SELENIUM AND IODINE CONTENT IN SHEEP MILK FROM FARMS IN CENTRAL AND EAST BOHEMIA*

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Selenium and iodine content was analyzed in sheep milk from 4 and 6 different farms in central and east Bohemia. Samples had been collected monthly in two years period. Iodine was determined by high performance liquid chromatography (HPLC) with electrochemical detection and selenium using electrothermal atomization atomic absorption spectrometry (ETA-AAS) with hydride generation. The average levels of selenium and iodine in milk from small family farms were 39.09 ± 12.77 µg/kg and 47.99 ± 13.24 µg/kg, respectively. On the big farm focused on commercial breeding the average values were significantly higher (576.7 ± 261.2 µg/kg). Significant differences in levels of both elements were found between summer and winter period. In the winter period, the average level of selenium was 68.33 ± 43.75 µg/kg and in the summer period 30.67 ± 8.31 µg/kg. Levels of iodine show the same tendency: 72.42 ± 64.37 µg/kg in the winter and 37.84 ± 16.09 µg/kg in the summer period. Moderately strong correlation (r = 0.39) was found between selenium and iodine content in sheep milk. The comparison of iodine and selenium content in sheep milk and whey implies that 69.4% of iodine is transferred to the whey fraction.

trace elements; ewe's milk; whey; HPLC with electrochemical detection; hydride generation

INTRODUCTION

Milk and milk products are considered a good source of selenium and iodine, i.e. minerals, which are highly important for thyroid gland function (Kvíčala, 2010).

Selenium, 40 years ago considered as toxic, nowadays is recognized as an essential trace element with a high impact in human health and currently is in the center of research focus. In organisms it appears in the form of proteins and, in some cases, as a sugar. Selenium is a part of enzymes glutathione peroxidase, deiodinase, thioredoxin reductase, selenium phosphate synthetase, selenium protein P and others, whose functions have not been yet clarified (Kvíčala, 2002). Selenium influences a wide range of hormones, especially the biochemistry of those in the thyroid gland, whereby, to a certain point, can influence the metabolism of iodine. Optimal intake of selenium is recommended in the range of 20–50 µg per day (Ošancová, 1990). A scientific committee for food suggests as a suitable daily intake 40 µg of selenium for men and 30 µg for women (Brown et al., 2001).

Iodine is a necessary element for thyroid hormones – thyroxine and triiodthyronine, which are participating in metabolism speed regulation. According to the newest attitude of the Interdepartmental commission for iodine deficiency solution (National Institute of Public Health in Prague) about half of European population is considered to have an insufficient iodine intake (Ryšavá, Kříž, 2010). In Czech Republic the iodine supply is satisfactory and according to WHO criteria the past iodine deficiency can now be considered as fixed, thanks to salt iodization and iodine supplementation of dairy cows (Zamrazil et al., 2004). EU regulations warn against health risks sequent to exceeding limit values of 0.008–0.010 mg/kg of body weight of daily iodine intake, or intake lower than daily optimum 150–300 µg (notice of the Ministry of Agriculture of the Czech Republic, No. 446/2004). The optimal concentration of iodine in milk for human nutrition is in the range from 200 to 300 µg/l.

The relationship between selenium and iodine was reviewed by Zimmermann, Köhrle (2002). Selenium deficiency leads to decreased activity of glutathione peroxidase (GPx) and deiodinase (DI). GPx causes increased levels of hydrogen peroxide and DI catalyzes the activation of prohormone T4 to the active thyroid hormone T3. Both enzymes are influencing the efficiency of thyroid hormone synthesis and thus metabolism of iodine.

Common levels of iodine found in sheep milk by different authors are in the range of 48–829 µg/l (Herzig et al., 1995; Ferri, 2003; Hampel et al., 2004; Paulíková, 2008). Number of studies, which are engaged in researching levels of selenium in sheep milk, is much lower. The range of values is 14–37 µg/l of selenium in sheep milk (Koutník et

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al., 1996; Yanardag and Orak, 1998; Hampel et al., 2004).

