

Essential and toxic elements concentrations in animal tissues of sheep from two different regions of Slovakia

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Article Details: Received: 2020-09-23 | Accepted: 2020-10-05 | Available online: 2020-12-31

<https://doi.org/10.15414/afz.2020.23.04.217-223>



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Animal products and meat from farm animals are consumed daily and it is very good source of animal proteins, but consumer's information about exposure to heavy metals in meat and its health risk is in general low. The main goal of the present work was to determine essential and toxic elements in offal and meat of sheep from two different regions of Slovakia with different environmental load. In present study 11 elements (essential elements: calcium, zinc, magnesium, selenium, iron, copper; toxic elements: arsenic, cadmium, mercury, lead, nickel) have been analysed. Statistically significant differences ($P < 0.01$) were noted between the concentrations of Ca, Zn, Cu in the liver, Zn and Se in the kidneys of animals, Ca, and Mg in muscle. In the liver of sheep, statistically significant differences ($P < 0.05$) was detected in concentration of Fe and in the case concentration of Se in the mammary gland and muscle of animals. Chemical analyses between the control and experimental group of animals indicated increased concentrations of Cd in the liver and kidney of animals in both monitored groups, which exceeded the maximum residual limit. Results of this also showed statistically significant correlations between some elements in animal tissue samples.

Keywords: tissues, sheep, essential elements, toxic elements

1 Introduction

The concentrations of different essential and toxic elements in livestock are interest from both human and animal health (Suttle, 2010; McLachlan et al., 2016). Exposure of farm animals to high concentrations of toxic metals such as lead (Pb), arsenic (As), cadmium (Cd), and nickel (Ni), or less than optimal concentrations of the essential elements, such as calcium (Ca), zinc (Zn), magnesium (Mg) or selenium (Se) can adversely affect the productivity of animals (Suttle, 2010). Meat from sheep can form an important part of the human diet. However, the consumption of this meat contributes to the exposure of consumer to toxic element which may be present in sheep tissues. There are several studies that have recorded levels of various elements in tissues of sheep (Gerber et al., 2009; Bilandžić et al., 2010; Ikem et al., 2015).

The intake of heavy metals by humans and animals is mainly through inhalation, in addition, the industrial activity greatly affects the concentrations of these elements in the food chain (Jarzyńska and Falandysz, 2011; Oymak et al., 2017). Essential and toxic elements, that animals get from feed and drinking water accumulate in the tissues of internal organs, such as kidney, liver, or muscle, which are consumed by humans worldwide. Ordinary consumers often do not know what health risks posed by toxic elements and they have no knowledge of the quantity of the heavy metals in these products (Lavery et al., 2008; Okareh, 2015). Therefore, monitoring the concentrations of heavy metals in organs for consumption is important from the point of view controlling of the effects of these metals on animal and human health (Giussani, 2011; Roggeman et al., 2014). In addition, sheep which are reared freely on pasture are also good indicators of the environmental pollution

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(Tunegová et al., 2016). As animal production changes over time, it is very important still update knowledge about concentrations and effects of elements in animal tissues. The aim of this study was to determine and compare the presence of essential and toxic metals in sheep's offal and meat in two different regions of Slovakia with different environmental load.

2 Material and methods

Sheep tissue samples were taken from two areas of Slovakia with different environmental load. A control group of animals (5 sheep, Tsigai breed; 7 years old) was obtained from area with undisturbed environment in the North of Slovakia and experimental group of animals (5 sheep, Tsigai breed; 7 years old) was obtained from area with slightly disturbed environment in the Western of Slovakia. The area with slightly disturbed environment is characterized by the load of energy emissions produced by the thermal power station in this area. The power station causes increased concentrations of heavy metals in soils in this region.

All animals were humanely killed in a registered slaughterhouse and samples of all tissues were taken immediately after slaughter. From each sheep, kidney (two kidneys per animal, 200 g), liver (200 g), muscle – musculus quadriceps femoris (200 g), and mammary gland (200 g) tissue of sheep were collected. The total number 40 samples of tissues were collected (20 samples in each group of animals). All samples were stored after collection in plastic bags in freezers at -18°C until analysis.

