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Selenium Content in Selected Organs of Roe Deer (*Capreolus capreolus*) as a Criterion to Evaluate Environmental Abundance of this Element in Poland

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ABSTRACT: Due to the fact that a considerable part of Europe, including some regions of Poland, is considered to be deficient in selenium, it seems necessary to develop a selenium map for different regions of Poland, which will be used in future to prepare a prophylaxis programme against selenium deficiency in animals. The aim of the study was to determine selenium content in selected organs (liver and kidneys) of roe deer from different regions of Poland as an indirect method of assessing environmental abundance of this element. Material for the study was collected from 28 sites located in 15 provinces of Poland. Selenium concentrations in organs were determined using spectrofluorometric methods. Mean selenium concentration in roe deer from Poland was 0.088 µg/g wet weight (w.w.) in liver and 0.503 µg/g w.w. in kidneys. Organ selenium concentrations in the studied animals varied considerably according to geographical location. Most areas of Poland are deficient in environmental selenium, as evidenced by the low content of this element in the organs of roe deer.

Key words: Selenium, Liver, Kidneys, Roe deer (*Capreolus capreolus*), Poland

INTRODUCTION

A considerable part of Europe, including some regions of Poland, is considered to be deficient in selenium (Flueck *et al.,* 2012; Kabata-Pendias & Pendias, 1999; Pilarczyk *et al*., 2008a, b; Pilarczyk *et al*., 2009; Zust *et al.,* 1996). Most authors highlight the need to monitor selenium status in animals, due to a very narrow margin of safety between deficient, optimal and toxic levels (Flueck *et al*., 2012; Witkowska *et al*., 1991). Therefore, it is necessary to develop a selenium map for different regions of Poland, which will be used in future to prepare a prophylaxis programme against selenium deficiency in animals. Studies on selenium concentrations in Poland and around the world have mainly focused on farm animals. There is scant scientific literature on animals living freely in the wild (roe deer, red deer, fallow deer, wild boar). Because free-living animals live in close association with their habitat throughout their lifetime, they are a good indicator of environmental selenium. Research on environmental selenium levels includes measurements of selenium concentrations in organs of free-living animals of the terrestrial ecosystem. Many different species of animals were used in such environmental studies, including wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) (Amici *et al*., 2012; Humann-Ziehank *et al*., 2008; Lazarus *et al*., 2008; Pilarczyk *et al*., 2010; Vikøren *et al*., 2005, 2011). Good bioindicators are those animals which meet specific conditions such as large population size, broad territorial range of a species, appropriate trophic level, availability and accessibility (Tataruch & Kierdorf, 2003). Roe deer is one of the most important game animals in Europe and meets the requirements describe above.The aim of the study was to evaluate selenium content in selected organs (liver and kidneys) of roe deer from different parts of 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 selenium 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 experimental material used in this study consisted of liver and kidney samples obtained from roe deer (*Capreolus capreolus*). A total of 328 (164 liver and 164 kidney) samples collected from 164 roe deer of both sexes were tested. Animals were shot

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during the hunting season between 15 August 2009 and 15 January 2010, in compliance with the hunting limits set. Material for the study was gathered from 27 sites located in 15 provinces of Poland. No selenium-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, No 48, Item 458, 2005). The animals harvested from different provinces were in the same age range of 3 to 4 years. After hunting, hunters determined their age using the key provided by Przybylski *et al.* (2010). All tissue samples 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 of the sample was converted into dry matter. Selenium concentrations in organs were determined using spectrofluorometric methods (Grzebula & Witkowski, 1977). The samples were digested in 3.5 ml HNO_3 at 230°C for 180 min and in 3 ml $HClO_4$ at 310°C for 20 min. Finally, 3 ml 9% HCl was added to reduce Se^{6+} to Se^{4+}. 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 ($\overline{X}$), 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 ($r_{x,y}$).

RESULTS & DISCUSSION

The results of tests for mean selenium concentrations in roe deer organs are shown in Tables 1 and 2. Mean selenium concentration in roe deer from Poland was 0.088 μg/g wet weight (w.w.) in liver and 0.503 μg/g w.w. in kidneys. Our study showed that selenium concentrations vary considerably according to the province of Poland. The highest mean concentration of selenium in liver, found in animals from the Świętokrzyskie province (0.129 μg/g w.w.), was three times as high as in the Pomorskie province (0.043 μg/g w.w.).

