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ORIGINAL PAPER

MUSCLE SELENIUM CONTENT IN RED DEER (*CERVUS ELAPHUS*), ROE DEER (*CAPREOLUS CAPREOLUS*) AND CATTLE (*BOS TAURUS*) FROM NORTH-EASTERN POLAND*

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Abstract

Being an essential micronutrient, selenium plays an important role in numerous metabolic processes, including the immune system's activity, reproduction and antioxidant defence. Although acute symptoms of its deficit are rare in free-living ruminants, its chronic deficiency may be a factor limiting the number of animals inhabiting certain territories. Nowadays, selenium is supplemented not only in farm animals but also in game species. Therefore, it is important to investigate its status in animals to monitor whether they suffer due to deficit or excess of selenium. The study aimed to evaluate the levels of selenium in the skeletal muscles of wild and domesticated ruminants inhabiting the north-eastern part of Poland. A total of 172 muscle samples were collected from red deer (*Cervus elaphus*), n = 94, European roe deer (*Capreolus capreolus*), n = 58, and domestic cattle (*Bos taurus*), n = 20. Mean selenium concentrations were 0.079, 0.075 and 0.149 mg kg^{-1} in red deer, roe deer and cattle, respectively. Bovine muscles did not vary greatly in terms of the selenium content, whereas high individual variability was found in the muscles of the studied cervids. The differences between the individuals with the highest and the lowest levels of selenium were nearly 11-fold and 12.5-fold for red deer and roe deer, respectively. Both groups included animals with a high level of selenium, corresponding to the upper limit of the reference values for cattle; these represented 13% of roe deer and 22% of red deer. Respectively 12 and 15% of roe deer and red deer had a low level of selenium. More than 80% of cervids in both groups studied remained within the reference range, which implies a good level of selenium supply in wild ruminants inhabiting the region. Due to the high variability in selenium levels among cervids studied, venison is a poorer source of this mineral than beef.

Keywords: selenium, muscles, red deer, roe deer, cattle.

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INTRODUCTION

Metals play an important role in the biochemical processes occurring in living organisms. The list of essential nutrients needed for the proper functioning of animal cells includes selenium, Se^{34}_{79} (Husakova et al. 2014, Li et al. 2016, Mehdi, Dufrasne 2016, Tan et al. 2016). So far, 35 selenoproteins have been identified, which in their active sites have L-selenocysteine, a naturally occurring amino acid (Flueck et al. 2012). Their most important biological functions include protection against the outcomes of cell structure peroxidation – which affects mainly the cell membranes – caused by the toxic by-products of aerobic respiration (Shchedrina et al. 2010). The protection involves glutathione peroxidases 1-5, whose action consists in reducing H_2O_2 and organic hydroperoxides (Lubos et al. 2011). Selenium represents an integral part of the catalytic sites of these enzymes, therefore changes in its concentration have a significant effect on their functioning. The selenoenzyme thioredoxin reductase, which participates in the reduction of disulfides, plays a similar role (Ahsan et al. 2014, Humann-Ziehank 2016). Selenium is also a key factor in the metabolism of thyroid hormones and in the immune response (Burk, Hill 2015, Zimmerman et al. 2015). The mineral is also necessary for the process of reproduction and acts to maintain the proper level of bone tissue metabolism (Flueck et al. 2012). Due to its biological properties, selenium is increasingly more often used in synthesizing organic compounds, also those with anti-neoplastic properties (Banerjee, Koketsu 2017).

Like many other essential metals, also selenium may be toxic if taken in excessive quantities. Whether there is a deficit or excess of available selenium depends primarily on the environmental conditions, the most important of which is the level of selenium in soils (El-Ramady et al. 2014). If we look at wild animals, which are entirely dependent on the environmental availability of micro- and macronutrients, any dietary deficiency may be what affects the size of a given population (Skibniewski et al. 2010, 2015, 2017, Stoebe et al. 2015, Durkalec et al. 2017). One may expect that under natural conditions the free-living ruminants that exhibit selenium deficiency symptoms will be quickly eliminated from the population due to increased susceptibility to diseases and predation. Consequently, it seems significant to detect animals with sub-clinical, periodical deficiencies of selenium, which in the long run may affect the health of a local population. This is due to the fact that selenium is often a rare element in soils of many regions of the world, and Poland is among the countries with selenium-deficient regions (Tan et al. 2016). Determination of the selenium supply in local cervid populations is also important because these game animals provide meat considered to be of high quality, fully satisfying the human nutritional needs. Venison is also often treated as a more noble product compared to meats of farmed animals.

