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Selenium levels in organs and tissues of domestic dog (*Canis lupus familiaris*) from the northwest area of Poland

Stężenie selenu w narządach i tkankach psa domowego (*Canis lupus familiaris*) z terenu północno-zachodniej Polski

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ABSTRACT

Introduction: Selenium (Se) is a component of oxidoreductases and cytochromes. It also influences the growth and proliferation of cells, and it is essential for the proper functioning of the immune system.

The aim of the study was to determine selenium concentration in the liver, kidneys, lungs, heart and muscles of dog (*Canis lupus familiaris*) from northwestern Poland.

Materials and methods: Samples of organs and tissues were collected from 15 dogs at the age of 1 month to 17 years. The dogs were fed a diet similar to humans, with a small amount of commercial feed. Selenium concentration in the organs was determined spectrofluorometrically.

Results: The highest Se concentration was found in the kidneys (0.57 ±0.42 mg/kg of wet weight – ww) and the lowest in muscles (0.11 ±0.09 mg/kg, ww). Mean selenium concentrations in the examined organs followed the order: kidneys>liver>lungs >heart>muscles.

Conclusions: Our study shows that the Se concentration in the studied materials was below optimal level in 75% of the analysed dogs. This means that 75% of dogs had a deficiency of this element. The results obtained in this research suggest that the animal diet, which was very similar to the human diet, was not sufficient to ensure an optimum Selenium level in the bodies of these animals.

Keywords: selenium; dog; Poland.

ABSTRAKT

Wstęp: Selen (Se) jest składnikiem enzymów oksydoredukcyjnych i cytochromów. Wpływa również na wzrost i proliferację komórek oraz jest niezbędny do prawidłowego funkcjonowania układu immunologicznego.

Celem badań było oznaczenie stężenia selenu w wątrobie, nerkach, płucach, sercu i tkance mięśniowej psa domowego (*Canis lupus familiaris*) z terenu północno-zachodniej Polski.

Materiały i metody: Próbki narządów i tkanek pobrano od 15 psów w wieku od 1 miesiąca do 17 lat. Dieta zwierząt była podobna do diety człowieka, z niewielką ilością komercyjnej karmy. Stężenia selenu były oznaczane metodą spektrofluorymetryczną.

Wyniki: Największe średnie stężenie Se u badanych psów stwierdzono w nerkach (0,57 ±0,42 mg/kg mokrej masy – m.m.), a najmniejsze w mięśniach (0,11 ±0,09 mg/kg m.m.). Średnie stężenie Se w badanych narządach i tkankach psów można ułożyć w następujący szereg: nerki>wątroba>płuca>serce>mięśnie.

Wnioski: U 75% badanych psów wartości stężenia Se w badanych materiałach znajdowały się poniżej optymalnego poziomu, co świadczy o niedoborze Se. Wyniki są dowodem na to, że dieta psów bardzo podobna do diety ludzkiej nie jest wystarczająca dla optymalnego poziomu Se w organizmach zwierząt.

Słowa kluczowe: selen; pies domowy; Polska.

INTRODUCTION

Every organism's diet, apart from nutritious components such as water, proteins, sugars, vitamins and fats, also includes essential macro- and microelements such as selenium (Se) [1]. Selenium is a non-metallic element widely distributed in the environment. However, its distribution and concentration in the Earth's crust is uneven. Poland belongs to Se deficiency areas because of the types of soils which are present in this region [2, 3]. The average Se content in soils is in the range of 0.1–2.0 mg/kg. Most selenium in selenite form, which is best absorbed by plants, is found in alkaline soils [4, 5].

Selenium is an essential element, ensuring proper functioning of the body, and this is probably why it is called the element of life. It is present in glutathione peroxidase and performs a similar function to vitamin E [3, 4, 6]. Selenium is a component of selenoproteins, which are part of many enzymes. These enzymes have beneficial effects on the growth and proliferation of cells and antibody production, and also increase the activity of immune cells and detoxify the body of heavy metals [1, 3, 5, 6, 7]. Interactions between Se and cadmium (Cd), mercury (Hg) and lead neutralize the toxic effects of heavy metals [8, 9].