The lowest mean concentration of selenium in the liver of roe deer from the Pomorskie province, was significantly ($Pd \leq 0.01$; $Pd \leq 0.05$) lower than that of roe deer from the other provinces except the Kujawsko-Pomorskie and Podkarpackie. In addition, mean liver selenium concentrations of roe deer from the Kujawsko-Pomorskie and Podkarpackie provinces were significantly ($Pd \leq 0.01$) lower than those of roe deer from the Śląskie ($Pd \leq 0.01$), Lubelskie and Wielkopolskie provinces ($Pd \leq 0.05$); mean liver selenium concentrations of roe deer from the Podlaskie province were significantly ($Pd \leq 0.05$) lower than those of roe deer from the Opolskie, Zachodniopomorskie, Warmińsko-Mazurskie and Małopolskie provinces; and mean liver selenium concentrations of roe deer from the Wielkopolskie province were significantly (Pd”0.05) lower than those of roe deer from the Łódzkie province (Table 1).The highest mean selenium concentrations in kidneys, found in roe deer (Table 2) from the Warmińsko-Mazurskie (0.868 μg/g w.w.), Łódzkie (0.845 μg/g w.w.), Świętokrzyskie (0.749 μg/g w.w.) and Opolskie provinces (0.604 μg/g w.w.), were significantly ($Pd \leq 0.01$; $Pd \leq 0.05$) higher than those of roe deer from the other provinces, with mean kidney selenium concentrations of roe deer from the Opolskie province being significantly ($Pd \leq 0.01$) lower compared to animals from the Warmińsko-Mazurskie, Łódzkie and Świętokrzyskie provinces, but not significantly different from mean kidney selenium concentrations of roe deer from the Małopolskie province (0.545 μg/g w.w.). Mean selenium concentrations in the kidneys of roe deer from the Warmińsko-Mazurskie province were also significantly ($Pd \leq 0.05$) higher than those of roe deer from the Świętokrzyskie province.The lowest mean selenium concentrations were found in the kidneys of roe deer from the Pomorskie (0.314 μg/g w.w.), Wielkopolskie (0.319 μg/g w.w.) and Podkarpackie (0.329 μg/g w.w.) provinces and they were significantly ($Pd \leq 0.05$; $Pd \leq 0.01$) lower than in the kidneys of roe deer from the Warmińsko-Mazurskie, Łódzkie, Świętokrzyskie, Opolskie, Małopolskie, Mazowieckie (0.483 μg/g w.w.), Śląskie (0.473 μg/g w.w.), Lubelskie (0.459 μg/g w.w.), Zachodniopomorskie (0.452 μg/g w.w.) and Podlaskie provinces (0.447 μg/g w.w.).

Furthermore, mean selenium concentrations in the kidneys of roe deer from the Małopolskie province were also significantly ($Pd \leq 0.01$) higher than those of roe deer from the Kujawsko-Pomorskie (0.389 μg/g w.w.) and Lubuskie provinces (0.393 μg/g w.w.).

Table. 1. Mean selenium concentrations in the liver of roe deer from different provinces

Province	N	Se content µg/g w.w.			Se content µg/g d.w.		
		$\overline{X}$	SEM	Range	$\overline{X}$	SEM	Range
Kujawsko-Pomorskie (1)	10	0.057	0.003	0.043-0.078	0.203	0.011	0.152-0.276
Lubelskie (2)	13	0.084	0.007	0.053-0.144	0.300	0.024	0.187-0.512
Lubuskie (3)	10	0.072	0.006	0.046-0.107	0.257	0.022	0.163-0.381
Łódzkie (4)	10	0.114	0.008	0.073-0.154	0.406	0.029	0.262-0.550
Małopolskie (5)	10	0.102	0.006	0.068-0.133	0.363	0.022	0.241-0.473
Mazowieckie (6)	10	0.071	0.003	0.040-0.081	0.253	0.010	0.143-0.288
Opolskie (7)	10	0.106	0.006	0.071-0.144	0.376	0.020	0.227-0.511
Podkarpackie (8)	10	0.062	0.010	0.026-0.117	0.222	0.034	0.094-0.416
Podlaskie (9)	10	0.078	0.008	0.037-0.132	0.278	0.029	0.131-0.470
Pomorskie (10)	10	0.043	0.005	0.023-0.069	0.155	0.019	0.082-0.246
Śląskie (11)	10	0.097	0.006	0.069-0.131	0.346	0.023	0.247-0.467
Świętokrzyskie (12)	10	0.129	0.005	0.103-0.159	0.460	0.018	0.367-0.568
Warmińsko-Mazurskie (13)	10	0.103	0.011	0.060-0.174	0.368	0.040	0.213-0.618
Wielkopolskie (14)	12	0.087	0.014	0.033-0.177	0.309	0.052	0.119-0.629
Zachodniopomorskie (15)	20	0.106	0.006	0.062-0.167	0.377	0.021	0.220-0.595
Total	164	0.088	0.002	0.023-0.177	0.312	0.009	0.082-0.629
Significant differences:	4,5,7,12,13,15 : 1,2,3,6,8,9,10**; 12 : 11,14**; 10 : 2,9,11,14**; 1,8 : 11**;9,12 : 5,7,13,15*; 10,11 : 3,6*; 1,8 : 2,14*; 4 : 14*						

