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OCCURRENCE OF SELECTED METALS IN FEED AND SHEEP´S MILK FROM AREAS WITH DIFFERENT ENVIRONMENTAL BURDEN

Martina Tunegová, Róbert Toman, Vladimír Tančin, Martin Janíček

ABSTRACT

The content of selected essential elements and toxic metals in feed and sheep's milk from areas with different parts of Slovak Republic was analyzed. Region of Novoť (undisturbed environment; North Slovakia) and region of Klátova Nová Ves (widely disturbed environment; Western Slovakia) were under investigation. Eleven metals have been analyzed (essential elements - calcium, zinc, selenium, iron, magnesium, copper; toxic elements – arsenic, mercury, lead, cadmium, nickel). Samples of feeds and milk were collected five-times during the year (spring and autumn season). Analyses of samples were performed by certified testing laboratory Eurofins Bel/Novamann (Nové Zámky, Slovak Republic). Analyses were performed by routine methods, according to the valid methodologies. The results showed significantly higher content of selected essential elements in feed in spring season from area with widely disturbed environment (Klátova Nová Ves). Significantly higher content of essential elements in milk was on farm of Novoť (undisturbed environment). Occurrence of toxic metals in feed from area with widely disturbed environment in spring season did not affect their content in milk. It can be concluded, that the use of milk of sheep from these areas for direct use or for dairy products processing is appropriate, safe and poses no health risk for the consumers.

Keywords: sheep's milk; toxic metal; essential element; feed; environment

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 **(Licata et al., 2004)**. The importance of milk in 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 human diet, not just for infants but also for adults and especially nursing mothers **(Sanz Ceballos et al., 2009; Kapila et al., 2013)**.

Sheep milk may contain various elements of nutritional or toxicological importance, and their levels can vary according to intrinsic factors (season, feeding and environment). Because 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 **(Llobet et al., 2003)**, 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 Middle East, where the livestock of these species is more common **(Sanal et al, 2011; Hilali et al., 2011; Najarnezhadand and Akbarabadi, 2013; Rahimi, 2013)**. 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 **(Khan et al., 2008)**. 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 **(WHO, 2007; Toman and Tunegová, 2017)**. 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 **(Bilandžić et al., 2011)**.

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 **(WHO, 2007; Chovancová et al., 2014)**. Lead and cadmium are considered potential carcinogens and are associated with etiology of a number of diseases in the cardiovascular system, kidneys, nervous system, blood and skeletal system **(Zhuang et al., 2007)**.

Micronutrient elements such as Fe, Cu, Zn, Se and macronutrients Ca, Mg, are essential for many biological functions **(Kazi et al., 2009; Lukačínová et al., 2012)**. 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 **(Kazi et al., 2009)**. 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 **(Cashman, 2011)**.

The presence of heavy metals and trace elements in milk has been reported in different countries and regions **(Simsek et al., 2000; Maas et al., 2011; Temiz et al., 2012; Rahimi et al., 2013)**. Moreover, an additional insight into metal uptake and assessment of human risks associated with the consumption of milk are still needed.

Scientific hypothesis

Contamination of environment with heavy metals and the insidious nature of their adverse ill health effects have become a matter of growing concern. The aim of this study was to determine the content of essential elements and toxic elements in feed and milk samples collected from farms of Slovakia, to find the actual contamination of selected areas, in view of its environmental character, and to refer to the suitability of the use of milk from these areas, to other food processing.

MATERIAL AND METHODOLOGY

Monitoring areas

The monitoring of areas was realized in 2016 during spring and autumn seasons on selected farms of Slovakia. According to the Ministry of Environment of Slovak Republic (SR), regions of SR are divided into three types of environmental quality: 1st environmental quality – regions with undisturbed environment and convenient environment; 2nd environmental quality – regions with a slightly disturbed environment, areas with disturbed environment and areas with widely disturbed environment; 3rd environmental quality – regions with heavily disturbed environment (Figure 1).

For monitoring in this study, the village Klátova Nová Ves (Western Slovakia) which is characterized as area with widely disturbed of environment was selected. This region, also called Horná Nitra, is typical with contamination of soil by heavy metals (As, Hg). Contamination soil and environment of this region is caused by power station in Nováky, where the coal is burned. The ash from the burning of low quality coal contains high amounts of As.

The second monitoring area is Novoť (Northern Slovakia) with undisturbed environment. On cow's farms of Novoť (750 ewes; Tsigai breed) and Klátova Nová Ves (300 ewes; Tsigai breed) 11 compounds, including 6 essential elements (Ca, Se, Zn, Mg, Fe, Cu) and 5 toxic elements (Cd, As, Hg, Ni, Pb) were analyzed.