There are various factors, which can influence the content of selenium and iodine in milk, e.g., the way of feeding, including mineral supplementation, locality, breed, application of disinfectants containing iodine etc. Levels of iodine in milk taken from animals in winter period are higher than in summer period (Herzig et al., 1995; Paulíková, 2008). Levels of iodine in milk are influenced also by presence of goitrogens in fodder. For example rapeseed cake, which is known for its high content of goitrogenic substances, was proved to cause decreased absorption of iodine (Hermansen et al., 1995; Třináctý et al., 2001).

Due to the increasing popularity of farmers markets and interest in local groceries, sheep milk is recently more available. However, the intake of selenium and iodine from sheep milk, while used as a substitute of cow milk, is not well known yet. This situation leads us to believe that there is an urgent need for analytical determination of sheep milk nutrients, especially trace elements. The aim of this study is to observe the natural occurrence of selenium and iodine in sheep milk of the most commonly kept breeds in the different localities of the Czech Republic.

MATERIAL AND METHODS

Levels of selenium and iodine were determined in sheep milk samples taken from four different farms (A–D) and from two more farms (E and F) only for iodine, of central and east Bohemia. Samples had been collected repeatedly once a month during the years 2007–2008, including the winter period. In the farms A–D the samples were obtained every time from the same animals, while in the farms E and F the samples were taken from pool milk. From the farm F, simultaneously with milk, samples of whey were also collected. In total, 77 samples were collected to the clean, plastic sampling flasks of 100 ml volume, frozen as soon as possible, transferred to the lab in thermo boxes and stored in the freezers with highest temperature less than –20°C.

Farm characteristics

Farm A: small family farm, herd of 30 heads (12 lactating ewes), breed Merino. Keeping: all year outdoor, with free entrance to the barn. Feeding: pasture, old bread, beet root, apples, hay and straw freely available.

Farm B: small family farm, herd of 25 heads (8 lactating ewes), breed Merino. Keeping: all year outdoor, free entrance to the shed, in a barn overnight. Feeding: pasture, old bread, straw, occasionally apples.

Farm C: small family farm, herd of 15 heads (6 lactating ewes), breed Merino. Keeping: during the summer period outdoor, overnight in a barn, in the winter period continually in a stable, occasionally outside. Feeding: pasture, grains, salt licks, hay, straw, old bread, occasionally beet root and apples.

Farm D: small family farm, herd of 20 heads (8 lactating sheep), breed Romanov sheep. Keeping: all year outdoor with possibility of a shed. Feeding: pasture, grains, salt licks, hay, straw, old bread, beet root, apples, corn.

Farms A–D are localized in central Bohemia, loamy sand to sand soils, typical crop potatoes, altitude 400–600 m a.s.l.

Farm E: big commercially focused farm with 300 lactating sheep and 100 lambs, breed East Friesian Milk sheep, localized in east Bohemia. Keeping: during the summer period all day long outdoor, in the winter period in a stable. Feeding: in the summer pasture, in the winter hay and grains. Molassesed mineral lick for organic farming available: Natumel (Iframix). Iodine content: 110 mg/kg, selenium content: 45 mg/kg.

Farm F: commercially focused farm localized in east Bohemia, keeping the breed East Friesian Milk sheep. Feeding: hay, straw, pasture, pressed oat and barley. Mineral lick Biosaxon was available, with iodine content 120 mg/kg and selenium content 30 mg/kg.

Samples of milk for iodine determination were dried and prepared using alkalic ashing, in the presence of potassium hydroxide and zinc sulfate, in accordance with a method after Fiedlerová (1998). Supernatants were filtered and iodine was analyzed using high performance liquid chromatography with electrochemical detection (HPLC-ED, Waters) on reversed phase column NOVA-PAK C-18 (Hejtmánková et al., 2005), due to modified procedure in accordance with IDF technical standard 167:1994, which describes direct determination of iodine content in fresh or dried refreshed milk.