2.1 Analysis of biological samples

Animal tissue samples were analysed in collaboration with a certified testing laboratory – Eurofins Bel/Novamann Nové Zámky (Slovakia). All tissue samples were homogenized using commercial food processor prior to analysis. From each tissue, samples (1 g) were mineralised by HNO₃ and H₂O₂ microwave decomposition (Microwave oven, MARS 6 240/50) for determination of essential and toxic elements – Ca, Mg, Zn, Se, Cu, Fe, Cd, As, Hg, Ni, Pb.

As and Se in animal tissues were analysed using HG-AAS (hydride generation atomic absorption spectroscopy; Spectr AA-220 FS, Netherlands), Ca, Fe, Mg using ICP-AES (inductively coupled plasma-atomic emission spectrometry; Varian 720-ES, USA), Cd, Pb, Ni using ETA-AAS (electro thermal atomization, atomic-absorption spectroscopy; Agilent DUO AA 240Z/240FS, USA), Zn and Cu using F-AAS (flame atomic-absorption spectroscopy; Agilent DUO AA 240Z/240FS, USA) and Hg using AMA-AAS (Advanced Mercury analyser, atomic-absorption spectrometry; Altec CR).

The limits of detection (LOD) in animal tissues were as follows: As, 0.010 mg kg⁻¹; Cd, 0.003 mg kg⁻¹; Ni, 0.03 mg kg⁻¹; Pb, 0.017 mg kg⁻¹; Hg, 0.003 mg kg⁻¹; Ca, 2.0 mg kg⁻¹; Mg, 0.3 mg kg⁻¹; Se, 0.010 mg kg⁻¹; Zn, 0.17 mg kg⁻¹; Cu, 0.17 mg kg⁻¹; Fe, 0.17 mg kg⁻¹.

The limit of quantification (LOQ) in animal tissues were as follows: As, 0.030 mg kg⁻¹; Cd, 0.010 mg kg⁻¹; Ni, 0.10 mg kg⁻¹; Pb, 0.050 mg kg⁻¹; Hg, 0.010 mg kg⁻¹; Ca, 6 mg kg⁻¹; Mg, 1.0 mg kg⁻¹; Se, 0.030 mg kg⁻¹; Zn, 0.50 mg kg⁻¹; Cu, 0.50 mg kg⁻¹; Fe, 0.05 mg kg⁻¹.

2.2 Statistical analysis

All results were statistically evaluated with statistical package IBM SPSS v.20. Descriptive statistics (mean, standard deviation, minimum values, maximum values) using one-way ANOVA were calculated. Then, statistical significance of results between control and experimental group of animals were separated using Student's *T*-test. Pearson's correlation analysis was used to determine the interactions between the concentrations of selected essential and toxic elements in animal tissues.

3 Results and discussion

Results of concentrations of selected essential and toxic elements in animal tissues are summarized in Table 1–4.

Since the liver and kidney are organs directly involved in the metabolism of essential as well as toxic elements, it was assumed that these elements would be detected in these organs in this study as well. In addition, these organs are typical organs for the accumulation of toxic elements. Concentrations of these elements could be influenced mainly by the age of sheep (7 years), as with increasing age the accumulation increases. Comparison of control and experimental group of animals showed statistically significant higher concentrations of Zn and Cu ($P < 0.01$) and Fe ($P < 0.05$) and significantly lower ($P < 0.01$) concentration of Ca in the liver of sheep from experimental group (slightly disturbed environment). Of the toxic elements, the highest concentration was recorded in the case of Cd in the liver of the experimental group and in the case of As in the liver of the control group of animals (Table 1). In addition, a concentration of Cd in liver in both of groups of animals exceeding the MRL (maximum residual limit; 0.05 mg kg⁻¹) according to Commission Regulation (EC) No 1881/2006 was recorded. MacLachlan et al. (2016) in the monitoring of selected elements in the organs of Australian sheep recorded in the liver 0.0280 mg Cd kg⁻¹, 0.01 mg As kg⁻¹, 0.040 mg Cu kg⁻¹, 0.31 mg Se kg⁻¹ and 37.2 mg Zn kg⁻¹. Compared to this work, were found a higher concentration of Cd in the liver of animals in both groups, a higher concentration of As in the liver of sheep from control group and

a significantly higher concentration of Cu in the liver of sheep from experimental group, where was found a concentration of 179.6 mg kg⁻¹. Cd is an element that, with its abilities can cause serious pathological conditions in various organs and produces a variety of health risks in humans and animals (El-Boshy et al., 2015). Some studies have shown that exposure to Cd can lead to liver, ovarian and testicular toxicity in poultry (Yang et al., 2012).