Samples collection

Milk

Total number samples of milk were 20. Samples of milk were obtained from sheeps at farms. About 500 mL sample of milk was collected five times during the production of milk, on spring in April (beginning of lactation) and in autumn in September (the end of lactation) on each farm. Despite the fact, that there was large number of animals on the farms, average milk samples were obtained from milk tanks. After collection, samples of milk were stored in PET bottles in deep-freezers at -18 ºC until they were analyzed.

Feed

Total number samples of feed were 20. Five average samples of feeds were obtained in spring season (April) and five average samples in autumn (September) on each farm. This feed was used for feeding the studied animals. Samples were stored in plastic bags in deep-freezers at -18 ºC until they were analyzed. Analyzed feed at sheep farm of Novoť was TMR (total mixed ration) in spring season and pasture, where the animals grazed during the autumn season. From sheep farm of Klátova Nová Ves, the analyzed feed in the both of season was pasture.

Elements analyses

Milk samples for determination of Ca, Zn, Fe, Mg, Cu, Ni, Pb, Cd were prepared by mineralization with microwave decomposition with HNO_3 and H_2O_2 (microwave oven MARS 6 240/50). Milk sample for determination of Se was prepared by mineralization with microwave oven with HNO_3 and H_2O_2 (microwave oven MARS 6 240/50), removal nitrous gases, cooling, followed addition solution of HCl, reduction from Se^{6+} to Se^{4+} by heated at 90°C. Milk samples for determination of As were prepared by dry mineralization with oxidation mixture (oxygen, oxides of nitrogen, ozone), heated at 300 – 400°C. The ash was re-diluted in solution of HCl.

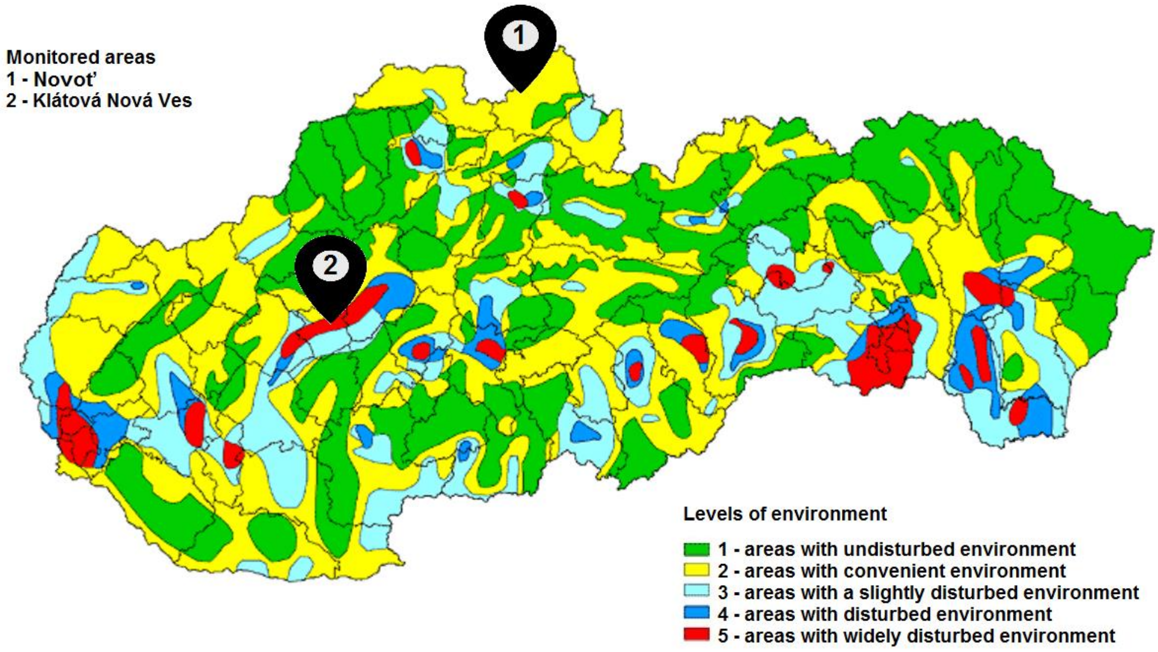

Figure 1 Environmental regionalization of Slovak Republic.

As and Se in milk and feed 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 ware 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 electro thermal 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 feeds was analyzedusing 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).

Statisical analysis

Statistical analysis of the data was performed using SAS 9.2 (SAS Institute Inc., USA). The results were analyzed by one-way analysis of variance (ANOVA) followed by Student's t-test. Statistical significance was set at $p < 0.05$. All data were expressed as mean, minimum values, maximum values, standard deviation and coefficient of variation.