The study aimed to determine the levels of selenium in the muscles of two species of free-living ruminants, red deer and roe deer, from the north--eastern part of Poland and to compare the data with the muscles of domestic cattle from the same area, eventually relating these results to human nutritional demands.

MATERIAL AND METHODS

The material consisted of 172 muscle samples collected from females representing three ruminant species living in north-eastern Poland, i.e. red deer (*Cervus elaphus*), 94 samples, roe deer (*Capreolus capreolus*), 58 samples, and domestic cattle (*Bos taurus taurus*), 20 samples. North-eastern Poland is an area with little industrialization, where the intact nature with numerous lakes, river valleys and vast forested areas provide a refuge for wild ruminants. These values make the land very attractive for tourism. Material for the study was collected from animals hunted in a few sites located in three neighbouring provinces of north-eastern Poland (Figure 1). Analyses involved samples of the masseter muscle (*musculus masseter*) dissected in a way to obtain a representative sample of its superficial and deep portion. Samples were collected in slaughterhouses and in meat processing plants from the heads of animals whose carcasses had been examined by the veterinary services, and their meat was classified as fit for

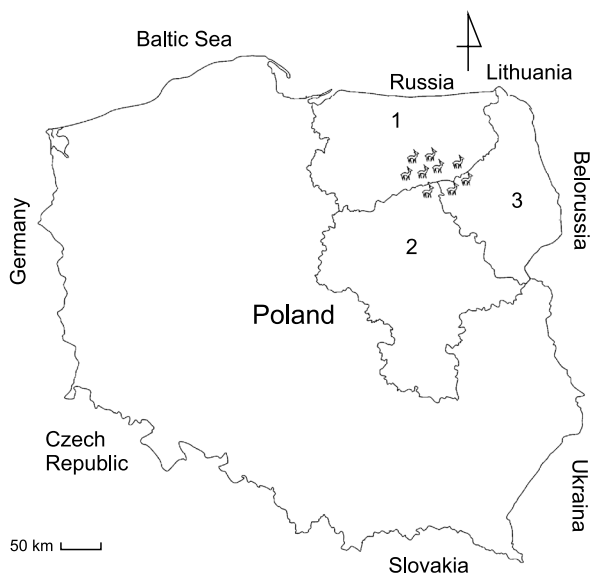

Fig. 1. Locations of sample collection, with accordance to the geographic regions:
1 – Warmińsko-Mazurskie Voivodeship, 2 – Mazowieckie Voivodeship, 3 – Podlaskie Voivodeship

human consumption. The free-living animals were divided into age groups on the basis of their mandibular dentition analysis. The first group comprised animals less than 2 years old, the second group was composed of animals older than three years. All cows were fully mature animals.

Until analyses, the samples were stored at -20°C. The selenium content was determined by spectrofluorimetric method. The muscle samples were digested in HNO_3 at 230°C for 180 min and in $HClO_4$ at 310°C for 20 min. Subsequently, the analytical procedure involved hydrolyzation of the samples with 9% HCl. Selenium was derivatised using 2,3-diaminonaphthalene (Sigma-Aldrich) under controlled pH with the formation of selenodiazole complex extracted into cyclohexane. The Se concentration was determined with a RF-5001 PC Shimadzu spectrophotofluorimeter. The excitation wavelength was 376 nm, and the fluorescence emission wavelength was 518 nm. The detection limit was 0.003 mg kg^{-1} wet weight. The precision of the method was tested using the certified reference material (CRM), i.e. bovine liver BCR-185R (European Commission Joint Research Centre Institute for Reference Materials and Measurements – LGC Standards GmbH, Wesel, Germany) – Table 1. For each sample, three repetitions were performed,

Table 1

The concentration of selenium in the BCR–185R reference material (mg kg^{-1} w.w.)

