Int. J. Environ. Res., 9(4):1287-1292, Autumn 2015
ISSN: 1735-6865

The Differences in the Level of Selenium in the Organs of Red Deer (Cervus elaphus) from Various Regions of Poland

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Received 30 July 2014; Revised 20 Jun 2015; Accepted 30 Jun. 2015

ABSTRACT: The aim of the study was to assess regional differences in selenium content in the environment based on analysis of the Se concentrations in liver and kidney of red deer from central, northern, south-eastern and south-western Poland. The level of selenium in organs was determined using spectrofluorometric methods. The mean concentration of selenium in the liver and kidney of red deer was 0.084 µg/g w.w. and 0.621 µg/g w.w., respectively. The highest mean concentration of selenium was found in the liver of red deer from the south-eastern region (0.120 µg/g w.w.), and it was significantly higher than the mean level of selenium in the liver of red deer from the northern (0.068 µg/g w.w.), central (0.070 µg/g w.w.) and south-western (0.079 µg/g w.w.) regions. The highest mean concentration of selenium in the kidney was also found in red deer from the south-eastern region (0.784 µg/g w.w.), and it was significantly higher than the mean level of selenium in the kidney of red deer from the northern (0.542 µg/g w.w.), central (0.561 µg/g w.w.) and south-western (0.606 µg/g w.w.) regions. Our results indicate that there are deficits of selenium in the environment in Poland.

Key words: Selenium, liver, Kidney, Red deer (Cervus elaphus), Poland

INTRODUCTION

Selenium (Se) is an essential element, important for human and animal health. The biological role of Se stems mainly from its presence in selenium-dependent enzymes which play a key role in many metabolic processes. Selenium is actively involved in the antioxidant defense systems, thyroid hormone metabolism and reproduction. It also plays an important role in neutralizing and removing a variety of toxic substances from the body (Kohrle, 2004; Florianczyk, 1999).

Studies on the concentration of selenium in Poland as well as in the world focused mainly on livestock animals. In the scientific literature there are only a few reports concerning red deer (Cervus elaphus).

In nature, Se is not uniformly distributed as there are areas with soils rich in this element as well as regions poor in Se. Majority of soils in the world has low selenium content (Borowska, 2002; Broadley *et al.*, 2006; Cartes *et al.*, 2005), and large part of Europe is considered to be low in Se (Flueck *et al.*, 2012; Flueck, 1994; Flueck & Smith-Flueck, 1990; Reglero *et al.*, 2009; Rodríguez-Estival *et al.*, 2011; Zust *et al.*, 1996).

The chemical composition of the soil is conditioned by numerous natural and anthropogenic factors. The level of Se in the soil and plants varies, which results from geographical location, climatic conditions (temperature, precipitation), the type of soil and its pH, soil salinity, intensification of leaching processes and Se displacement from the rock formations into water basins, absorption processes of iron oxides and soil clay minerals, organic matter content, the chemical form in which Se occurs, the plant species, the degree of environmental pollution (antagonistic elements such as lead, cadmium and sulphur) (Borowska, 1994; Borowska & Koper, 2006; Pezzarossa *et al.*, 2007; Pyrzynska, 2002). Therefore, there is a need to develop the so-called Se map for particular regions of the country to be able to develop in the future a program of preventive supplementation of this element in animals. D?bski et al. (1993) demonstrated the relationship between the concentration of selenium in the milk of cows and the concentra-

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tion of Se in the animals living in the wild. Therefore, free-living animals can be a good indicator of the content of this element in the environment for the animals fed with regional feeds (e.g., cows, sheep and goats grazing in pastures).

The aim of the study was to assess regional differences in Se content in the environment based on analysis of the Se concentrations in liver and kidney of red deer from central, northern, south-eastern and south-western Poland.

MATERIALS & METHODS

Most chemicals were obtained from Chempur® apart from 2,3-diaminonaphthalene (DAN) which was obtained from Sigma-Aldrich. The analytical procedure was verified by determining Se concentration in the reference material NCS ZC 71001 (bovine liver) (China National Analysis Center for Iron and Steel, Beijing, China). All chemicals used were of analytical reagent grade.

The study was performed in the area of Poland (central Europe), which devided on four regions: central, northern, south-eastern and south-western (Fig.1).

