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Original article

Evaluation of selenium status and its distribution in organs of free living foxes (*Vulpes vulpes*) from an Se deficient area

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Abstract

The objective of the study was to determine selenium status and its distribution in the organs of free living foxes from selenium deficient areas of north-western Poland. Samples of organs harvested from 40 foxes shot during the 2008-2009 hunting seasons served as experimental material. Selenium concentration in the organs was determined spectrofluorometrically. Selenium distribution in tissues depends largely on its dietary content. Our study indicated that concentrations of selenium in the examined organs followed the order: kidney>liver>spleen>lung>heart and kidneys were the organ with the highest retention of this element. Mean selenium concentration in fox kidneys was 0.60 ± 0.15 μg/g wet weight. Several times less selenium on average was found in the liver (0.27 ± 0.09 μg/g w.w.), lungs (0.17 ± 0.06 μg/g w.w.), spleen (0.19 ± 0.06 μg/g w.w.) and heart (0.13 ± 0.05 μg/g w.w.). All the animals studied were deficient in selenium.

Key words: selenium, organ distribution, free living fox, Se deficient area

Introduction

Free living animals are often used in environmental studies as indicators of environmental pollution. They may also act as indicators of the environmental abundance of valuable bioelements and serve to monitor changes in terms of Se amounts and availability in different ecosystems (Pilarczyk et al. 2008, 2009, 2010b).

Selenium is unevenly distributed in the environment. Some areas have soils with high selenium content (considerable areas of North and South America and part of China) and other have soils deficient in this element (several provinces of China, New Zealand, Finland, a considerable part of Europe, including some regions of Poland) (Kabata-Pendias and Pendias 1993, Zust et al. 1996, Gupta and Gupta 2000, Pilarczyk et al. 2009). In Poland, due to the low selenium content in the environment, some animals become ill as a result of dietary selenium deficiency. Low selenium intake gives rise to degenerative diseases of some organs (e.g. heart and liver) and malformation of hard tissue (Turan at al. 2000). Inadequate selenium supply may cause all body tis-

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sues to be deficient in this element (Gabbedy et al. 1977, Pinkiewicz et al. 1986, Radostits et al. 2000).

Selenium distribution in tissues depends largely on its dietary content. In general, the highest amounts of selenium occur in the liver and kidney, and in the spleen as selenoprotein W (Kuczyńska and Biziuk 2007). Because the brain, endocrine glands and reproductive glands are supplied with selenium before the liver, heart and skeletal muscles, the latter organs suffer from disturbances in case of selenium deficiency (Behne et al. 1995).

The objective of the study was to evaluate the status of selenium and its distribution in the organs of free living foxes from a selenium deficient area.ą

Materials and Methods

Samples

Research material included liver (n=40), kidney (n=38), lung (n=20), heart (n=28) and spleen (n=18) samples obtained from red foxes originating from north-western Poland. The foxes were shot during the hunting seasons of 2008-2009 in compliance with the hunting limits set. The age and sex of animals were not determined. All tissue samples were homogenized and frozen (- 20°C), and stored in the laboratory until analysis.

Reagents

All chemicals used were of analytical reagent grade. Most of the chemicals were obtained from Chempur® except 2,3-diaminonaphtalene (DAN), which was obtained from Sigma Aldrich. Certified reference material BCR-185R (bovine liver) (European Commission Joint Research Centre Institute for Reference Materials and Measurements) was obtained from LGC Standards GmbH Wesel, Germany.

Analysis

Concentrations of selenium in samples were determined using the spectrofluorometric method described by Grzebuła and Witkowski (1977). The tissues were digested in HNO_3 at 230°C for 180 min. and in $HClO_4$ at 310°C for 20 min. The samples were then hydrolyzed with 9% HCl. The selenium was derivatized with 2,3-diaminonaphtalene (Sigma-Aldrich) under controlled pH (pH 1-2) conditions with the formation of selenodiazole complex. This complex was extracted into cyclohexane. EDTA and hydroxylamine hydrochlorine were used as masking agents. Se concentration was determined fluorimetrically using an RF-5001 PC Shimadzu spectrophotofluorimeter. The excitation wavelength was 376 nm, the fluorescence emission wavelength was 518 nm.

The accuracy of the analyses was verified using certified reference material BCR 185R (bovine liver). A reference sample was analyzed in triplicate. The mean Se concentration was 93.8% ± 5.2% of the reference values (range between 90.2% and 103.9%). The detection limit was 0.003 µg/g wet weight.

