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ORIGINAL PAPER

Liver and kidney concentrations of selenium in wild boars (*Sus scrofa*) from northwestern Poland

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Abstract The aim of this study was to determine liver and kidney concentrations of selenium in wild boars from the northwest part of Poland, depending on season of the year, age, sex, and body weight. Altogether, samples of livers and kidneys from 172 wild boars that were shot in 2005–2008 were investigated. Liver and kidney concentrations of selenium were determined using spectrofluorometric method. In all the animals studied, selenium concentration was several times lower in the liver than in the kidneys. Selenium concentration averaged 0.19 μg/g wet weight (w.w.) in the liver and 1.20 μg/g w.w. in kidneys. The present study showed that season ($P \leq 0.05$), age ($P \leq 0.01$), and body weight ($P \leq 0.01$) have a significant effect on selenium concentration in the liver of wild boars. Liver selenium concentration was the highest in spring (0.23 μg/g w.w.) and the lowest in autumn (0.16 μg/g w.w). Young animals (up to 1 year of age) and those with the lowest body weight (up to 20 kg) were characterized by a slightly lower selenium concentration in the liver compared to older and heavier animals. No significant differences were found in organ selenium concentration between males and females. According to biochemical criteria for the diagnosis of selenium deficiency in pig liver, which were used to evaluate selenium concentration in the liver of wild boars, no individuals were found to have optimal levels. Considering that in Se deficiency higher selenium concentrations are found in kidneys than in the liver, it can be presumed that the wild boars had Se deficiency. However, this is difficult to state conclusively because there are no reference values for this species.

Keywords Selenium · Trace elements · Wild boar · *Sus scrofa* · Kidney · Liver · Poland

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Introduction

Selenium is an essential trace element that determines the normal growth and development of humans and animals. The biological role of selenium stems mainly from its presence in selenium-dependent enzymes (glutathione peroxidase, iodothyronine deiodinase, selenoproteins P and W, thioredoxin reductase) which play a key role in many metabolic processes (Musik et al. 2003; Kohrle 2004; Floriańczyk 1999).

In nature, selenium occurs irregularly, with some areas rich in selenium (considerable areas of North and South America and part of China) and some deficient in this element (a considerable part of Europe, including some regions of Poland, several provinces of China, and New Zealand; Kabata-Pendias and Pendias 1993; Zust et al. 1996; Pilarczyk et al. 2008a, 2009).

Until recently, research on Se levels in animals and the health consequences of its deficiency was limited to farm animals only. Polish research in this area (Jaśkowski et al. 1993; Balicka-Ramisz et al. 2006; Pilarczyk et al. 2007, 2008b) demonstrated low serum concentrations of selenium in animals, possibly suggesting that these animals are raised in selenium-deficient areas.

Thanks to a full integration with their habitat, free-living animals are a good indicator of environmental selenium concentrations throughout their lives. Game animals have been examined relatively seldom (McDowell et al. 1995; Vikøren et al. 2005; Pilarczyk et al. 2008a, 2009). Selenium deficiency in free-living animals is strictly associated with the amount and availability of this element in the soil. Selenium is taken up from the soil environment by plants, from which it reaches animals. The amount of selenium absorbed by plants depends on a number of factors such as soil pH, oxidation and reduction conditions of the soil environment, and degree of selenium oxidation. As a result of low amounts of available selenium in the soil, it is low in plants and deficient in animals (Kabata-Pendias and Pendias 1993).

The wild boar (*Sus scrofa*) is an omnivorous animal that ingests over 90% of its food from plants and around 10% from animals. Wild boars consume acorns, beech nuts, nuts, berries and other fruits, some grasses and herbs, rootlets and rhizomes, imagoes, their pupae and larvae, small rodents, frogs, eggs and chicks of birds nesting on the ground, young mammals that let themselves be caught, and carrion (Genov 1981; Pinna et al. 2007).

At present, selenium is acknowledged to be a microelement that is vital for the normal height and development of animals and humans. Selenium deficiency in farm and free-living animals may cause large economic losses due to its associated white muscle disease, mulberry heart disease, and hepatosis dietetica in hogs and wild boars; in females, it can lead to the death of fetuses due to their arrested development, retained placenta after delivery, inflammation of the genital tract, the MMA syndrome, and a decrease in the breeding abilities of males (Grela and Sembratowicz 1997; Radostits et al. 2000). Although there is no official information about the incidence of selenium deficiency diseases in wild boars from the northwest part of Poland, hunters reported seeing smaller litters from time to time.

Jacyno et al. (2005) observed that animals receiving higher dietary amounts of Se were characterized by better libido, higher concentration and total number of spermatozoa in ejaculate, and lower percentage of spermatozoa with morphological defects. Likewise, Reglero et al. (2009a) found semen quality to be strictly related to selenium concentration in testes. This shows that selenium deficiency may be one of the reasons for a decline in the wild boar population.