Differences significant at: ** Pd"0.01; * Pd"0.05

Table 2. Mean selenium concentrations in the kidneys of roe deer from different provinces

Province	N	Se content µg/g w.w.			Se content µg/g d.w.		
		$\overline{X}$	SEM	Range	$\overline{X}$	SEM	Range
Kujawsko-Pomorskie (1)	10	0.389	0.023	0.277-0.482	1.384	0.083	0.988-1.172
Lubelskie (2)	13	0.459	0.020	0.297-0.603	1.634	0.072	1.059-2.146
Lubuskie (3)	10	0.393	0.024	0.284-0.535	1.399	0.087	1.012-1.906
Łódzkie (4)	10	0.845	0.088	0.430-1.173	3.009	0.313	1.531-4.176
Małopolskie (5)	10	0.545	0.014	0.479-0.644	1.941	0.052	1.705-2.293
Mazowieckie (6)	10	0.483	0.015	0.355-0.611	1.719	0.054	1.265-2.176
Opolskie (7)	10	0.604	0.046	0.363-0.929	2.149	0.163	1.292-3.306
Podkarpackie (8)	10	0.329	0.044	0.146-0.521	1.170	0.156	0.519-1.854
Podlaskie (9)	10	0.447	0.022	0.336-0.574	1.593	0.079	1.198-2.044
Pomorskie (10)	10	0.314	0.018	0.223-0.369	1.118	0.062	0.796-1.314
Śląskie (11)	10	0.473	0.016	0.386-0.528	1.685	0.056	1.373-1.879
Świętokrzyskie (12)	10	0.749	0.031	0.500-0.860	2.668	0.112	1.777-3.062
Warmińsko-Mazurskie (13)	10	0.868	0.051	0.553-1.057	3.091	0.180	1.971-3.766
Wielkopolskie (14)	12	0.319	0.044	0.035-0.497	1.135	0.155	0.126-1.769
Zachodniopomorskie (15)	20	0.452	0.021	0.304-0.711	1.611	0.074	1.082-2.533
Total	164	0.503	0.015	0.035-1.173	1.792	0.054	0.126-4.176
Significant differences:	4,7,12,13 : 1,2,3,8,9,10,14,15**; 4,12,13 : 5,6,7,11**; 12 : 13*; 7 : 6,11*;8,10,14 : 5,6,11**; 8,10,14 : 2,9,15*; 5 : 1,3**;						

