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Monitoring of selected essential elements and contaminants at sheep and cow farms in Eastern Slovakia

Monitoring vybraných esenciálnych prvkov a kontaminantov na farme oviec a hovädzieho dobytka na východnom Slovensku

Martina TUNEGOVÁ*, Róbert TOMAN and Vladimír TANČIN

Department of Veterinary Disciplines, Slovak University of Agriculture, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic. *correspondence: tunegova.martina@gmail.com

Abstract

The aim of this study was to determinate the actual contamination of selected area of Slovakia, in view of its environmental character referred both to the suitability or unsuitability of the use of milk from this area, to other food processing. This article deals with analysis of the content of selected compounds in soil, feed and milk, at the cow and sheep farms. Village in Eastern Slovakia, Tulčík, was the area of investigation. This area is characterized as an area with mild disturbance of environment. 11 compounds have been analyzed (calcium, selenium, cadmium, arsenic, polychlorinated biphenyls – congeners 138, 153, 180, and pesticides - p,p´ DDE, Endosulfan I., Beta-HCH, aflatoxin M_1). Samples of soil were collected once a year (spring season), samples of feeds and milk were collected two-times a year (spring and autumn season). Analysis of samples was performed in Eurofins Bel/Novamann (Nové Zámky, Slovak Republic). Analyses were performed by routine methods, according to the valid methodologies. Levels of compounds were obtained and then results have been compared with the most acceptable limits in according to applicable legislation. At both farms, 73.08% (38 samples) of analyzed compounds were below the limit of quantification (LOQ) and 26.92% (14 samples) of compounds were quantifiable. The most significant differences between monitored farms were recorded in soil (27 720 mg·kg^{-1} Ca), feed (27 620 mg·kg^{-1} Ca) and milk (960 mg·kg^{-1} Ca). The high content of calcium in soil and feed did not affect the content of calcium in milk. The results showed that the content of toxic elements, polychorinated biphenyls, pesticides and aflatoxin M_1 in analyzed area of Eastern Slovakia was very low and under the limit of quantification. It can be concluded, that the use of milk from this area for direct use or for dairy products is appropriate and poses no health risk to the consumers.

Keywords: essential elements, feed, milk, monitoring, soil, xenobiotics

Abstrakt

Cieľom tejto práce bolo zistiť skutočnú kontamináciu vybranej oblasti Slovenska, vzhľadom na jej charakter prostredia a poukázať tak na vhodnosť alebo nevhodnosť využívania mlieka z tejto oblasti na ďalšie potravinárske spracovanie. Predkladaná práca sa zaoberá analýzou obsahu vybraných látok v pôde, krmive a mlieku na ovčej farme a na farme hovädzieho dobytka v oblasti s mierne narušeným prostredím na východnom Slovensku. Celkovo bolo analyzovaných 11 látok (vápnik, selén, cadmium, arzén, polychlórované bifenyly - kongenéry 138, 153, 180 a pesticídy - p,p´ DDE, Endosulfan I., Beta-HCH, aflatoxín M_1). Vzorky pôdy boli odoberané raz ročne (jarné obdobie), vzorky krmiva a mlieka dvakrát ročne (jarné a jesenné obdobie). Analýzy vzoriek boli vykonávané v laboratóriách EurofinsBel/Novamann v Nových Zámkoch (Slovenská republika). Analýzy sa vykonávali rutinnými metódami podľa sledovaných látok na kalibrovaných alebo overených meracích zariadeniach podľa platných metodologických predpisov. Získané hodnoty obsahu vybraných látok boli porovnávané s najvyššími prípustnými limitmi podľa platnej legislatívy. V prípade oboch fariem sme zaznamenali, že 73.08% (38 vzoriek) vzoriek sledovaných látok bolo pod limitom kvantifikácie (LOQ) a 26.92% (14 vzoriek) vzoriek bolo kvantifikovateľných. Najvýraznejšie rozdiely sme zaznamenali v obsahu vápnika v pôde (27 720 $mg \cdot kg^{-1}$ Ca), krmive (27 620 $mg \cdot kg^{-1}$ Ca) a mlieku (960 $mg \cdot kg^{-1}$ Ca) medzi monitorovanými farmami. Vysoký obsah vápnika v pôde a krmive nemal vplyv na obsah vápnika v mlieku. Naše výsledky poukázali na nízky obsah toxických prvkov, polychlórovaných bifenylov, pesticídov a aflatoxínu M_1 v analyzovanej oblasti východného Slovenska a boli pod limitom kvantifikácie. Záverom môžeme konštatovať, že používanie mlieka z tejto oblasti na priamu spotrebu alebo pre ďalšie spracovanie na mliečne produkty nepredstavuje zdravotné riziko pre konzumentov.