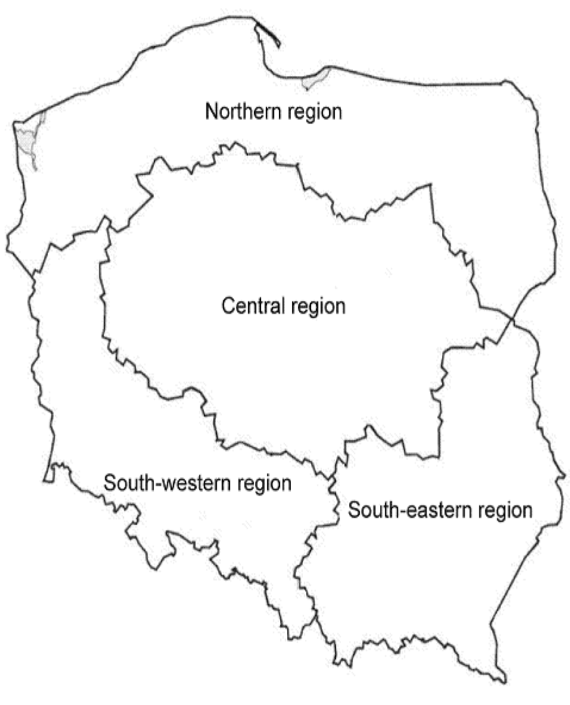

Fig.1. Sampling sites according to the geographical location

Northern region of Poland constitute lands that have long been considered poor in Se, which is reflected in its content in the tissues of free-living animals (Pilarczyk *et al.*, 2009). Se deficient soils contain from 0.1 to 0.6 mg/kg of Se (Gupta & Gupta 2000). According to Zab?ocki (1990), the Se level in the soil in Western Pomerania ranged from 0.026 to 0.293 mg/kg, while the soils in the southern Poland contained even 3-fold higher concentration (0.04 - 0.64 mg/kg) of this element (Piotrowska, 1984). Even higher content of Se in the soils of southern Poland was measured by Biernacka & Ma?uszy?ski (2006) (0.060 - 0.818 mg/kg). The experimental material used in this study consisted of liver and kidney samples obtained from red deer (Cervus elaphus). A total of 354 (177 liver and 177 kidney) samples collected from 177 red deer of both sexes were tested. Animals were shot during the hunting season between 15 August 2009 and 15 January 2010, in compliance with the hunting limits set. Experimental material for the study was acquired in 4 geographical regions of Poland: northern (n=52), central (n=41), south-eastern (n=44) and south-western (n=40) (Fig. 1). No Se-supplemented salt licks were used in the Hunting Clubs, from which the material was obtained. The timing of hunting conformed to the Regulation of the Minister of Environment of 16 March 2005 establishing seasons of hunting warrantable animals (Journal of Laws, 25 March 2005). The animals harvested from different provinces were in the same age range of 4 to 6 years.

All tissue samples (one lobe of the liver and whole kidney) were homogenized and then frozen (-20°C), and stored in the laboratory until analysis. During collection of samples for chemical determinations we also collected samples for dry matter determination (samples of 1 g were dried at 105°C until they became a solid mass). The result of chemical determination obtained for wet weight (w.w.) of the sample was converted into dry matter (d.w.).

Selenium concentrations in organs were determined using spectrofluorometric methods (Grzebula & Witkowski 1977). The samples were digested in 3.5 ml HNO3 at 230°C for 180 min and in 3 ml HClO4 at 310°C for 20 min. Finally, 3 ml 9% HCl was added to reduce Se6+ to Se4+. Selenium was derived with 2,3-diaminonaphthalene (Sigma-Aldrich) under the conditions of controlled pH (pH 1-2) 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 fluorometrically using a Shimadzu RF-5001 PC spectrofluorophotometer. The excitation wavelength was 376 nm, and the fluorescence emission wavelength was 518 nm.

The accuracy of the method was verified using certified reference material NCS ZC 71001 (bovine liver) (China National Analysis Center for Iron and Steel, Beijing, China). The Se concentrations obtained were

91.1% of the reference value.

The results were statistically analysed using STATISTICA 9.0 PL. Tables give arithmetic means ($\bar{x}$), standard error of the mean (SEM), and minimal and maximal values (range). The normality of variable distribution was tested by the Shapiro-Wilk test, after which variables not normally distributed were adjusted to normal distribution by logarithmic transformation. Statistical analysis was performed with log-transformed data using one-way analysis of variance to test the effect of each variable separately. Significant differences were determined using Duncan's test. The correlation between liver and kidney selenium concentrations in the studied animals was determined by calculating Pearson's coefficient of correlation (rx,y).