Differences significant at: ** Pd”0.01; * Pd”0.05

In all the roe deer analysed collectively, a highly significant correlation was observed between selenium concentrations in liver and kidneys ($r_{x,y}$=0.50; Pd"0.001), but no significant correlations were found in particular provinces (Table 3). It can be assumed from our results that the geochemical characteristics of the soil substratum (soil parent rock) has a decisive effect. In general, selenium content of plants reflects its soil levels and in many cases it is positively correlated to the total content of this element in the environment (Kabata-Pendias & Pendias, 1999; Szymańska & Hawrylak, 2007). The highest liver selenium concentrations were found in roe deer from the Świętokrzyskie province. This area is dominated by chernozem soils, which are considered the most fertile, have a high humus content, and occur in compact areas. The parent material for chernozems are primarily compacted sediments (loess and clay). Kabata-Pendias & Pendias (1999) states that increased amounts of selenium typically occur in soils rich in organic matter and iron compounds. According to the same author, the concentration of selenium available to plants depends mainly on the type of soil. However, one cannot ignore such factors as soil contamination with heavy metals and sulphur, the use of phosphate and nitrogen fertilizers, and low pH of soils (Gupta & Watkinson 1985). In our study, animals in the Świętokrzyskie province were obtained from the Końskie County, in which soils are known to be least contaminated with sulphur (1.9%) (Kamiński 2006). Elevated soil sulphur and phosphorus concentrations reduce the availability of selenium for uptake by plants, and thus decrease selenium levels in food. The low selenium content in roe deer from the Pomorskie province might be also attributed to the fact that the sampling area has a chemical plant that manufactures cadmium- and lead-containing phosphate fertilizers as well as Poland's second largest refinery, which produces fuels, oils and lubricants. These plants are the largest air polluters in the area. Heavy metals enter the animal body mainly through ingestion, and to a lesser extent through inhalation (Silva *et al.*, 2005; Cizmecioglu & Muezzinoglu 2008; Popiołek-Pyrz *et al.*, 2003). Selenium absorption in the body depends not only on the chemical composition of food, but also on the interactions between Se and other elements. Floriańczyk (1999) reports that Se acts antagonistically to Cd and Pb. In the presence of these metals, absorption of selenium from food is decreased. This explains why the wild animals living in this area have such low organ concentrations of selenium. This theory is supported by Dudka (1992) who found high soil selenium concentrations (0.4-0.6 mg/kg) in the area under study (vicinity of Wejherowo). The high concentration of selenium in the surface layer of the soil shows that the anthropogenic factor has played a considerable role.

Table 3. Correlations between kidney and liver selenium concentrations in roe deer from different provinces

Province	N	Coefficient of correlation ($r_{x,y}$)
Kujawsko-Pomorskie	10	ns
Lubelskie	13	ns
Lubuskie	10	ns
Łódzkie	10	ns
Małopolskie	10	ns
Mazowieckie	10	ns
Opolskie	10	ns
Podkarpackie	10	ns
Podlaskie	10	ns
Pomorskie	10	ns
Śląskie	10	ns
Świętokrzyskie	10	ns
Warmińsko-Mazurskie	10	ns
Wielkopolskie	12	ns
Zachodniopomorskie	20	ns
Total	164	0.50***

Coefficient of correlation significant at: *** Pd0.001
ns – not significant

According to Pollock (2005), the biochemical criteria used to detect selenium deficiency based on liver Se concentrations in deer are as follows: below 0.6 µg/g d.w. – deficient level; 0.6-0.88 µg/g d.w. – marginal level; above 0.88 µg/g d.w. – optimal level for deer. McDowell *et al.*, (1995) hold that selenium concentrations below 3.0 µg/g d.w. in the kidneys of game animals indicate that this element is deficient. Considering the above ranges, it can be stated that liver Se deficiency affects roe deer in all the Polish provinces studied (Fig. 1). One possible reason is the low concentration of bioavailable forms of selenium in the soil, which significantly determines the concentration of this element in plants consumed by roe deer. Selenium deficiency in roe deer from Poland was reported by Wieczorek-Dąbrowska (2009) and Pilarczyk *et al.* (2008a; 2009; 2011) in Western Pomerania, and by Borawska *et al.* (2005) in the Podlaskie province. An almost 3-fold higher liver selenium concentration, compared to our results (0.088 µg/g w.w.), was observed by Humann-Ziehank *et al.* (2008) in roe deer from Germany (0.27 µg/g w.w.). In Norway, selenium concentrations in the organs of animals were also found to vary widely according to their region of origin (Vikøren *et al.*, 2011). These authors found the lowest Se concentration in the liver of roe deer from the Etnedal region (0.46 µg/g d.w.), and the highest in roe deer from Afjord (4.07 µg/g d.w.).

Twenty-eight percent of the studied roe deer were deficient in this element. Wilson & Grace (2001) reported some cases of white muscle disease in young red deer and roe deer for selenium concentrations of d”0.035 µg/g w.w., which they demonstrated in 12.16% of the roe deer studied. When comparing our results for kidney Se concentrations in roe deer to the criteria used to evaluate the environmental abundance, we found the level of this element to be optimal only in the Łódzkie (3.009 µg/g d.w.) and Warmińsko-Mazurskie provinces (3.091 µg/g d.w.), and deficient in the other provinces (Fig. 2).