Kľúčové slová: esenciálne prvky, krmivo, mlieko, monitoring pôda, xenobiotiká

Introduction

The enormous and rapid development of chemical and agrochemical industries during the last century has resulted in the release of a large number of chemical compounds into the environment. In fact, a lot of different chemical compounds are currently used in the daily life of human beings or result from human activities and many of these are frequently being detected in numerous environmental monitoring studies (Bell et al., 2011; Haarstad et al., 2011; Pal et al., 2010; Gavrilescu, 2005). Most of pollutants are chemical substances that persist in the environment, can bioaccumulate throughout the food chain, and may be toxic to biotic communities, thus posing a risk to human health and the environment (El-Shahawi et al., 2010; Jones and de Voogt, 1999).

Nutritional essential elements such as calcium (Ca) and selenium (Se) are coactivators of several important enzymes and proteins which are necessary for health maintenance (Zhao et al., 2013; Wu at al., 2011). Selenium is a naturally occurring micronutrient that is essential for several known major metabolic pathways, including thyroid hormone metabolism and antioxidant defense systems. Selenium is incorporated into antioxidant enzymes (Schmutzler et al., 2007), such as intracellular glutathione peroxidase, which protect the thyroid from H_2O_2-induced peroxidative damage during hormone synthesis (Howie et al., 1995). Milk and dairy products in diets represent an optimum source of Ca and other limiting nutrients such as potassium (K) and magnesium (Mg) involved in bone health. In particular Ca intake positively affects bone mass, ensuring adequate bone development in childhood and youth (Caroli et al., 2011).

Heavy metals are widely dispersed in the environment. The toxicity induced by excessive levels of some of these elements, such as cadmium (Cd), arsenic (As) are well known (Llobet et al., 2003). Metals like cadmium and arsenic are the major toxic elements posing a treat to human health. Their ecosystem accumulation (water-soil-plant-animal) makes them very toxic and leads to undesirable consequences for live organisms (Piskorová et al., 2003; Bogut et al., 2000). Sheep and cattle reared freely on pasture are also indicators of the environmental pollution like the wild animals (Gallo et al., 1996). Increased concentration of heavy metals in the body of domestic animals results in low fitness of animals and reproduction problems as well as in immunity decline and occurrence of cancerous and teratogenic diseases (Bires et al., 1995).

Organochlorine insecticides are important environmental pollutants as they are applied widely in agriculture, livestock, forestry, and used for domestic and industrial activities. Physicochemical properties of these compounds especially their high lipophilicity, facilitate their absorption and storage of in human and animal bodies (Kampire et al., 2011). Residues in milk, which is one of the most widely used foodstuffs containing lipids, can be a quantitative and qualitative index for the presence of these toxins in animal bodies. Accumulation has the potential to adversely affect the food chain (Bulut et al., 2011). Many developed countries have banned the use of some types of organochlorine, for example the USA which banned the use of DDT in 1970. Environmental studies have found that pesticide residues such as DDT, PCBs, and other organochlorine compounds continue to be present in humans and other mammals around the world many years after production and use have been limited. Due to their persistence it is difficult to eliminate the organochlorines by simply avoiding their use, as many years are required for them to disappear from the environment (Subir and Mukesh, 2008).These pesticides are also still being used in some parts of the world due to their potent and wide spectrum effects against harmful organisms. The main health hazards associated with exposure to these compound are abdominal pains, diarrhea, hypertension, respiratory diseases, and dysfunction of the reproductive system, pre/postnatal damage, carcinogenesis and mutagenesis (Aktar et al., 2009).

The intake of contaminated feed and fodder by animals is the main source of entry of pesticides to the bodies of animals, which ultimately results in the contamination of milk, meat consumed by humans (Hernández et al., 2010) and inevitably humans also can be contaminated. Milk is considered as nearly complete food since it is a good source of protein, fat and major minerals. Also, milk is one of the main constituents of the daily diet, especially for vulnerable groups such infants, school age children and older people (Kampire et al., 2011). Certain environmental chemicals, including pesticides termed as endocrine disruptors, are known to elicit their adverse effects by mimicking or antagonizing natural hormones in the body and it had been postulated that their long-term, low-dose exposure is increasingly linked to human health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Aktar et al., 2009).