Selenium has been found in every mammal's tissues. The highest concentration was observed in the liver, kidneys and

blood plasma. The borderline between optimal Se concentration and the deficiency or excess of this element is small [2, 4, 7, 10]. Selenium deficiency may result in muscle dystrophy, pulmonary oedema, hypothyroidism and cardiac insufficiency. However, excess Se levels may cause internal organ atrophy and neuritis [3]. It was also found that both deficiency and excess of Se increases the risk of cancer development [2].

The majority of biomonitoring studies concern the concentration of elements in wild animals [8, 11, 12, 13, 14]. There are few studies that have used domesticated warm-blooded animals, such as dogs [15]. Domestic dog (*Canis lupus familiaris*) is a good model organism on account of, among others things, its lifespan and exposure to similar environmental factors as humans. Furthermore, the occurrence of many human diseases such as tumours, cardiomyopathy, epilepsy and hip dysplasia has also been observed in dogs [16, 17].

So far, there have been few literature data concerning Se concentration in the organs and tissues of dogs. Therefore, the aim of this study was to determine the Se concentration in the liver, kidneys, lungs, heart and muscles of dogs from northwest Poland.

MATERIALS AND METHODS

This study was conducted on 15 dogs aged 1 month to 17 years. The dogs came from the Veterinary Clinic of the Animal Shelter in Szczecin. The diet of the studied dogs was similar to humans' diet, with a small amount of commercial feed. The animals were collected with veterinary surgeons' help, who performed euthanasia on dogs for various reasons. The dogs were not terminated specifically for this study. The main reason for the euthanasia was the old age of the dogs, which was accompanied by respiratory failures and tumours. The ages of these animals were determined by the veterinary surgeons based on information in medical documentation and from the owners of the animals. The dogs were divided into two age categories: young dogs <9 years (n = 11) and old dogs >9 years (n = 4).

Obtaining biological material from dogs was approved by the District Veterinary Surgeon in Szczecin. The studied materials were collected from dead animals, and therefore it was not required to have the agreement of the Local Animal Research Ethics Committee.

The samples of the liver, kidneys, lungs, heart and muscles were collected from domestic dogs. Tissue samples were kept frozen at –20°C in a laboratory until analysed. Selenium concentrations were determined by the spectrofluorimetric method according to Pilarczyk et al. [10]. The dried samples (0.1–0.3 g) were wet mineralized in a mixture of concentrated acids: nitric acid (HNO_3) for 3 hours at 230°C and perchloric acid ($HClO_4$) for 20 minutes at 310°C. Then, Se^{6+} was reduced to Se^{4+} using 3 mL 9% HCl. Selenium samples were obtained with 2,3-diamoninaphtalene under controlled pH (pH 1–2) with the formation of selenodiazole complex. This complex was extracted using cyclohexane. EDTA and hydroxylamine hydrochlorine were used as masking agents. Total Se concentration was determined fluorometrically using a Shimadzu RF-5001 PC spectrofluorophotometer (detection limit 0.003 $\mu g/g^{-1}$). The excitation and the fluorescence emission wavelengths were 376 and 518 nm, respectively. The accuracy of the method was verified using certified reference material BCR-185R.

Statistical studies were performed using StatSoft Statistica 10.0 and Microsoft Excel 2003. Statistical analysis of the results involved the calculation of arithmetic mean (AM), standard deviation of the AM, and the minimum and maximum ranges. Results of selenium concentrations are presented in mg/kg wet weight – ww.

In order to verify the significant differences between the mean concentrations in organs and tissues in dogs, we used Student's t-test. Differences were rated significant at $p < 0.05$.

RESULTS

Levels of Se in the examined organs and tissues of dogs, considering their age and sex, are presented in Table 1. In the entire study group of dogs, the highest selenium concentration was in kidneys (0.57 ±0.42 mg/kg ww), and the lowest was in muscles (0.11 ±0.09 mg/kg ww). Mean selenium concentrations in the examined organs and tissues followed the order: kidneys >liver>lungs>heart>muscles.