RESULTS AND DISCUSSION

The results of our study summarize Table 1 and Table 2. During the spring seasons, content of essential elements in feed (Ca, Zn, Fe, Mg, Cu) were significantly higher ($p < 0.001$) on farm of Klátova Nová Ves (widely disturbed environment). Mean content of Se (0.16 $mg.kg^{-1}$) was higher on farm of Novoť (undisturbed environment), but this difference was not statistically significant. Content of toxic metals As, Hg, Ni, Pb, Cd in feed on farm of Klátova Nová Ves was found. Significantly higher ($p < 0.001$) content of As and Ni was present on farm of Klátova Nová Ves. Amount of Hg, Pb and Cd in feed was below the LOQ (limit of quantification) on farm with undisturbed environment (Novoť). **Bushra et al. (2014)** studied concentrations of toxic elements in feed from rural and urban areas. They found, that the content of Cd, Ni and Pb was 0.27, 1.68, 4.11 $mg.kg^{-1}$ in urban areas and in rural areas 0.037, 0.024, 4.52 $mg.kg^{-1}$, respectively. Compared to results of their study, on farm of Novoť (undisturbed environment) and Klátova Nova Ves (widely disturbed environment), content of Pb and Cd was lower and content of Ni was higher. Lower content of Ni, As, Cd, Pb in feed state **Zhou et al. (2017)** compared to our results. Higher value of Ni may be due to low soil pH, use of synthetic fertilizers and contamination of water used for irrigation **(Tahir et al., 2017)**. High quantity of Ni is known to be injurious for animal and human health. Its effects on various aspects of reproduction have been previously described. Animal studies demonstrate that nickel has negative effects on the structure and function of the testis, seminal vesicles and prostate gland; there is similar report on adverse effect on spermatozoa **(Pandey et al., 2000; Forgacs et al., 2001)**. **Lukáč et al. (2014)** reported the negative effect of nickel on spermatogenesis. The decrease in the relative volume of germinal epithelium indicates on alterations of the spermatozoa production. Cadmium causes tissue damage in humans and animals and many toxicological studies have found the functional and structural changes in the kidneys, liver, lungs, bones, ovaries and fetal effects **(Kukner et al., 2007; Massányi et al., 2007)**.

During the autumn season, in case of both farms, decrease content of the all studied toxic metals was found compared to spring season. Content of all toxic metals was below the LOQ (Table 1). Significantly higher content of microelements Zn, Fe, Cu was observed on farm of Novoť (p <0.001). Content of macroelements Ca and Mg was significantly higher (p <0.001) on farm of Klátova Nová Ves. On farm of Klátova Nová Ves, content of Se (<0.03 $mg.kg^{-1}$) was reported. The seasonal variations of essential trace elements in feed are presented in Table 1. In our study, a significant decrease in essential elements in feed between spring and autumn season was found in both farms. This decline could be due to the season at which the feed was taken. **Tomáš (2007)** states that the input of elements from the soil into the plant could be affected by the quality of organic matter, plant nutrition, microbial activity, the way of soil management but also fertilization, presence of other elements (synergism, antagonism), plant species and variety **(Tkáč et al., 2008)**. In ruminants, mineral deficiency can impair or even inhibit metabolic pathways required for normal body function, and produces clinical symptoms of different intensity. Severe macroelement or microelement deficiencies are manifested by symptoms corresponding to the function of the deficient element in the body, thus contributing to an accurate diagnosis of the health problem. In a minor deficiency, the symptoms are non-specific, often transient and difficult to diagnose due to low intensity. Mineral deficiency generally leads to impaired immunity, inhibited growth, reproductive disorder and lower productivity in animals **(Radwińska and Žarczyńska, 2014)**.

A positive result of our study was that content of toxic metals, which detected in feeds on both farms, did not affect on their occurrence in sheep milk. Content of all toxic elements (As, Hg, Ni, Pb, Cd) in milk was below the LOQ (Table 2) even in an area with a widely disturbed environment (Klátová Nová Ves). The content of Pb in milk in this work was significantly lower than in milk samples from Iran **(Najarnezhadand and Akbarabadi, 2013; Rahimi, 2013)** and very low in comparison to the results of **Anastasio et al. (2006)** and **Licata et al. (2012)**. **Ayar et al. (2009)** analyzed Pb concentration in dairy products and milk consumed in Turkey. According to their study, the concentrations range of Pb was reported as 0.09-0.19 $mg.kg^{-1}$. In other study, conducted by **Tajkarimi et al. (2008)** lead content was estimated in raw milk collected from different regions of Iran. Accordingly, the mean level of Pb content obtained from 97 samples had a range from 1.0 - 46 $ng.ml^{-1}$. **Issa et al. (2016)** recorded levels of Pb in dairy products from 0.018 – 7.421 $mg.kg^{-1}$ in their study. The Cd levels found in milk in this study were significantly lower than data found in literature **(Rahimi, 2013)**, while **Coni et al. (1996)** reported levels of Cd much higher. It must be underlined that, to this day, EU Commission does not established yet a maximum admitted limit for Cd in milk. Hence, the only reference value for