Humans and animals are subjected to "biological hazard" from natural toxicants that occur in food and feed. Mycotoxins are secondary metabolites produced by a few fungal species belonging mainly to the *Aspergillus*, *Penicillium* and *Fusarium genera*. Such compounds may be formed by these mycotoxigenic molds when growing in contaminated foods at production, processing, transportation and also during storage (Bhat et al., 2010; Murphy et al., 2006). Aflatoxin is one of the most important mycotoxins and it can be produced by different species of *Aspergillus genus*, mainly *Aspergillus flavus* and *Aspergillus parasiticus.* The main economic source of this mycotoxin are cereal-based foods; however aflatoxin can also be found in foods of animal origin such as milk and dairy products (Elsanhoty et al., 2014). The contamination of milk and dairy products with mycotoxins can occur by indirect contamination when lactating animals ingest aflatoxin B_1 (AFB_1) contaminated feed which will pass to the milk as aflatoxin M_1 (AFM_1) and also by direct contamination, when molds can grow in milk (very unlikely) or on dairy products as intentional additives or accidental contamination (Sengun et al., 2008). Aflatoxins are compounds that have strong effect on human and animal health because they lead to serious damage to the liver induction of tumors as well as immunosuppressive, mutagenic, teratogenic and carcinogenic effects (Hernandez-Mendoza et al., 2009).

Materials and methods

Monitoring area

The monitoring of area was realized in 2015 during spring and autumn season. The village Tulčík (Eastern Slovakia) is characterized as area with mild disturbance of environment. In Tulčík, cow and sheep farms were monitored. 11 compounds, including 2 essential elements (calcium, selenium), 2 toxic elements (cadmium, arsenic), 3 polychlorinated biphenyls (congeners 138,153, 180), 3 pesticides (p,p´ DDE, Endosulfan I., Beta-HCH) and one mycotoxin (aflatoxin M_1) were analyzed. Aflatoxin M_1 was analyzed only in the milk and other compounds were detected in feed, soil, and cow and sheep milk.

Milk

Samples of milk were obtained from the dairy cows and ewes at farms. Samples were collected two times during the production of milk, on spring in April (beginning of lactation) and in autumn in September (the end of lactation). Nevertheless, that on the farms was big number of animals (450 sheeps, 420 cows), average milk samples were obtained from milk tanks. Milk was stored in PET bottles in deep-freezers at -18 ºC until they were analyzed.

Soil

One average sample of soil was collected in spring period on beginning season. The sample was collected from area where the feed was grown or from pastures where the studied animals were grazing during the season. The samples were stored in plastic bags in deep-freezers at -18 ºC until they were analyzed.

Feed

One average sample of feeds was obtained in spring season (April) and one average sample in autumn (September) at both farms. These feeds were 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 was meadow hay and at cow farm total mixed ration (TMR).

Compounds analysis methods

Arsenic and selenium in milk, soil and feed were analyzed using the hydride generation atomic absorption spectroscopy (HG-AAS) method with Spectr AA-220 FS (The Netherlands). Calcium in milk, soil and feed was detected using the inductively coupled plasma-atomic emission spectrometry (ICP-AES, Varian 720-ES, USA). Cadmium in milk and feed was analyzed using the electrothermal atomization atomic-absorption spectrometry (ETA-AAS, Agilent DUO AA 240Z/240FS, USA) and in soil using the flame atomic absorption spectrometry (F-AAS, Agilent DUO AA 240Z/240FS, USA). PCBs and pesticides were analyzed in milk using the gas chromatography/electron capture detection (GC/ECD, HP Agilent 6890N, USA), in soil using the GC/ECD (HP 5890A, USA) and in feed using the gas chromatography–tandem mass spectrometry (GC-MS/MS, Agilent 7890 B and Agilent 7000 Triple Quad, USA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS, Agilent 1260 Infinity II LC System and Agilent 6400 QQQ LC/MS System, USA). Aflatoxin M_1 in milk was detected using the high performance liquid chromatography fluorescence detection in UV spectrum (HPLC/UV/FLD, AGILENT 1260, USA).

Analyses of results

Results of analyses were compared with the acceptable limits in valid Slovak and European Union (EU) legislation.

Results

The content of eleven selected compounds in samples of soil, feed and milk from sheep and cow farms is shown in Table 1 and Table 2. In case of both farms, 73.08% (38 samples) of compounds were found below the limit of quantification (LOQ) and 26.92% (14 samples) of analyzed compounds were quantifiable.