The aim of this study was to determine selenium concentrations in the liver and kidneys of wild boars from the northwest part of Poland, depending on the season, sex, and body weight.

Materials and methods

Samples

Research material included liver and kidney samples obtained from 172 wild boars originating from northwestern Poland. Wild boars were shot during the hunting seasons of 2005–2008 in compliance with the hunting limits set. Thirty-seven, 56, and 49 wild boars were harvested during the 2005/2006, 2006/2007, and 2007/2008 hunting seasons, respectively. After the shooting, carcasses and organs were transported to a wild game collection center, where the carcasses were marked and the age, sex, and weight of animals were determined. The age of animals was estimated using fur characteristics (Lochman 1987). After these procedures, the prepared organs (n_{liver}=172, n_{kidney}=172) were transported to a laboratory and stored at −20°C until chemical analyses. Whole livers and kidneys were homogenized to obtain a uniform material, from which samples were collected for selenium determination.

Reagents

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

Analysis

Concentrations of selenium in liver and kidneys were determined using spectrofluorometric method (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. Then, the samples were hydrolyzed with 9% HCl. Selenium was derivatized with 2,3-diaminonaphtalene (Sigma Aldrich) under the conditions of controlled pH (pH 1–2) with the formation of selenadiazole complex. This complex was extracted into cyclohexane. EDTA and hydroxylamine hydrochlorine were used as masking agents. Se concentration was determined fluorimetrically using 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 Se concentration obtained ranged between 89.4% and 101.7% of the reference values (mean 95.5%±5.9%). The detection limit was 0.003 μg/g wet weight.

Statistical data analyses

Statistical calculations were performed using procedures of STATISTICA PL 7.1. All data are expressed throughout as an arithmetic mean ± standard deviation and also geometric mean and median. The concentrations of the Se were log-transformed to attain or approach a normal distribution of the data. The effects of season, sex, age, and body weight on selenium concentration in the liver and kidneys of wild boars were analyzed using generalized linear models. Differences were considered as significant at the level of $P<0.05$ and $P<0.01$. Relationships between concentrations of selenium in the liver and in the kidneys were evaluated by calculating the coefficients of correlation. Pearson's correlation coefficient ($r_{x,y}$) was counted for every season separately and together for all data. Statistical significance of coefficients of correlation was tested at the level $P<0.05$ and $P<0.01$.

Results

The present study showed that season ($P\leq0.05$), age ($P\leq0.01$), and body weight ($P\leq0.01$) have a significant effect on selenium concentration in the liver of wild boars, while kidney selenium concentration was significantly influenced only by season ($P\leq0.05$; Table 1). In all the animals studied, selenium concentration was higher in kidneys than in livers (Table 2). Liver selenium concentration averaged 0.19 μg/g w.w. in the liver and was several times higher in kidneys (1.20 μg/g w.w.). The lowest selenium concentration in the liver was found in the autumn period and the highest in the spring and summer periods, with statistically significant differences ($P\leq0.05$). In kidneys, selenium concentration was also the lowest in the autumn. In the summer period, a statistically significant ($P\leq0.05$) positive mean correlation of $r=0.36$ was found between selenium concentration in the liver and in kidneys, with no such correlations observed in the other periods.

Table 1 Effect of season, sex, age, and body weight on selenium concentration in the liver and kidneys of wild boars

Treatment	Liver *P* values	Kidneys *P* values
Season	0.02	0.03
Sex	–	–
Age	<0.001	–
Body weight	<0.001	–

– no correlation (non significant correlation)

Our results showed that the sex of animals had no significant effect on Se concentration in the organs. Mean selenium concentration in kidneys and liver was 1.23 and 0.19 μg/g w.w. in females and 1.14 and 0.20 μg/g w.w. in males.

Young animals up to 1 year of age (Table 2) were characterized by lower selenium concentration in the liver ($P\leq0.01$) compared to older animals. In addition, a statistically significant ($P\leq0.05$) positive mean correlation of $r=0.36$ was found between liver and kidney selenium concentration of young animals.

The analysis of the effect of body weight on selenium concentration in the liver of wild boars revealed similar relationships as for the effect of age. Animals weighing up to 20 kg were characterized by significantly ($P\leq0.01$) lower selenium concentration in the liver (0.15 μg/g w.w. on average) compared to animals weighing above 40 kg. In animals weighing between 41 and 60 kg, there was a statistically significant ($P\leq0.05$) positive mean correlation of $r=0.30$ between selenium concentration in the liver and kidneys.