Table 1 Comparison between mean content of mineral and toxic elements in feed on farm of Novoť and Klátova Nová Ves ($mg.kg^{-1}$).

NOVOŤ – spring season					**KLÁTOVA NOVÁ VES – spring season**			
Element	Mean ±SD	Min	Max	CV	Mean ±SD	Min	Max	CV
Ca	2450 ±1.58	2448.00	2452.00	0.065	7400 ±1.58***	7398.00	7402.00	0.02
Zn	40.2 ±0.02	40.18	40.22	0.04	42.20 ±0.16***	42.00	42.40	0.37
Se	0.16 ±0.02[NS]	0.14	0.18	9.88	0.065 ±0.002	0.063	0.067	0.001
Fe	129 ±1.58	127.00	131.00	1.22	3330 ±1.58***	3328.00	3332.00	0.05
Mg	1180 ±1.58	1178.00	1182.00	0.13	3050 ±1.58***	3048.00	3052.00	0.05
Cu	7.30 ±0.15	7.100	7.500	2.16	11.2 ±0.16***	11.00	11.40	1.41
As	0.042 ±0.002	0.04	0.044	3.76	1.20 ±0.16***	1.00	1.40	13.1
Hg	<0.01[a]	-	-	-	0.02 ±0.01	0.04	0.009	0.001
Ni	0.16 ±0.02	0.14	0.18	9.88	6.80 ±0.16***	6.60	7.00	2.32
Pb	<0.30[a]	-	-	-	3.80 ±0.16	3.60	4.00	4.16
Cd	<0.10[a]	-	-	-	0.15 ±0.02	0.13	0.17	10.54
NOVOŤ – autumn season					**KLÁTOVA NOVÁ VES – autumn season**			
Ca	914 ±1.58	912.00	916.00	0.17	1140 ±1.58***	1138	1142	0.13
Zn	7.5 ±0.16***	7.30	7.70	2.11	5.30 ±0.16	5.10	5.50	2.98
Se	0.08 ± 0.002	0.079	0.083	0.00	<0.03	-	-	-
Fe	99.00 ±1.58***	97.00	101.00	1.59	72.00 ±1.58	70.00	74.00	2.19
Mg	303 ±1.58	301.00	305.00	0.52	314 ±1.58***	312.00	316.00	0.50
Cu	2.60 ±0.16***	2.40	2.80	6.08	1.60 ±0.16	1.40	1.80	9.88
As	<0.03	-	-	-	<0.03	-	-	-
Hg	<0.01	-	-	-	<0.01	-	-	-
Ni	<0.01	-	-	-	<0.1	-	-	-
Pb	<0.3	-	-	-	<0.3	-	-	-
Cd	<0.1	-	-	-	<0.1	-	-	-

Note: SD: standard deviation; min.: Minimum values; Max.: maximum values; CV: coefficient of variation; *** values in the same line present significant differences p <0.001; NS: not significant; [a] Values below LOQ (limit of quantification).

Table 2 Comparison between mean content of essential and toxic elements in sheep´s milk on farm of Novoť and Klátova Nová Ves (mg.kg^{-1}).