In case of calcium, quantifiable values in all samples (soil, feed and milk) were found at both farms. Detectable selenium values were recorded in sample of soil (spring season) and in sample of feed (autumn season) at the sheep farm. At cow farm, detectable values of selenium in sample of soil and in both samples of feed (spring and autumn season) were found. Arsenic was detected in sample of soil and in both samples of feed at both farms. Polychlorinated biphenyls (congeners 138, 153, 180) have been also recorded in sample of feed in autumn season at both farms.

The highest differences were recorded in content of calcium in soil, feed and milk. In sample of soil at the sheep farm, content of calcium 31 330 mg·kg^{-1}was recorded. At the cow farm, content of calcium was 3 610 mg·kg^{-1}. The difference in soil calcium content between both monitored farms was 27 720 mg·kg^{-1}. At the sheep farm 36 490 mg·kg^{-1}, and at the cow farm 8 870 mg·kg^{-1} of calcium during the spring season were detected in animal feed. Difference in content of calcium between both farms was 27 620 mg·kg^{-1}.The difference in content of calcium in milk between both monitored farms was 960 mg.kg^{-1}in autumn season. Content of calcium in milk at the sheep farm was 2 170 mg·kg^{-1} and 1 210 mg·kg^{-1}in cow milk.

The content of calcium was higher in all three cases (soil, feed, milk) at the sheep farm when compared to the cow farm. The same value of Ca, 7 960 mg·kg^{-1} was found in samples of feed in autumn season at both farms. In case of selenium, arsenic and polychlorinated biphenyls, no differences were detected between the monitored farms.

Table 1. The content of selected compounds in soil, feed and milk at the sheep farm (mg·kg^{-1})

Tabuľka 1. Obsah vybraných látok vo vzorkách pôdy, krmiva a mlieka na frame oviec

Sheeps	Season	Ca*	Se*	Cd*	As*	PCB 138*	PCB 153*	PCB 180*	p, p´ DDE*	Endosulfan I.*	Beta- HCH*	Aflatoxin M1*
Soil	spring	31330	0.2	< 0.40[a]	4.3	<0.0001[a]	<0.0001[a]	<0.0001[a]	0.0065	<0.0005[a]	<0.0001[a]	-
Feed	spring	36490	<0.030[a]	<0.10[a]	0.11	<0.05[a]	<0.05[a]	<0.05[a]	<0.05[a]	<0.01[a]	<0.05[a]	-
	fall	7960	0.27	<0.10[a]	0.19	0.05	0.05	0.05	<0.05[a]	<0,01[a]	<0.05[a]	-
Milk[b]	spring	1770	<0.030[a]	<0.0040[a]	<0.030[a]	<0.02[a]	<0.02[a]	<0.02[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.013[a]
	fall	2170	<0.030[a]	<0.0040[a]	<0.030[a]	<0.02[a]	<0.02[a]	<0.02[a]	<0.001[a]	<0,001[a]	<0.001[a]	<0.013[a]

[a] Values below LOQ (limit of quantification), Ca: calcium, Se: selenium, Cd: cadmium, As: arsenic, PCB: polychlorinated biphenyls, p,p´ DDE: dichlorodiphenyldichloroethylene, Beta- HCH: hexachlorocyclohexan [b] Average sample of milk from 450 sheeps

Table 2. The content of selected compounds in soil, feed and milk at the cow farm (mg·kg^{-1})

Tabuľka 2. Obsah vybraných látok vo vzorkách pôdy, krmiva a mlieka na farme hovädzieho dobytka

Cows	Season	Ca*	Se*	Cd*	As*	PCB 138*	PCB 153*	PCB 180*	p, p´ DDE*	Endosulfan I.*	Beta- HCH*	Aflatoxin M1*
Soil	spring	3610	0.21	<0.40[a]	8.8	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0001[a]	<0.0002[a]	<0.0001[a]	-
Feed	spring	8870	0.09	<0.10[a]	0.12	<0.05[a]	<0.05[a]	<0.05[a]	<0.05[a]	<0.01[a]	<0.05[a]	-
	fall	7960	0.27	<0.10[a]	0.19	0.05	0.05	0.05	<0.05[a]	<0.01[a]	<0.05[a]	-
Milk[b]	spring	915	<0.030[a]	<0.0040[a]	<0.030	<0.02[a]	<0.02[a]	<0.02[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.013[a]
	fall	1210	<0.030[a]	<0.0040[a]	<0.030	<0.02[a]	<0.02[a]	<0.02[a]	<0.001[a]	<0.001[a]	<0.001[a]	<0.013[a]