Milk samples for determination of selenium were exposed, after lyophilization, to acid treatment of concentrated nitric acid (2 ml) and hydrogen peroxide 30% (3 ml) overnight. Afterwards were wet-ashed in microwave mineralizer MW-3^+ Speedwave (Berghof). Selenium was reduced from oxidation form VI to IV by exposition to concentrated hydrochloric acid. The analysis was performed by atomic absorption spectrometry with electrothermal atomization (Varian) and generator of hydrides (GH-AAS).

All the samples were measured in triplicates. Results were processed in Microsoft Excel and statistically evaluated in Statgraphics. For the evaluation of average variability of selenium and iodine values, the One-Way ANOVA was used. For the comparison of samples and for the evaluation of correlations, Multiple Sample Comparison and Multiple Variable Analysis were respectively used.

Table 1. Analytical results of certified reference material

	R	*BCR 063* I (mg/kg)	*H 281* Se (mg/kg)
Measured values (n = 6)	average	0.82	0.022
	uncertainty	0.07	0.001
Certified values	average	0.81	0.028
	uncertainty	0.05	0.004

Table 2. Iodine content (µg/kg) in sheep milk from the different farms

	Mean	Min. value	Max. value	St. dev.	CV (%)	Median	Samples
Farm A	34.92	15.04	49.63	12.71	36.4	37.52	23
Farm B	62.23	22.7	141.1	38.05	61.15	52.14	14
Farm C	56.12	20.23	197.7	45.00	80.2	38.14	12
Farm D	38.69	23.32	89.6	20.7	53.6	29.52	5
Farm E	576.7	348.8	1398	261.2	45.3	503.9	13
Farm F	123.5	47.08	203.9	51.06	41.7	65.16	10

Table 3. Selenium content (µg/kg) in sheep milk from different farms

	Mean	Min. value	Max. value	St. dev.	CV (%)	Median	Samples
Farm A	27.36	15.07	58.78	14.39	52.6	20.61	23
Farm B	40.41	30.00	50.86	7.84	19.4	40.4	14
Farm C	32.13	22.94	44.54	6.72	20.94	31.75	12
Farm D	56.46	18.37	173.0	41.37	73.27	39.37	5

RESULTS AND DISCUSSION

Quality control of analytical data was verified by simultaneous analysis of certified reference material BCR 063 (skim milk, iodine) and BCR H281 (ray grass, selenium) (Table 1).

Determined average levels of selenium and iodine in sheep milk, obtained from different farms, are shown in the Tables 2 and 3. Average content of selenium in milk from small family farms A–D was 39.09 ± 12.77 µg/kg and average content of iodine was 47.99 ± 13.24 µg/kg. According to Groppel (1993), levels of iodine in sheep milk below 79 µg/l are already deficient. Thus, on farms A–D, the status of iodine was below recommended limit. Differences in content of iodine and selenium in sheep milk from small farms were not statistically significant, even though on the farm D a different breed was kept (Romanov sheep, farm A–C Merino).

The determined content of iodine in sheep milk was found within the range 15.04–203.9 µg/kg whereas the selenium content interval was 15.07–173 µg/kg. On both commercially focused farms E and F the same breed (East Friesian Milk sheep) was kept, but the content of iodine was significantly different. The high levels of iodine in milk of ewes from farm E are apparently a consequence of iodine mineral lick intake, which was not applied on the small farms. It is highly probable that the crucial factor for iodine content in milk is the way of keeping and feeding, regardless of the kept breed.

Studies about iodine in sheep milk were conducted also by Trávníček, Kursa (2001), who determined an average concentration of iodine of 105.5 µg/l, Hejtmánková et al. (2008) found out on average 214.3 µg/kg, Hampel (2004) 520 ± 60 µg/kg and Ferri (2003) even 675 ± 154 µg/kg. Azuolas, Caple (1984) published results from measuring iodine in sheep milk in range 79–1931 µg/l. On commercially oriented farms E and F the determined average levels of iodine in sheep milk were higher, namely 576.7 ± 261.2 µg/kg and 123.5 ± 51.06 µg/kg and significantly higher ($P < 0.05$) content of iodine in milk from farm E in comparison with other farms was confirmed. However, Schöne, Rajendram (2009) consider iodine concentrations in milk higher than 500 µg/l as undesirable.