	NOVOŤ – spring season				**KLÁTOVA NOVÁ VES – spring season**			
Element	Mean ±SD	Min	Max	CV	Mean ±SD	Min	Max	CV
Ca	1410 ±1.58	1408.00	1412.00	0.11	2560 ±1.58***	2558.00	2562.00	0.06
Zn	4.90 ±0.16	4.70	5.10	3.22	8.00 ±0.58**	6.00	10.00	19.76
Se	0.031±0.002	0.029	0.033	5.10	<0.03 [a]	-	-	-
Fe	0.59 ±0.02	0.57	0.61	2.67	<0.50 [a]	-	-	-
Mg	107.00 ±1.58	105.00	1099.00	1.47	187.0 ±1.58***	185.00	189.00	0.084
Cu	<0.50[a]	-	-	-	<0.5[a]	-	-	-
As	<0.03[a]	-	-	-	<0.03[a]	-	-	-
Hg	<0.002[a]	-	-	-	<0.002[a]	-	-	-
Ni	<0.1[a]	-	-	-	<0.1[a]	-	-	-
Pb	<0.0[a]	-	-	-	<0.01[a]	-	-	-
Cd	<0.004[a]	-	-	-	<0.004[a]	-	-	-
	NOVOŤ – autumn season				**KLÁTOVA NOVÁ VES – autumn season**			
Ca	1890 ±1.58***	1888.00	1892.00	0.08	1590 ±1.58	1588.00	1592	0.09
Zn	4.00 ±0.58*	2.00	6.00	39.53	2.20 ±1.16	2.00	2.40	7.18
Se	0.06 ±0.001	0.061	0.065	2.50	<0.03[a]	-	-	-
Fe	0.81±0.02***	0.79	0.83	1.95	0.74 ±0.02	0.72	0.76	2.13
Mg	162.00 ±1.58***	160.00	164.00	0.97	179 ±1.58	177.00	181.00	0.88
Cu	<0.50[a]	-	-	-	<0.5[a]	-	-	-
As	<0.03[a]	-	-	-	<0.03[a]	-	-	-
Hg	<0.002[a]	-	-	-	<0.002[a]	-	-	-
Ni	<0.1 [a]	-	-	-	<0.1[a]	-	-	-
Pb	<0.01[a]	-	-	-	<0.01[a]	-	-	-
Cd	<0.004 [a]	-	-	-	<0.004[a]	-	-	-

SD: standard deviation; Min.: minimum values; Max.: maximum values; CV: coefficient of variation; *** Values in the same line present significant differences (p <0.001); ** Values in the same line present significant differences (p <0.01); * Values in the same line present significant differences (p <0.05); [a] Values below LOQ (limit of quantification).

Cd in milk has been set by FAO/WHO standard **(Codex Alimentarius Commission, 2011)**, that states an authorized limit equal to 10 ng.g^{-1}. Scientific data about As contamination in ovine milk are poor, in exception of **Sanal et al. (2011)**, that reported a higher As content in sheep milk compared to our results. In spring season, content of essential elements (Ca, Zn, Mg) in sheep milk was higher on farm of Klátova Nová Ves in case of Ca (p <0.001), Zn (p <0.01), Mg (p <0.001) statistically significant too. In milk from this area, low content of Se (<0.03 mg.kg^{-1}), Fe (<0.05 mg.kg^{-1}), Cu (<0.05 mg.kg^{-1}) was found. On the other hand, statistically significant higher content of essential elements (Ca, Zn, Se, Fe, Mg) was recorded on farm from area with undisturbed environment (Novoť) compared to farm of Klátova Nová Ves. The contents of essential elements Zn, Fe, Cu in ovine milk in this study were slightly lower than values of these elements as reported **Medico et al. (2016)**.

Regarding Ca content, in the present study a low level was found in case of farm of Novoť, in comparison to our previously published results **(Tunegová et al., 2016),** that showed levels of Ca in sheep milk in spring season 1770 mg.kg^{-1} and in autumn season 2170 mg.kg^{-1}.

The concentrations of elements in raw milk are also affected by animal forage, feed and water **(Dobrzański et al., 2005; Al-Wabel, 2008)**. Animal feed with elevated levels of these elements causes also an increase of their level in milk **(Bushra et al., 2014)**. 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 **(Herwing et al., 2011)**. Changes in 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) **(Komperej et al., 1999)**.

CONCLUSION

The results indicate that content of selected essential elements and toxic metals in feed and milk changes depending on the season of year. The work showed significantly higher content of selected essential elements in feed in spring season from area with widely disturbed environment (Klátova Nová Ves). Significantly higher content of essential elements in milk was found on farm of Novoť (undisturbed environment). In this work occurrence of toxic metals in feed from area with widely disturbed environment in spring season did not affect their milk content. It can be concluded, that the use of milk of sheep from these areas for direct use or for dairy products processing is appropriate, safe and pose no health risk for the consumers.

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Contact address:

Martina Tunegová, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Veterinary Disciplines, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: tunegova.martina@gmail.com

Róbert Toman, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Veterinary Disciplines, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: robert.toman@uniag.sk

Vladimír Tančin, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Veterinary Disciplines, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; NPPC- Research Institue for Animal Production, Hlohovecká 2, 951 41 Lužianky, Slovakia, E-mail: vladimir.tancin@uniag.sk

Martin Janíček, Slovak University of Agriculture, Faculty of Agrobiology and Food Resources, Department of Animal Husbandy, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: xjanicek@is.uniag.sk