RESULTS & DISCUSSION

The mean concentration of Se in the liver of red deer studied from Poland was 0.084 ug/g w.w., while in the kidney it amounted to 0.621 ug/g w.w. (Table. 1 and 2). The highest mean Se concentration was found in the liver of red deer from the south-eastern region (0.120 µg/g w.w.), and it was significantly ($P \leq 0.01$) higher than the mean Se level in the liver of red deer from the northern (0.068 µg/g w.w.), central (0.070µg/g w.w.) and south-western (0.079 µg/g w.w.) regions (Table 1).

The highest mean Se concentration in the kidney was also found in red deer from the south-eastern region (0.784 µg/g w.w.), and it was significantly ($P \leq 0.01$; $P \leq 0.05$) higher than the mean Se level in the kidney of red deer from the northern (0.542 µg/g w.w.), central (0.561 µg/g w.w.) and south-western (0.606 µg/g w.w.) regions (Table 2).

In the current study, the mean Se concentration of selenium in the liver of red deer studied from the region of Poland was 0.294 µg/g d.m., while in the kidney it amounted to 2.171µg/g d.m. Red deer from south-eastern Poland had over 1.5-fold higher concentration of Se in the liver (0.120 µg/g w.w.) compared to animals from northern region (0.068 µg/g w.w.). The highest level of Se in kidneys was also found in deer (0.784 mg/g w.w.) from the south-eastern region, while the lowest in deer (0.542 µg/g w.w.) from the northern region.

There are scarce publications in the Polish literature that relate to the Se concentration in the organs of

Table 1. The mean concentration of selenium in the liver of red deer tested according to the geographical location

Region	N	Se content µg/g w.w.			Se content µg/g d.m.		
		Mean	SEM	Range	Mean	SEM	Range
Northern region (1)	52	0.068	0.007	0.014-0.214	0.238	0.023	0.048-0.746
Central region (2)	41	0.070	0.007	0.035-0.272	0.246	0.024	0.124-0.951
South-eastern region (3)	44	0.120	0.017	0.016-0.474	0.420	0.061	0.057-1.658
South-western region (4)	40	0.079	0.006	0.016-0.173	0.276	0.022	0.057-0.605
Total	177	0.084	0.005	0.014-0.474	0.294	0.019	0.048-1.658
Significance:	3 : 1, 2, 4*						

Statistically significant at: * P<0.05

SEM - standard error of mean

Table 2. The mean concentration of selenium in the kidney of red deer tested according to the geographical location

Region	N	Se content µg/g w.w.			Se content µg/g d.m.		
		Mean	SEM	Range	Mean	SEM	Range
Northern region (1)	52	0.542	0.062	0.124-3.027	1.895	0.216	0.430-10.577
Central region (2)	41	0.561	0.027	0.255-1.126	1.960	0.095	0.889-3.934
South-eastern region (3)	44	0.784	0.070	0.179-1.971	2.740	0.245	0.627-6.886
South-western region (4)	40	0.606	0.044	0.275-1.522	2.119	0.155	0.962-5.318
Total	177	0.621	0.029	0.124-3.027	2.171	0.100	0.430-10.577
Significance:	3 : 1, 2, 4*						

Statistically significant at: * P<0.05

SEM - standard error of mean

red deer. These are fragmentary data and refer only to the northern Poland (Western Pomerania and Warmia and Mazury voivodeships). The study of Wieczorek-D?browska (2009) found a comparable Se level in the liver (0.23 µg/g d.m.) of red deer derived from the West Pomeranian region compared to the values obtained in our study for the northern Poland. In the case of kidney, values obtained in our study were slightly lower when compared to the results of Wieczorek-Dabrowska (2009) (2.62 µg/g d.m.). Pilarczyk et al. (2009) measured over 1.5-fold higher Se concentration in the liver of red deer from Western Pomerania (0.37 µg/g d.m.) in comparison to the results of the current study. These authors have also demonstrated a greater concentration of this element in the kidneys of deer studied (2.72 µg/g d.m.) in relation to our investigations. Jarzynska & Falandysz (2011) detected a comparable Se content, to our results, in the liver of red deer (0.20 µg/g d.m.) in the north-eastern region (Warmia and Mazury). Further, low Se level in our study was found in the organs of free-living animals in the central region. According to Debski et al. (2001), in these regions predominant are low peat and sandy soils or soils formed from the sands of various origin. These soils have a low content of Se. The bioavailability of Se from peat soils is very low due to the significant acidification.