Special consideration should be given to our results obtained for roe deer from the Łódzkie province. Se levels in animals from this area were observed to be deficient in liver but optimal in kidneys. This relationship probably results from the fact that liver and kidneys play a major role in trace element metabolism. The largest number of positive correlations between toxic and essential elements was reported in these organs (López Alonso *et al.*, 2004). Upon entering the body, metals are bound by plasma proteins and move to the liver and kidneys, which are the target accumulation organs (Orłowski, 2008). In the organs, metals are bound by metallothionein (MT) whose synthesis is stimulated by selenium-containing glutathione peroxidase (GSHPx) (Shimizu & Morita, 1990). Glutathione peroxidase participates in binding of metal ions (especially Cd), leading to a reduction of its activity in organ cells, which is associated with the displacement of Se ions from the active centre (Jurczuk *et al*,. 2004). For this reason the body is first depleted of selenium reserves, which are stored mainly in the liver. Therefore, selenium deficiency first affects the liver. Selenium reacts with toxic metals (Pb, Cd, Hg) to form metal selenides, which are excluded from biochemical processes (liver). Because these compounds are poorly soluble (kidney excretion problems), they are accumulated in the kidneys (increased Se) (Orłowski, 2008; Shimizu & Morita, 1990). This is probably why selenium concentrations appear to be optimal in the kidneys of roe deer from the Łódzkie province.

Pollock (2005) believes that hepatic selenium concentrations are a better indicator of animal selenium status compared to renal selenium concentrations. Liver is the principal organ responsible for selenium homeostasis in the organism. Selenoproteins (including selenium dependent glutathione peroxidase) are synthesized and distributed in liver cells. Due to its high metabolic activity, the liver is particularly susceptible to the effects of free radicals and their