[a] Values below LOQ (limit of quantification), Ca: calcium, Se: selenium, Cd: cadmium, As: arsenic, PCB: polychlorinated biphenyls, p,p´ DDE: dichlorodiphenyldichloroethylene, Beta- HCH: hexachlorocyclohexane [b] Average sample of milk from 420 cows

Discussion

Increasing of agricultural production efficiency entails using of large amounts of chemical products not only in production of animal feed, but also on farms with milk production. In study, the content of selected compounds in soil, feed and milk have been examined in area of eastern Slovakia, Tulčík. This area is considered as an area with mild disturbed environment.

Comparing the calcium content in soil at the sheep farm (31 330 mg·kg^{-1}) with content of calcium in soil at the cow farm (3 610 mg·kg^{-1}), high difference was recorded. High content of calcium at the sheep farm was caused by subsoil in this area. High difference was observed by comparing the calcium content in feed in spring on both farms.

The high calcium content in feed at the sheep farm was affected, similarly to the calcium content of soil by the subsoil on which the feed was grown. As sheep were fed in the spring with meadow hay, the higher calcium content of the feed was caused by the presence of alfalfa, that are in livestock nutrition (especially in ruminants) considered to be one of the most important sources of calcium (Gálik et al., 2011). Content of selenium in the feed of sheep in sample from spring was below the LOQ (<0.030 mg·kg^{-1}) and in the autumn it was 0.27 mg·kg^{-1}.

Hay is particularly appreciated in terms of calcium (Ca) and potassium (K) in animal nutrition (Rayburn, 1997; Buxton and Fales, 1994). It is important natural volumetric feed for sheep and particularly important for rearing of lambs. It has beneficial dietary effects, improves feed intake and rumen activity (Zeman et al., 2006). Good hay has a positive effect on intestinal peristalsis, fertility and reproduction. It is an important stabilizing factor of production performance of ewes (Labuda et al., 1982).

Cows were fed using a total mixed ration (TMR). Dvořáček (2010) states, that the calcium content of TMR should be 9 –13 g·kg^{-1} (9000 -13000 mg·kg^{-1}) and selenium content 0.5 mg·kg^{-1}. Compared to results, a deficit of both elements in TMR was recorded.

Špánik et al. (2006) states, that the composition of sheep milk (1950 mg·kg^{-1} of Ca; 0.17 mg·kg-1 of Se) is different than composition of cow milk (1150 mg·kg^{-1} of Ca; 0.14 mg·kg^{-1} of Se). In study, the calcium content was in both periods in cow milk and sheep milk lower than indicated by Špánik et al. (2006). Antunovič et al. (2001) indicates in his work, that the highest concentrations of calcium were found in colostrums during the 10th and 30th day of lactation with a gradual decrease, and it increased again on 60th day of lactation. This argument also corresponds to results, because lower calcium content was found in spring, comparing to autumn. Changes in composition of milk also affects many genetic (breed, unique, herd) and physiological factors (lactation, age, animal health), but also the environment (food, climate, season, method of milking) (Komperej et al., 1999).

The content of selenium in both periods and in both types of milk in study was below LOQ (<0,030 mg·kg^{-1}). Government Ordinance of Slovak Republic no. 438/2006 indicated maximum permissible values of arsenic: 4 mg·kg^{-1}, cadmium: 1 mg·kg^{-1}, DDT: 0.05 mg·kg^{-1}, Endosulfan: 0.1 mg·kg^{-1}, β-HCH: 0.02 mg·kg^{-1}, PCB: 1.25 TEQ·kg^{-1}. Compared to results, founded arsenic in feed at both farms in both periods corresponds to the maximum permissible level.

The contents of all three PCBs congeners in feed at the sheep and cow farms in autumn were 0.5 mg·kg^{-1}. In other samples, their content was below the LOQ. The content of toxic elements, polychlorinated biphenyls, pesticides and aflatoxin M_1 in sheep milk and cow milk was below the LOQ.

Conclusions

The results show that the content of toxic elements, polychlorinated biphenyls, pesticides and aflatoxin M_1 in analyzed area of Eastern Slovakia was very low and under the limit of quantification. High difference in content of calcium in soil and feed in spring season at the sheep farm was recorded. These results did not affect the content of calcium in milk. It can be concluded that the use of milk from this area for direct use or for dairy products is appropriate and poses no health risk to the consumers.

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