By comparing the concentrations of elements in the kidneys of sheep, was found a statistically significantly higher ($P < 0.01$) concentration of Zn in sheep from experimental group and a lower concentration of Se ($P < 0.01$) (Table 2). It was also recorded the occurrence of toxic elements Cd, As in the kidneys of animals, while the higher concentration of Cd was in group of experimental

animals and the concentration of As in control group. As in the case of the liver, Cd concentration also was found in the kidneys in both of groups of animals, that significantly exceeded the MRL limit according to Commission Regulation (EC) No 1881/2006. In addition, an average concentration of Ni (0.16 mg kg⁻¹) in the kidneys of animals from experimental group was recorded. The kidney is well known to be the most critical organ affected by chronic cadmium exposure. Wang (2013) states that Cd can cause various histopathological changes in various renal segments, damage to the tubular system, and renal dysfunction (Johannes et al., 2006).

Slamečka et al. (1994) studied the accumulation of heavy metals in the organs of the hare. In the analysis of the liver and kidneys of rabbits, the Cd content in the liver

Table 1 Levels of essential and toxic elements in liver of control and experimental group of animals (mg kg⁻¹)

Control group				Experimental group			
Element	mean ±SD	min	max	element	mean ±SD	min	max
Ca	92.20 ±20.32	69.0	125,0	Ca	57.02 ±5.17**	52.0	65.0
Mg	116.80 ±58.023	14.0	150,0	Mg	134.20 ±5.72	128.0	139.0
Zn	14.04 ±6.69	6.2	22,1	Zn	35.08 ±5.18**	31.3	43.9
Se	0.33 ±0.18	0.16	0.59	Se	0.115 ±0.06	0.08	0.23
Cu	11.78 ±8.11	0.58	19.3	Cu	179.6 ±82.50**	89.0	313.0
Fe	80.14 ±12.59	61.0	94.0	Fe	150.60 ±59.23*	95.0	251.0
Cd	0.08 ±0.05	0.06	0.17	Cd	0.12 ±0.05	0.037	0.15
As	0.05 ±0.02	0.034	0.069	As	<0.030		
Hg	<0.002			Hg	<0.002		
Ni	<0.10			Ni	<0.10		
Pb	<0.010			Pb	<0.010		

SD – standard deviation; Min – minimum; Max – maximum; * $P < 0.05$; ** $P < 0.01$

Table 2 Levels of essential and toxic elements in kidney of control and experimental group of animals (mg kg⁻¹)

Control group				Experimental group			
Element	mean ±SD	min	max	element	mean ±SD	min	max
Ca	129.40 ±27.88	88.0	151.0	Ca	132.40 ±5.31	78.0	158.0
Mg	130.20 ±4.55	123.0	135.0	Mg	134.40 ±21.17	117.0	170.0
Zn	9.42 ±4.36	4.5	12.8	Zn	18.84 ±2.46**	15.5	22.3
Se	1.42 ±0.25	1.1	1.8	Se	0.85 ±0.07**	0.7	0.9
Cu	1.75 ±0.95	0.64	2.6	Cu	14.82 ±25.82	3.0	61.0
Fe	40.42 ±10.19	26.0	52.0	Fe	64.14 ±46.26	3.0	113.0
Cd	0.47 ±0.19	0.32	0.78	Cd	0.64 ±0.15	0.47	0.84
As	0.06 ±0.02	0.038	0.075	As	0.03 ±0.002	0.031	0.034
Ni	<0.10			Ni	0.16 ±0.06	0.12	0.26
Hg	<0.002			Hg	<0.002		
Pb	<0.010			Pb	<0.010		

SD – standard deviation; Min – minimum; Max – maximum; * $P < 0.05$; ** $P < 0.01$

of rabbits recorded 0.262 mg kg⁻¹ and in the kidney 3.603 mg kg⁻¹ and also reported that Cd accumulates more in the kidneys and its levels tend to increase strongly with age. Compared to the liver and kidney analyzes of sheep in our work, we recorded lower Cd values in the liver and kidneys.