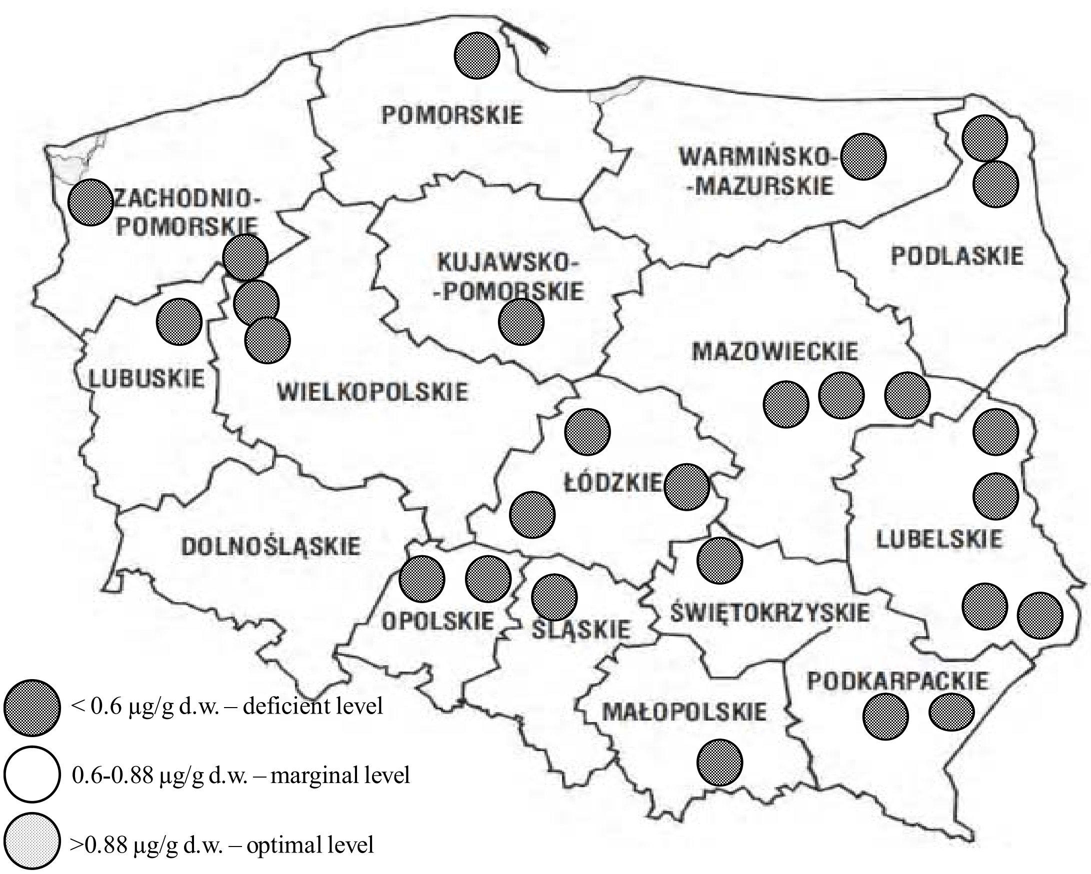

Fig. 1. Mean selenium concentrations (µg/g d.w.) in the liver of roe deer

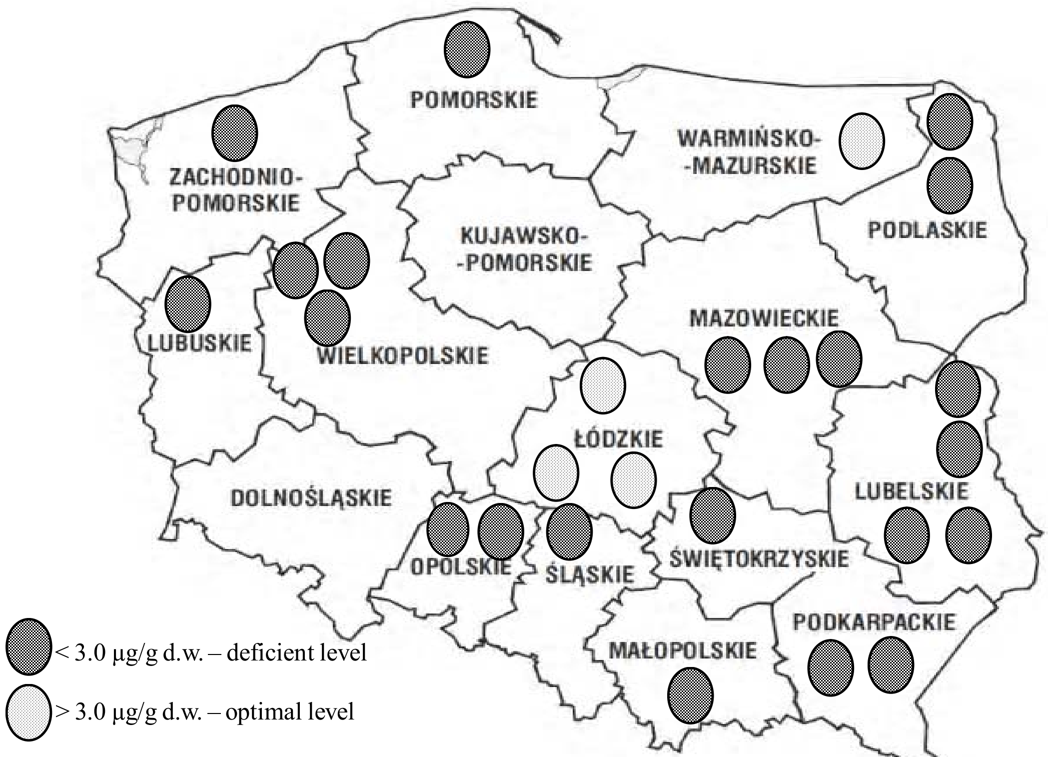

Fig. 2. Mean selenium concentrations (µg/g d.w.) in the kidneys of roe deer

derivatives. When selenium is deficient, the reserves of this trace element, which is mainly stored in the liver, are depleted from the body. In case of selenium deficiency, selenium is first mobilized from the liver. In turn, Oh *et al.* (1976) report that the ratios between kidney and liver selenium concentrations are important. According to these authors, lambs fed a low selenium diet always had a higher concentration of this element in kidneys than in liver. The opposite situation was observed when lambs received a high selenium diet, after which they had higher selenium concentrations in liver than in kidneys. In our study, liver selenium concentrations were not higher than kidney selenium concentrations in any of the provinces under study.

CONCLUSION

We have shown selenium content in the organs of roe deer to vary considerably according to geographical location. Most areas of Poland are deficient in environmental selenium, as evidenced by the low content of this element in the organs of roe deer. Mean selenium concentration in roe deer from Poland was 0.088 µg/g wet weight (w.w.) in liver and 0.503 µg/g w.w. in kidneys. Organ selenium concentrations in the studied animals varied considerably according to geographical location. Most areas of Poland are deficient in environmental selenium, as evidenced by the low content of this element in the organs of roe deer.

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