Discussion

At present, no standards are available for selenium concentrations in the liver and kidneys of wild boars. The results of environmental studies should not be compared with the results obtained for pigs because of major differences in the feeding of these animals, which translates directly into Se concentration in their bodies. Because there are no reference values concerning selenium content in the organs of wild boars, we used the standards designed for pigs. According to Puls (1994), biochemical criteria used in the diagnosis of selenium deficiency in the liver of pigs are as follows: below 0.11 μg/g w.w., deficiency; 0.12–0.39 μg/g w.w., marginal level; above 0.40 μg/g w.w., suitable (optimal) level for animals. Using these criteria to evaluate selenium concentration in the liver of wild boars, no individuals were found to have optimal levels. Se deficiency was observed in 31.0% of animals in autumn, 20.9% in summer, 15.2% in winter, and 12.0% in spring. Marginal levels were found in the other animals.

In the examined wild boars, liver selenium concentration ranged from 0.05 to 0.58 μg/g w.w. The highest mean concentration of selenium in the liver (0.23 μg/g w.w.) was found in spring, during the period when the lowest proportion of animals had Se deficiency.

Table 2 Selenium concentration

	N	Se concentration (μg/g w.w.)										
		Liver					Kidney					Value of the coefficient of correlation ($r_{x,y}$) between Se concentrations in the liver and kidney
		Mean	SD	Range	GM	Median	Mean	SD	Range	GM	Median	
Season												
Winter	46	0.17	0.07	0.08–0.38	0.16	0.16	1.20	0.26	0.70–2.03	1.17	1.21	–
Spring	25	0.23a	0.11	0.10–0.40	0.21	0.21	1.16	0.32	0.14–1.56	1.08	1.22	–
Summer	43	0.21b	0.09	0.09–0.40	0.19	0.18	1.39a	0.36	0.22–1.98	1.32	1.41	0.36*
Autumn	58	0.16ab	0.07	0.05–0.36	0.15	0.16	1.11a	0.31	0.24–1.62	1.04	1.21	–
Total	172	0.19	0.09	0.05–0.58	0.17	0.17	1.21	0.33	0.14–2.03	1.15	1.22	0.21**
Sex												
Male	83	0.20	0.10	0.05–0.40	0.17	0.19	1.14	0.36	0.14–1.97	1.09	1.22	0.29**
Female	89	0.19	0.08	0.06–0.39	0.17	0.18	1.23	0.32	0.24–2.03	1.19	1.22	–
Total	172	0.19	0.09	0.05–0.58	0.17	0.17	1.21	0.33	0.14–2.03	1.15	1.22	0.21**
Age [year]												
<1	49	0.14AB	0.06	0.05–0.39	0.13	0.13	1.19	0.35	0.30–2.03	1.13	1.22	0.36*
1–2 years	99	0.20A	0.09	0.06–0.40	0.18	0.19	1.24	0.30	0.22–1.97	1.19	1.22	–
>2	24	0.24B	0.08	0.09–0.39	0.22	0.23	1.13	0.40	0.14–1.80	1.01	1.17	–
Total	172	0.19	0.09	0.05–0.58	0.17	0.17	1.21	0.33	0.14–2.03	1.15	1.22	0.21**
Weight (kg)												
≤20	29	0.15AB	0.07	0.06–0.39	0.13	0.13	1.23	0.36	0.53–2.03	1.21	1.21	–
21–40	41	0.18	0.07	0.05–0.38	0.16	0.15	1.15	0.36	0.24–1.97	1.11	1.22	–
41–60	54	0.21A	0.08	0.07–0.40	0.19	0.20	1.24	0.33	0.22–1.97	1.22	1.22	0.30*
>60	48	0.21B	0.10	0.06–0.40	0.17	0.20	1.14	0.36	0.14–1.80	1.07	1.20	–
Total	172	0.19	0.09	0.05–0.58	0.17	0.17	1.21	0.33	0.14–2.03	1.15	1.22	0.21**

The same lower case letters denote statistically significant differences at $p<0.05$; the same upper case letters denote statistically significant differences at $p<0.01$

GM geometric mean, – no correlation (non significant correlation)

*$p<0.05$ (statistically significant coefficient of correlation); **$p<0.01$ (statistically significant coefficient of correlation)

Our study has shown that, in all seasons of the year, mean selenium concentrations in the wild boars reached only marginal levels. The animals' habitat has long been considered deficient in selenium. Zabłocki (1990) reported soil selenium concentrations in northwest part of Poland to range from 0.026 to 0.293 mg/kg d.w., while even a threefold higher concentration of this element was found in the soils of south Poland (Piotrowska 1984). Research in the northwest part of Poland on Se concentration in wild ruminants showed Se deficiency (based on Se status criteria for ruminants; Puls 1994) in more than half of the animals studied (Pilarczyk et al. 2008a, 2009).