All results were expressed on a wet and dry weight basis.

Data analyses

Statistical calculations were performed using Statistica PL 7.1. All data are expressed throughout as an arithmetic mean ± standard deviation (SD), and also geometric mean and median. The concentrations of Se were $\log_{10}$-transformed to attain or approach a normal distribution of the data. Data were compared by one-way ANOVA, and Tukey's test was applied to test for differences in Se concentrations between organs examined. Differences were considered as significant at the level of $P < 0.05$ and $P < 0.01$. Relationships between concentrations of selenium in the liver, kidneys, lungs, heart and spleen were evaluated by calculating the Pearson's correlation coefficient ($r_{x,y}$). Statistical significance of coefficients of correlation was tested at the level $P < 0.05$ and $P < 0.01$. All statistical analyses were performed on wet weight concentration.

Results

The wet weight (w.w.) and dry weight (d.w.) concentrations of selenium in analyzed tissues are listed in Table 1 and 2.

The highest concentration of selenium was found in fox kidneys, where it averaged 0.60 ± 0.15 µg/g wet weight (2.36 ± 0.34 µg/g dry weight). These concentrations ranged widely from 0.34 to 0.93 µg/g w.w. (1.33-3.64 µg/g d.w.). Statistical analysis showed that Se concentration in kidneys was significantly ($P < 0.01$) higher compared to the other organs. Selenium concentration in liver (0.27 ± 0.09 µg/g w.w. on average) was half that in kidneys and almost three times as low in terms of dry matter. At the same time, Se liver concentrations were significantly ($P < 0.01$) higher than in the lungs, spleen and heart. The mean concentration of selenium in these organs was 0.17 ± 0.06, 0.19 ± 0.06 and 0.13 ± 0.05 µg/g w.w., respectively. The content of Se in the spleen did not differ significantly in relation to that in the lungs ($P=0.1402$) and heart ($P=0.0750$).

Table 1. Selenium concentration in micrograms per gram of wet weight of liver, kidney, lung, spleen and heart of red foxes.

Organs	N	Se concentration (µg/g w.w.)				
		Mean	SD	Range	GM	Median
Liver	40	0.266^{A}	0.093	0.112-0.436	0.250	0.259
Kidneys	38	0.603^{B}	0.145	0.340-0.934	0.586	0.588
Heart	20	0.127^{CF}	0.054	0.032-0.258	0.115	0.135
Lungs	28	0.174^{DE}	0.062	0.058-0.296	0.163	0.170
Spleen	18	0.189^{EF}	0.062	0.105- 0.391	0.181	0.171

GM – geometric mean
A,B – the different letters denote statistically significant differences at $p < 0.01$

Table 2. Selenium concentration in micrograms per gram of dry weight of liver, kidney, lung, spleen and heart of red foxes.

Organs	N	Se concentration (µg/g d.w.)				
		Mean	SD	Range	GM	Median
Liver	40	0.883^{A}	0.339	0.100-1.482	0.801	0.865
Kidneys	38	2.356^{B}	0.578	1.326-3.642	2.285	2.292
Heart	20	0.493^{CF}	0.233	0.128-1.033	0.432	0.541
Lungs	28	0.748^{DE}	0.266	0.249-1.272	0.697	0.729
Spleen	18	0.740^{EF}	0.069	0.692-0.789	0.739	0.731

GM – geometric mean
A,B – the different letters denote statistically significant differences at $p < 0.01$

Positive and very high correlations ($P < 0.01$) were also found between selenium levels in liver and lungs ($r=0.74$), liver and heart ($r=0.83$), and lungs and heart ($r=0.74$) (Table 3).

Table 3. Correlation coefficients between examined organs of foxes.