Several Czech authors analyzed the iodine content in cow milk. For instance Třináctý et al. (2001) found an average content of iodine in cow milk 594 ± 178.1 µg/kg, Trávníček et al. (2006) published 442.5 ± 185.6 µg/l, Hejtmánková et al. (2006) 225 ± 109 µg/kg and Paulíková et al. (2008) 136.9 ± 258.2 µg/l. Thus, it was not confirmed that levels of iodine in sheep milk could be so significantly higher than in cow milk, as Hampel et al. (2004) presented in his work.

Table 4. Selenium and iodine content (µg/kg) in sheep milk in summer and winter period

Element	Period	Mean	Min. value	Max. value	St. dev.	CV (%)	Median	Samples
Selenium	winter	68.33	25.74	173.0	43.75	64.04	51.66	15
	summer	30.67	15.06	50.86	8.31	27.11	29.44	38
Iodine	winter	75.42	29.08	197.6	46.37	61.48	60.00	15
	summer	37.84	15.04	89.66	16.09	46.64	29.93	38

On the other hand, the average content of selenium in sheep milk 14 ± 3 µg/kg, determined by Hampel et al. (2004), is about half the average content of selenium in sheep milk determined in this work and which nicely corresponds with selenium content in cow milk 32.2–39.5 µg/l (Koutník et al., 2005).

Contents of iodine and selenium in sheep milk, obtained in the winter period (October–March) and in the summer period (April–September) are presented in Table 4. The results imply that in the winter period the levels of both elements are significantly higher ($P < 0.05$) and in the summer period decrease roughly to half. Groppel, Anke (1991), Dahl et al. (2003) and Trávníček et al. (2006) share the opinion that it is a consequence of differences in summer and winter fodder. The reason for these differences is a lower content of iodine in summer feeding (pasture). Concentration of iodine increases with decreasing concentration of water in plant tissues. Thus, hay and silage contain higher concentrations of iodine (Herzig, Suchý, 1996; Bobek, 1998).

Another reason could be the presence of goitrogens in the summer fodder. It is expectable, that fresh fodder contains goitrogenic substances, which are responsible for decreased levels of iodine in milk. The values found in milk in this work are in accordance with those investigated by Paulíková (2008), who determined an average iodine concentration of 197.6 µg/kg in sheep milk in the winter period and of 56.0 µg/kg in the summer period. Levels of selenium determined in sheep milk were significantly higher ($P < 0.05$) in the winter period than in the summer period as well.

Fig. 1 shows the correlation between the contents of selenium and iodine in sheep milk. The graph is interlaid with linear regression line. Statistical evaluation of data resulted to moderately strong correlation ($r = 0.39$).

The distribution of iodine in milk fractions was also observed. For this purpose, the milk and whey obtained from the farm F was analyzed. In milk and whey was found, on average, 143.1 ± 113.1 µg/kg and 99.38 ± 58.58 µg/kg of iodine, respectively. This implies that 69.4% of iodine in the milk moved to the whey fraction. Thus, whey could be considered not only as a source of nutritionally valuable proteins, but of iodine as well. Transfer of iodine to whey was confirmed also by Rudolfová et al. (2000). The relationship between iodine content found in sheep milk and whey is depicted in Fig. 2. The slope of the line and values of regression coefficient ($b = 1.74$) implies that the values of iodine determined in milk and whey are significantly correlated.