Analysis of the concentrations of elements in the muscle between the observed groups of animals showed a statistically significantly higher ($P < 0.01$) concentration of Zn in sheep from experimental group. The other analyzed elements Ca, Se, Fe occurred in a higher concentration in the muscle tissue of sheep in control group and the differences between the groups were statistically significant (Ca: $P < 0.01$; Se and Fe: $P < 0.01$) (Table 3). In addition, concentration of As (0.05 mg kg⁻¹)

was found in the muscle of sheep in control group and a concentration of Ni (0.12 mg kg⁻¹) was also recorded in one sample of sheep muscle tissue from experimental group. The most significant effects of Ni exposure are carcinogenicity and neurotoxicity (Zhao et al., 2009). Inhaled or ingested Ni from the environment can lead to multiple toxic effects in organs, including the lung, liver, kidney, and brain (Costa et al., 2002; Das et al., 2008). Nickel could replace some other metals (especially Zn) in various enzymes of the body, which can lead to changes in the function of proteins in the body (Chen et al., 2017). In work of MacLachlan et al. (2016), they pointed to lower concentrations of As, Cu and higher concentrations of Zn in sheep muscle compared to results of present work.

Table 3 Levels of essential and toxic elements in muscle of control and experimental group of animals (mg kg⁻¹)

Control group				Experimental group			
Element	mean ±SD	min	max	element	mean ±SD	min	max
Ca	196.40 ±274.03	117.0	296.0	Ca	63.42 ±8.68**	51.0	75.0
Mg	188.00 ±12.98	167.0	202.0	Mg	166.60 ±18.46	144.0	195.0
Zn	14.96 ±3.19	9.5	17.3	Zn	27.36 ±3.24**	21.9	29.8
Se	0.31 ±0.20	0.07	0.59	Se	0.04 ±0.005*	0.038	0.049
Cu	1.05 ±0.27	0.82	1.5	Cu	6.64 ±10.69	0.66	25.4
Fe	21.58 ±6.97	12.0	29.0	Fe	9.99 ±5.29*	1.0	13.0
As	0.05 ±0.009	0.035	0.058	As	<0.030		
Ni	<0.10			Ni	0.12a		
Cd	<0.0040			Cd	<0.0040		
Hg	<0.002			Hg	<0.002		
Pb	<0.010			Pb	<0.010		

SD – standard deviation; min – minimum; max – maximum; * $P < 0.05$; ** $P < 0.01$; a concentration of metal only in one sample of tissue

Table 4 Levels of essential and toxic elements in mammary gland of control and experimental group of animals (mg kg⁻¹)

Control group				Experimental group			
Element	mean ±SD	min	max	element	mean ±SD	min	max
Ca	2,224.80 ±1,598.11	943.00	4,311.00	Ca	1,474.00 ±1,223.04	354.0	2,840.0
Mg	386.40 ±483.82	147.00	225.00	Mg	176.20 ±50.33	134.0	254.0
Zn	21.06 ±29.65	6.10	74.00	Zn	13.26 ±3.35	10.20	17.60
Se	0.28 ±0.08	0.07	0.39	Se	0.12 ±0.02**	0.09	0.15
Cu	0.97 ±0.16	0.82	1.20	Cu	1.36 ±0.33	1.0	1.8
Fe	35.7 ±8.41	27.50	47.7	Fe	51.52 ±8.05*	42.7	64.1
Cd	0.016a			Cd	<0.010		
As	0.031 ±0.001	0.03	0.032	As	<0.030		
Ni	<0.10			Ni	0.12a		
Pb	0.071a			Pb	<0.050		
Hg	<0.002			Hg	<0.002		

SD – standard deviation; min – minimum; max – maximum; * $P < 0.05$; ** $P < 0.01$; a concentration of metal only in one sample of tissue

Chemical analysis of element in the mammary gland (Table 4) of sheep showed a higher concentration of Fe in sheep from experimental group ($P < 0.05$) and a lower concentration of Se ($P < 0.01$) compared to sheep from control group. Concentrations of Ca, Mg, Zn were

significantly higher in sheep from the undisturbed area in control group, but the differences between groups of animals were not statistically significant. However, in the mammary glands of sheep were also recorded the occurrence of toxic elements, Cd (0.016 mg kg^{-1}) and in

Table 5 Correlations between concentrations of essential and toxic elements in animal tissues