TABLE 1. Concentration of selenium in organs and tissues of domestic dogs from northwestern Poland

Parameters		Selenium concentration (mg/kg w.w.)				
		liver	kidneys	lungs	heart	muscles
Total (n = 15)	n	15	15	11	11	12
	AM ±SD	0.30 ±0.24	0.57 ±0.42	0.13 ±0.11	0.11 ±0.07	0.11 ±0.09
	min.	0.06	0.14	0.05	0.05	0.04
	max.	0.69	1.16	0.32	0.23	0.31
Old dogs (n = 4)	n	4	4	4	4	4
	AM ±SD	0.50 ±0.03	0.96 ±0.18	0.27 ±0.03	0.20 ±0.02	0.10 ±0.01
	min.	0.46	0.74	0.24	0.18	0.09
	max.	0.53	1.16	0.32	0.23	0.12
Young dogs (n = 11)	n	11	11	7	7	8
	AM ±SD	0.23 ±0.24	0.43 ±0.39	0.06 ±0.01	0.06 ±0.01	0.11 ±0.12
	min.	0.06	0.14	0.05	0.05	0.04
	max.	0.69	1.10	0.09	0.07	0.31
Female (n = 7)	n	7	7	5	5	5
	AM ±SD	0.27 ±0.19	0.59 ±0.42	0.14 ±0.10	0.12 ±0.07	0.07 ±0.03
	min.	0.09	0.15	0.05	0.05	0.04
	max.	0.53	1.16	0.25	0.21	0.10
Male (n = 8)	n	7	8	5	5	7
	AM ±SD	0.33 ±0.28	0.56 ±0.44	0.13 ±0.12	0.11 ±0.08	0.14 ±0.11
	min.	0.06	0.14	0.05	0.06	0.04
	max.	0.69	1.10	0.32	0.23	0.31

AM – arithmetic mean

Both in old (n = 4) and young (n = 11) dogs, the highest and lowest selenium levels were found in kidneys and muscles, respectively. A statistically significant difference between Se concentration and young and old dogs was found in liver and kidneys. Both in males (n = 8) and in females (n = 7), the highest Se concentration was observed in kidneys, and the lowest in muscles. There were no statistically significant differences in the levels of Se in males and females.

DISCUSSION

There are few data on Se concentrations in the organs and tissues of canines (Table 2). In the present studies kidneys had the highest Se concentration in all dogs, as they are the organs responsible for the elimination of the excess of this element from the body [18, 19]. It was reported that the optimal Se level in the kidneys and in the liver of *Canidae* is in the range of 1.0–1.5 mg/kg ww and 0.5–1.5 mg/kg ww, respectively [20]. Nevertheless, the average Se concentration in kidneys was 0.57 ±0.42 mg/kg ww and in the liver 0.30 ±0.24 mg/kg ww in the tested dogs from northwestern Poland. The appropriate concentration of Se in kidneys and liver was found only in 2 dogs, a young male and an old female. As the average levels of the studied element in the tested materials are below the optimal values, it should be concluded that the dogs were deficient in this element. Depression, muscle weakness, loss of appetite, dyspnoea and coma were reported in dogs with Se deficiency [21]. According to the available medical documentation, no such symptoms were observed in studied dogs from the northwest area of Poland.

Much higher Se levels than in the present study were found by Paßlack et al. [15] in dogs fed commercial feed. Paßlack et al. [15] registered Se concentration in the liver, renal medulla and cortex of dogs from Berlin to be 0.43, 0.63 and 0.20 mg/kg w.w., respectively. The levels of Se in the liver and kidneys of dogs observed by López-Alonso et al. [22] also exceeded those found in our study. The Se concentration in dogs bred by inhabitants of Lugo (Spain) was 0.76 and 1.45 mg/kg ww in liver and kidneys, respectively. Differences in the Se concentration in the organs of dogs can be caused by diet. It has been shown that the higher the Se levels in food, the higher the Se urinary excretion and accumulation in the liver [18, 23]. In the present study, the dogs were fed mainly the leftovers of owners' meals. This means that the diet did not provide adequate amounts of Se in their bodies.