We found that season of the year has a significant effect of selenium concentration in the organs of wild boars, especially in their livers. The highest concentration of this element was found in the spring and summer period and the lowest in the autumn. This phenomenon is probably due to seasonal variations in wild boar diets (Giménez-Anaya et al. 2008; Baubet et al. 2004). Baubet et al. (2004) observed that green parts of plants are dominant in the diet of European wild boars in the spring. Galeas et al. (2007) reported that research on seasonal fluctuations in Se content of plants reveal that the highest concentrations of this element in leaves are found during the spring period. According to Baubet et al. (2004), plant roots, seeds, and fruits form a considerable proportion of the diet in spring. Compared to spring and summer, an increased proportion of underground parts of plants (roots and bulbs) in the diet is observed in autumn. During this period, Se returns to the roots but in smaller amounts (Galeas et al. 2007) and, what is more, volatile forms of Se are formed in roots as a result of increased metabolism (Wójcik 2000). In summer, in addition to forest and meadow plants, wild boars eagerly use agricultural crops such as maize, cereals, and potatoes

(Genov 1981). Schley and Roper (2003) report that cultivated plants are often part of the diet and are consumed in large amounts.

It is also worth noting that, in the autumn (October and November), wild boars show increased sexual activity accompanied by no food intake (Weiler et al. 1996). In addition to seasonal changes in the availability of selenium-rich foods, this phenomenon probably influences selenium concentration in the organs of these animals.

The mean Se concentration in the liver (geometric mean ≈0.576 μg/g d.w., taking into account the mean d.w. of liver 31.2%) of wild boars, obtained in our study, was comparable with the findings of Reglero et al. (2009b), who reported that the liver of wild boars from south Spain contained 0.453 μg Se/g d.w. (Sierra Madrona) and 0.589 μg Se/g d.w. (Montes de Ciudad Real and eastern Sierra Morena). However, these results are lower compared to other species of animals. In roe deer from Western Pomerania (Poland), Pilarczyk et al. (2008a) observed that the mean selenium concentration was 0.71±0.64 μg/g d.w. in the liver and 3.09±1.73 μg/g d.w. in the kidneys. McDowell et al. (1995) found that average Se concentration in the liver of white-tailed red deer was 0.676 μg/g d.w. Millán et al. (2008) reported that the liver selenium concentration in red foxes and badgers from Spain averaged 1.72 and 4.88 μg/g d.w., respectively

Statistical analysis showed that young animals up to 1 year of age were characterized by significantly ($P \leq 0.01$) lower concentration of selenium in the liver compared to older animals. A similar relationship in other animal species was observed by Rush et al. (2008) and Conover and Vest (2009). We believe that such a low concentration of Se in young wild boars could result from more rapid metabolism in these animals. The increased metabolic rate may result in a release of greater amounts of free radicals, thus increasing the body's requirement for substances with antioxidant properties, including glutathione peroxidase that contains selenium, which is found in the liver. Based on results of experimental studies with rats, Sunde and Thompson (2009) concluded that selenium requirement in young animals can be half as large as in adult animals.

In our study, we observed that liver Se concentrations in males and females were similar. No correlation between Se concentration and sex was also reported by Nicpoń et al. (2005) in dogs, Conover and Vest (2009) in eared grebes (*Podiceps nigricollis*), and Rush et al. (2008) in polar bears. Meanwhile, Millán et al. (2008) found that the liver of female red foxes and mongooses had higher Se concentrations compared to the males. Perhaps, such differences are observed when there are distinct differences in feeding habits between sexes.

Pollock (2005) holds that selenium concentrations in the liver are a better indicator of Se status than the kidneys. However, considering the lack of proper reference values, knowledge of the content of this element also in kidneys can help diagnose Se deficiency in animals. Oh et al. (1976) observed that kidney selenium concentrations in animals receiving selenium-deficient feeds were much higher than in the liver and found an opposite situation in animals receiving selenium-rich feeds. In our study, we observed that liver Se concentration was several times lower than in kidneys, possibly suggesting that the dietary content of this element was too low or the form of Se was poorly available.

Comparison of the Se concentrations in wild boar kidneys, obtained in the present study, with the criteria used in the diagnosis of selenium deficiency in the kidneys of swine (Puls 1994), shows that optimal concentrations in wild boar kidneys were shown only in 4.0% of animals in spring, 37.2% in summer, 5.2% in autumn, and 8.7% in winter.

Conclusion

Because of limited literature data on selenium concentration in the organs of wild boars and the lack of reference values, it is difficult to state conclusively if the content of this element fell within the range that could be considered appropriate. The selenium deficiency could be evidenced by the fact that Se concentration in the liver of wild boars was several times lower than in kidneys. It seems necessary to conduct further research in this area while analyzing the health status and reproductive parameters of wild boars to determine the reference values for this species.

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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