacokinetic models for multi-element exposure would further enhance the precision of cumulative risk assessment. Therefore, generating additional occurrence data on Li in dairy products through periodic surveys would improve the robustness of cumulative exposure assessments, especially for young children with high milk intake.

The comprehensive risk assessment performed in this study provides critical insights into the potential health risks associated with the long-term consumption of sheep milk contaminated with toxic elements. The findings are particularly relevant for: 1) Public health policymakers, who need data for setting regulatory limits on heavy metal concentrations in dairy products. 2) Nutritionists and healthcare professionals, for advising at-risk populations (children, pregnant women, elderly) on safe dairy consumption levels. 3) Food safety authorities, for monitoring and enforcing strict quality control in the dairy industry. If HQ, HI, CR, or TCR values exceed safe thresholds, additional toxicological studies and risk mitigation strategies should be considered.

5. CONCLUSIONS

Sheep milk from a moderately disturbed region of Slovakia delivers a substantial source of calcium and provides a moderate contribution to dietary magnesium intake, particularly in younger age groups, with its mineral profile being comparable or more favorable than that of other dairy types. In contrast, the contribution of sheep milk to dietary iron intake remains low across all population groups and should be considered nutritionally limited rather than substantial. On the other hand, lithium and strontium levels remain within safe limits; however, highly sensitive groups (particularly young children) reach a significant portion of the tolerable limits from a single source, underscoring the need for comprehensive exposure assessment. With respect to arsenic, no potential health risk associated with sheep milk consumption was identified in the studied region. In practice, this means that producers should consider ways to further optimize magnesium and iron content (e.g., by selecting specific feed or supplementing trace elements), and regulatory authorities may consider periodic screening of lithium and strontium in dairy products to support cumulative dietary exposure assessments in high consuming and vulnerable subgroups, particularly young children. Consumers, especially parents of young children, should include sheep milk in a balanced diet to maximize its nutritional benefits while minimizing cumulative exposure.

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