Organs	N	Value of coefficient of correlation ($r_{x,y}$)
Liver – kidney	37	nd
Liver – lung	28	0.74**
Liver – heart	20	0.83**
Liver – spleen	18	nd
Kidney – lung	28	nd
Kidney – heart	20	nd
Kidney – spleen	18	nd
Lung – heart	18	0.74**
Lung – spleen	16	nd
Heart – spleen	8	nd

** – coefficient of correlation significant at $p < 0.01$
nd – not detected (non-significant correlation)

Discussion

Foxes are opportunistic predators and will feed on whatever is available. For this reason, the composition of food consumed by foxes depends mainly on geographical location and species diversity of local prey. Foxes also consume plant food (fruit, berries, nuts), the availability of which also depends on geographical location. Goszczyński (1986) reports that a fox's diet in Poland includes small rodents (33-62%), hares (16.6-46%), carrion (3-21.2%), birds (1.9-26%) and plant food (1-6%). Polish foxes also consume scraps of food left by humans. Foxes from north-western Poland are characterized by a particularly high consumption of voles and other field rodents. In this part of Poland, food composition does not vary from season to season as much as in other areas (Goszczyński 1986).

Food is the main source of selenium and its body content depends on the amount and form of this element in the environment (Navarro-Alarcon and Cabrera-Vique 2008). No soil selenium map of Poland has been made to date, but the results of a few studies indicate that this element is deficient in Poland (Zabłocki 1990, Borowska 1996). West Pomerania (north-eastern Poland) is considered particularly deficient in selenium. Soil selenium concentration ranged from 0.02 to 0.29 in West Pomerania (Zabłocki 1990) but was reported to be higher in south Poland, where it ranged from 0.04 to 0.64 mg/kg (Piotrowska 1984). Low selenium concentration in the soils of Pomerania was also reported by Borowska et al. (2007) and Cieśla et al. (1994).

Selenium deficiency in Polish soils is responsible for the low selenium content of plants, which results in low selenium content of animal diets and tissues (Pilarczyk et al. 2009, 2010ab).

Selenium is absorbed in the gastrointestinal tract, where organic and inorganic selenium compounds are transported from the intestine to the liver. In this organ they are reduced to selenides, which are used for

the synthesis of selenoproteins. The remaining part is transported with blood to different tissues and organs (Alissa et al. 2003, Kuczyńska and Biziuk 2007).

The Se concentrations determined in fox organs in our study were lower than those obtained by other authors (Prestrud et al. 1994, Hoekstra et al. 2003, Millán et al. 2008). Hoekstra et al. (2003) reported that mean hepatic Se concentration in arctic foxes from the Canadian Arctic varied between 0.79 and 0.88 μg/g w.w. depending on sampling site. Higher Se concentrations were found in the liver of foxes from Svalbard (2.7 μg/g w.w. on average) by Prestrud et al. (1994) and in the liver of red foxes from southern Spain (1.72 μg/g d.w. on average, geometric mean) by Millán et al. (2008).

In our study, we found kidneys to have the highest content of selenium. The concentration of this trace element in liver, lungs and heart was several times lower.

Puls (1994) reported that selenium deficiency in *Canidae* occurs when Se liver concentration falls below 0.3 μg/g w.w. The level of 0.3-0.5 μg Se/g w.w. is marginal and that of 0.5-1.5 μg/g w.w. normal (optimal). When comparing Se liver concentrations in the foxes investigated in the present study with these criteria, it is clear that the free living foxes studied are deficient in this element. Similar conclusions can be drawn when analyzing Se concentration in kidneys, where selenium concentration in *Canidae* should be in the 1.0-1.5 μg/g w.w. range (Puls 1994) compared to just 0.34-0.93 μg/g w.w. found in our study.

Oh et al. (1976) found selenium concentration to be higher in kidneys than in liver when Se supply was inadequate. Based on the observations of these authors, it is evident that the foxes analyzed in our study were deficient in this trace element.

Se content in the heart was the lowest among all organs. Too small a concentration of Se in the heart may cause its dysfunction, especially since Se affects the aggregation and thrombotic processes in blood vessels and is found in the enzyme that controls the formation of cardiac muscle protein (Rayman 2000). Se deficiency in the heart results in degenerative lesions in the muscle, thus impairing normal blood flow in the body. One of the most common animal diseases caused by selenium deficiency is mulberry heart disease (MHD).

Conclusion

Comparing our results with the literature data and reference values for *Canidae* (Puls 1994), it can be assumed that all the animals studied were deficient in selenium. The highest retention of this element was characteristic of the kidneys. The concentrations of selenium in the organs examined followed the order: kidney>liver>spleen>lung>heart. The low Se concentration in the organs of foxes possibly suggests that this element is deficient in their diet and/or is present in a poorly available form.

Ethical Standards

The authors declare that the experiment complies with the current laws of Poland.

Conflict of interest

The authors declare that they have no conflict of interest.

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