Element	Ca	Zn	Se	Fe	Cu	Mg	Cd	Ni
LIVER								
Ca		0.667*	0.314	-0.513	-0.717*	0.110	-0.110	
Zn			-0.748*	0.603	0.851*	0.252	0.307	
Se				-0.516	-0.454	0.165	-0.311	
Fe					0.501	0.128	0.134	
Cu						0.183	0.502	
Mg							-0.586	
Cd								
Ni								
KIDNEY								
Ca		0.036	-0.311	0.250	0.032	0.071	-0.136	0.3
Zn			-0.574	0.562	0.277	0.358	0.511	0.684
Se				-0.316	-0.252	-0.056	-0.374	0.141
Fe					-0.479	0.654*	-0.251	0.617
Cu						-0.256	0.558	-0.366
Mg							-0.134	0.975**
Cd								-0.470
Ni								
MUSCLE								
Ca		-0.788**	0.434	0.849**	-0.292	0.511		
Zn			-0.560	-0.709	0.275	-0.387		
Se				0.492	-0.266	0.614		
Fe					-0.644*	0.623		
Cu						-0.647*		
Mg								
Cd								
Ni								
MAMMARY GLAND								
Ca		0.690*	0.058	-0.571	-0.119	0.698*		
Zn			-0.192	-0.431	-0.239	0.992**		
Se				-0.456	-0.498	-0.085		
Fe					0.654*	-0.516		
Cu						-0.313		
Mg								
Cd								
Ni								

* $P < 0.05$; ** $P < 0.01$; 0–0.33 low correlation; 0.34–0.66 middle correlation; 0.67–1.0 strong correlation

one sample of mammary gland Pb (0.071 mg kg⁻¹) and in one sample of the mammary gland the concentration of Ni (0.12 mg kg⁻¹) from experimental group. The concentration of toxic elements in the mammary gland, which were found in this work, could subsequently affect the occurrence of these elements in sheep's milk. But as we report in our work (Tunegová et al., 2018), despite the presence of toxic elements in the mammary gland of sheep, which came from the experimental group, we did not find any toxic elements in the milk of these animals.

In Table 5 are stated results of Pearson's correlation analyses between selected essential and toxic elements in tissues of sheep.

The available literature describes more frequent interactions between various elements, especially in kidneys and liver, or muscle tissue of animals (López-Alonso et al., 2004; Blanco-Penedo et al., 2006). Tomza-Marciniak et al. (2011) state the most numerous correlations between the essential elements and the toxic elements in the kidneys and followed by the liver. López-Alonso et al. (2004) state that interactions in organs such as the liver and kidneys occur because these organs playing a major role in the metabolism of trace elements. In this work were found significant correlations in sheep liver between Zn and Ca ($r = -0.667$; $P < 0.01$), Cu and Ca ($r = -0.717$; $P < 0.05$), Se and Zn ($r = -0.748$; $P < 0.01$) and Cu and Zn ($r = 0.851$; $P < 0.01$). In the kidney of sheep, Mg and Fe ($r = 0.654$; $P < 0.05$) and Ni and Mg ($r = 0.975$, $P < 0.05$) correlated positively and statistically significantly. Muscle tissue analysis showed significant correlations between Zn and Ca, Fe and Ca, Fe and Zn, Mg and Cu, Cu and Fe. We found strong positive correlations in the mammary gland of sheep between Zn and Ca, Mg and Ca, Mg and Zn, and Cu and Fe. Authors Wang et al. (2012) report correlations between essential elements (Z, Cu, Fe, Mn, Se) and toxic (Cd, Pb) in blood and in urine. Several studies suggest that interactions between Cd, Pb and Zn in the body result in a high affinity of these metals for metallothionein and their ability to induce its synthesis. They can induce its synthesis in various tissues, especially in the intestine, liver, and kidney (Cai et al., 2009). Metallothionein is synthesized in response to exposure to Cd, Zn, Cu or Hg (metallothionein inducers contribute to the accumulation of metals by their elimination from metabolism) (Olsson et al., 2010).

4 Conclusions

The results of this study indicate that the monitoring of toxic elements in animal products is important, as was found increased Cd levels according to MRL in the liver and kidneys of sheep in both of groups. Frequent consumption of such animal products can pose a risk

to the consumer, even though they are a good source of some essential elements. From the point of view of consumer health protection, it is better to give priority to the consumption of meat – muscle, where we recorded the average values of toxic elements below the LOQ in comparison with internal organs. Despite the different environmental loads of the monitored areas, toxic elements in animal tissues were also recorded in area with undisturbed environment. To find out the reasons, why they were elevated levels of some toxic elements in the body of animals in this area further research is needed.

Acknowledgments

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

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