Far higher Se levels in the liver were observed by Prestrud et al. [11] and Hoekstra et al. [12] in polar foxes (*Alopex lagopus*) compared to our study. Prestrud et al. [11] examined foxes from Norway, where the average Se concentration in soils is about 0.78 mg/kg. In contrast, Hoekstra et al. [12] conducted research in various regions of Canada. The average Se concentration in soils in Canada ranges from 0.1–1.67 mg/kg. Differences in Se levels in the liver between dogs from northwestern Poland and foxes from Norway and Canada were probably related to climatic factors and soil conditions in these regions. The average Se content in soils in Poland is 0.06–0.64 mg/kg [5]. Prestrud et al. [11] and Hoekstra et al. [12] found a positive correlation between Se levels as well as Hg and Cd concentrations, which confirmed the influence of selenium on the reduction of the toxic effects of the above-mentioned heavy metals in mammals.

Considerably higher Se concentrations in the liver and muscles were observed in foxes by Millán et al. [13] and Golubkina et al. [14], in the area of Doñana (southern Spain) and Khabarovsk (Russia), respectively, than in the dogs examined

TABLE 2. Comparison of selenium concentration between the present study and the results of other authors

Species	Place	Selenium concentration (mg/kg w.w.)											
		liver		kidneys			muscles		heart		lungs		Ref
		n	AM	n	renal cortex (AM)	renal medulla (AM)	n	AM	n	AM	n	AM	
Domestic dog (*Canis lupus familiaris*)	Northwestern Poland	15	0.30	15	0.57		11	0.13	11	0.11	11	0.11	
	Berlin, Germany	50	0.43	50	0.63	0.20							[15]
	Lugo, Spain	77	0.76	77	1.45								[22]
Red fox (*Vulpes vulpes*)	Northwestern Poland	40	0.27	38	0.60				20	0.13	28	0.17	[10]
	Doñana, Southern Spain	16	0.53				17	0.16					[13]
	Khabarovsk, Russia						3	0.24					[14]
Silver fox (*Vulpes vulpes*)	Northwestern Poland	81	0.25	81	0.597		81	0.07					[8]
Arctic fox (*Alopex lagopus*)	Svalbard, Norway	87	2.70										[11]
	Ulukhaqtuuq, Canada	11	0.79										[12]
	Arviat, Canada	43	0.88										[12]
Raccoon dog (*Nyctereutes procyonoides*)	Southeastern Poland	20	0.23	20	0.49		20	0.08	20	0.10	20	0.14	[24]

AM – arithmetic mean, Ref – reference

in our study. These differences may result from species identity and the different diets of these mammals. Foxes commonly eat rodents, birds and fish, which contain large amounts of Se [9, 14], whereas dogs from northwest Poland ate mainly leftovers of the owners' meals.

The normal action of the heart depends on the concentration of selenium, and low levels of this element may cause heart dysfunction. One of the most common animal diseases caused by Se deficiency is mulberry heart disease [10, 24]. The average Se concentration in heart and lungs was 0.11 mg/kg ww in the present study. A similar concentration of Se was found in red fox (*Vulpes vulpes*) from northwestern Poland [10] and raccoon dogs (*Nyctereutes procyonoides*) from southeastern Poland [24].

There was no statistically significant differences between the concentration of Se in the examined organs and the sex of dogs from northwest Poland and dogs from Berlin [15] or dogs bred by Lugo inhabitants [22].

The present study, similarly to the study by Paßlack et al. [15], and contrary to the study by López-Alonso et al. [22] found statistically significant differences in Se levels in the liver and kidneys of young and old dogs. There were also statistically significant differences in Se concentrations in the liver of young and old dogs from northwestern Poland, red and silver foxes from the same area [8, 10], and raccoon dogs from southeastern Poland [24]. This relationship was not found in kidneys in the foxes and raccoon dogs.

Comparing Se concentration determined in the present study to the results obtained by other authors and to biochemical criteria [20], it can be assumed that the examined dogs had selenium deficiency. The results of this study indicate the low concentration of Se in the dogs' diet or the inadequate content of an available form of this element in food.

CONCLUSIONS

1. In the study group of dogs, the highest selenium concentration was in kidneys, and the lowest was in muscles.
2. A statistically significant difference in Se concentration was found in the liver and kidneys and young and old dogs.
3. The results reported here suggest that the animals' diet, which was very similar to the human diet, is not sufficient to ensure optimum Se levels in the bodies of these animals.

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