There are only few publications in the world literature that relate to the Se concentration in the organs of red deer. These data are fragmentary and concern only selected regions of the country. In the study by Vikøren et al. (2005) conducted in Norway, the mean Se concentration in the liver of red deer studied was 0.11 µg/g w.w. The Se level detected was similar to the concentrations found in the present study in the south-eastern region (0.120 µg/g w.w.). Over 2-fold higher concentration of Se in the liver of red deer in Croatia was found by Lazarus et al. (2008) (0.24 µg/g w.w.). McDowell et al. (1995) found over two-fold higher content of Se in the liver of white-tailed red deer (Odocoileus virginianus)(0.68 µg/g d.m.) originating from the area of southern Florida.

According to Pollock (2005), biochemical criteria used for the diagnosis of Se deficit based on the concentration of Se in the liver of white-tailed red deer are as follows: below 0.6 µg/g d.m. - deficit; 0.6-0.88 µg/g d.m. - marginal level; above 0.88 µg/g d.m.- appropriate level (optimum) for deer. However, accoridng to McDowell et al. (1995), the Se level below 3.0 µg/g d.m. in the kidney of game animals indicate a deficit of this element.

Considering the above-given standards, Se deficiency can be identified in the liver and kidney of examined red deer from the whole Poland. One of the reasons for this phenomenon may be a low concentration of bioavailable forms of selenium in the soil, which significantly determines the concentration of this element in plants eaten by deer.

Distribution of Se in the plant as well as in plants of particular species is unequal and not uniform. Hartfiel & Bahners (1987) showed that the Se content in the deeper layers of the soil was decreasing, thus the plants with a deep root system had lower availability of that element. Distribution of Se in various parts of plants depends on the species as well as their developmental stage and physiological condition. Terry et al. (2000) reported that plants accumulated selenium primarily in young leaves in the early stages of development and in seeds during the reproductive phase. Then a decrease in the content of Se occurred in the leaves. These authors showed a comparable content of Se in caryopses and roots in mature cereal plants, while its level was lower in stems and leaves.

An essential part of the natural red deer food, in rich habitats, depending on the season, is a deciduous browse. In poorer habitats in the spring and summer red deer eat large quantities of grasses and herbs, while the food basis in autumn is the browse derived from coniferous species. In some hunting grounds, in addition to forest and meadow plants, crops such as potatoes, beets and oats are also a significant part of the red deer diet (Gebert & Verheyden-Tixier, 2001; Kamler & Homolka, 2005).

Pilarczyk et al. (2011) identified in the region of Western Pomerania during summer a deficit of Se in 100% of red deer studied, while in autumn and winter it amounted to 28.6 and 83.3%, respectively. These authors showed optimum Se concentration in the liver of red deer only during autumn (28.6% of the studied animals). Previous study of Pilarczyk et al. (2009), carried out again in Western Pomerania (years 2003-2007), also showed a deficiency of this element in red deer, and it affected all the animals tested in winter, 94% in summer and 75% in autumn. The optimum concentration of Se was found only in 10% of individuals in autumn and 6% in summer.

Significant positive correlations between the concentration of Se in the liver and kidney of red deer were found in the south-eastern ($r_{x,y}$=0.92; P≤0.001), south-western ($r_{x,y}$=0.75; P≤0.001), central ($r_{x,y}$=0.45; P≤0.01) and northern ($r_{x,y}$=0.38; P≤0.01) regions (Table 3).

In the few publications on selenium level in free-living animals (wild boar, roe deer and red deer), a relationship has been described between the concentration of this element in the liver and kidneys. In our study there was a significant high positive correlation observed between the concentration of Se in the liver and kidneys in all red deer investigated combined (rx,y

Table 3. Correlation between the concentration of selenium in the kidney and liver of red deer according to the geographical location

Region	N	Correlation coefficient ($r_{x,y}$)
Northern region (1)	52	0.38**
Central region (2)	41	0.45**
South-eastern region (3)	44	0.92**
South-western region (4)	40	0.75**
Total	177	0.70**

Correlation coefficient significant at: **P<0.01

= 0.70). Pilarczyk et al. (2009) documented the existence of a weak correlation between the Se concentration in the liver and kidneys of deer from the area of Western Pomerania (rs = 0.23). These authors analyzed particular seasons and found a statistically significant correlation (rs = 0.36) only in summer, whereas in other seasons the correlation was statistically irrelevant.

CONCLUSION

Our results indicate that there are deficits of Se in the environment in Poland. It is evidenced by the low content of this element in the organs of red deer. We have demonstrated marked differences in the selenium content in the organs of deer that depend on geographic location. The highest level of Se in the liver and kidneys of red deer was found in south-eastern Poland, while the lowest in the northern part of Poland. Due to the fact that red deer are fully integrated with the environment (throughout the life), they are a good indicator of the content of Se in the environment.

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