CONCLUSION

Statistically significant differences of iodine content in sheep milk from different farms were confirmed. Concentration of iodine in sheep milk obtained from small family farms were below the required limit for iodine in milk. On the other hand, the content of iodine in milk from farm E was above the optimal range. The levels of iodine and selenium determined in sheep milk were comparable with the amounts commonly found in cow milk. In the winter period the concentration

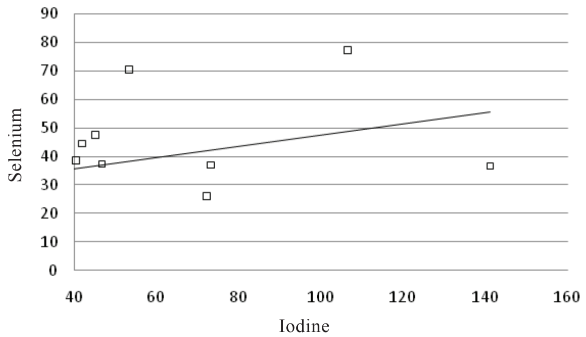

Fig. 1. Correlation of selenium and iodine contents in sheep milk (µg/kg)

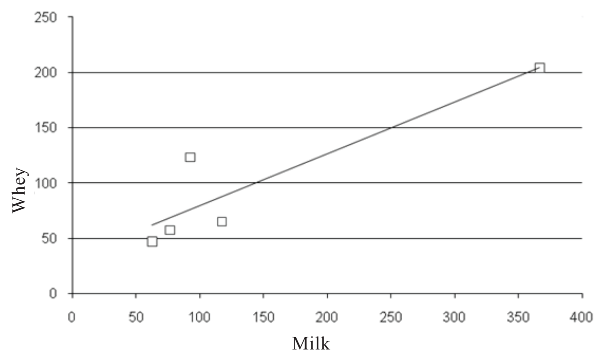

Fig. 2. Comparison of iodine values found in sheep milk and whey (µg/kg)

of both iodine and selenium was significantly higher than in the summer period. Moderately strong correlation was found between iodine and selenium contents. Approximately 70% of the iodine in milk transfers to the whey fraction, and thus whey, not only milk, is a good source of iodine in nutrition.

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Obsah selenu a jódu v ovčím mléce z farem ve středních a východních Čechách

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Ovčí mléko z různých farem ve středních a východních Čechách bylo analyzováno na obsah selenu (4 farmy) a jódu (6 farem). Vzorky byly odebírány jednou měsíčně ve dvouletém časovém rozsahu. Jód byl stanoven pomocí vysokoúčinné kapalinové chromatografie s elektrochemickou detekcí (HPLC-ED) a selen pomocí elektrotermické atomové absorpční spektrometrie s hydridovou technikou (ETA-AAS-HG). Průměrné obsahy selenu a jódu v mléce z malých rodinných farem byly 39,09 ± 12,77 μg.kg^{-1} resp. 47,99 ± 13,24 μg.kg^{-1}. Na velké farmě, zabývající se komerčním chovem, byly průměrné hodnoty obsahu jódu v mléce statisticky významně vyšší (576,7 ± 261,2 μg.kg^{-1}). Mezi letním a zimním obdobím byly nalezeny statisticky významné rozdíly v obsahu obou prvků. Hladina selenu v zimním období byla průměrně 68,33 ± 43,75 μg.kg^{-1} zatímco v letním období průměrně 30,67 ± 8,31 μg.kg^{-1}. Jód vykazuje podobný trend: 75,42 ± 46,37 μg.kg^{-1}v zimním období a 37,84 ± 16,09 μg.kg^{-1} v letním období. Mezi obsahem selenu a jódu v ovčím mléce byla zjištěna středně silná korelace (r = 0,39). Dále byl porovnán obsah jódu v ovčím mléce a syrovátce. Bylo zjištěno, že z mléka do syrovátky přejde 69,4 % jódu, což potvrzuje, že nejen mléko, ale i syrovátka je dobrý zdroj jódu v lidské výživě.

selen; jód; ovčí mléko; ovčí syrovátka; HPLC s elektrochemickou detekcí; hydridová technika

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