Se	Reference material bovine liver BCR–185R		Recovery (%)
	certified concentration	obtained concentration	
	1.68	1.56	93

and the averaged result was presented as a concentration in milligrams per kilogram of wet weight (mg kg^{-1}w.w.). Statistical analysis was carried out using Statistica™ (Statsoft Inc., version 12.0). Before analyses, data were investigated to determine their distribution using the Shapiro-Wilks' *W* test. As the data proved to be normally distributed, differences between the groups were estimated using one way ANOVA model. *Post hoc* tests were performed at a significance level of $p \leq 0.01$ using the Tukey's HSD test.

RESULTS

Statistical description of the selenium content in the tissues of the animals studied is presented in Table 2. The means in the red deer and roe deer groups were similar and did not differ significantly. Both groups included individuals with high levels of selenium in the muscles, close to the upper limit of reference levels for cattle. In roe deer, these represented 13% of the population, whereas 12% of roe deer had selenium levels in the muscles within the range 0.01 to 0.05 mg kg^{-1} wet weight, which is considered

Table 2

The content of selenium in muscles of the animals studied (mg kg^{-1} w.w.)

Species		*n*	AM	SD	Range
Red deer	animals less than 2 years old	39	0.069	0.023	0.019-0.111
	animals older than 3 years	55	0.085	0.036	0.017-0.186
	all animals	94	0.079 *A*	0.032	0.017-0.186
Roe deer	animals less than 2 years old	24	0.063	0.026	0.013-0.136
	animals older than 3 years	34	0.083	0.027	0.019-0.162
	all animals	58	0.075 *B*	0.028	0.013-0.162
Cattle	all animals	20	0.149 *AB*	0.023	0.089-0.190

AM – Arithmetic Mean, SD – Standard Deviation, *A*, *B* – differences significant at $p \leq 0.01$

as the deficiency range for cattle. Among red deer, 22% were characterized by a high Se level, whereas animals with its low level represented 18% of the studied population. The differences between the individuals with the highest and the lowest levels of selenium among red deer were nearly 11-fold, whereas among roe deer they reached 12.5-fold. Each group included two individuals with a selenium level well below 0.02 mg kg^{-1} wet weight. The concentrations of selenium in both free-living species were not affected by age. The levels of selenium in bovine muscles were more balanced and the extreme values differed only 2.13 times. Statistical analysis revealed the presence of highly significant differences between the free-living ruminants and cattle.

DISCUSSION

Various tissues of the body reveal different concentrations of selenium. Stoebe et al. (2015), who analyzed selenium in fallow deer farmed in a free--ranging system, ranked the following parenchymal organs in terms of the selenium content, in the decreasing order: the kidney, spleen, myocardium, liver, skeletal muscles. Despite the fact that muscles contained the least of selenium, the most common outcome of selenium deficiency in domesticated animals is muscular dystrophy called white muscle disease, WMD (Humann-Ziehank 2016). Its symptoms include stiffness and pain in the limbs and spine muscles. Apart from this, acute selenium deficiency may manifest through respiratory disorders and myocardial necrosis, which can lead to fatality. This condition may also be a result of vitamin E deficiency, which often correlates with selenium deficiencies (Flueck et al. 2012). Acute symptoms are usually the case in juvenile, fast-growing animals (Humann-Ziehank 2016). Diagnostically problematic are sub-clinical, chronic selenium deficiencies, which may cause reproductive disorders and immuno-

logical system dysfunctions (Stoebe et al. 2015). Flueck et al. (2012) claim that selenium deficiencies in cattle are not acute in character and develop in a sub-clinical way. Wild animals which develop any symptoms leading to a decrease in motor capacity are more susceptible to predation due to their weakness. For this reason, in recent years, attention has been paid to the importance of selenium for game animals, since selenium deficiencies may be a factor affecting the size of their populations in the areas poor in selenium. Reports on selenium levels in free-living ruminants inhabiting Poland's ecosystems have been published by Jarzyńska and Falandysz (2011), Pilarczyk et al. (2011) and Nowakowska et al. (2014, 2015).

Whole blood, serum, liver, kidney or spleen are the tissues most often analyzed for selenium levels in the body. Muscle tissue of free-living cervids is relatively rarely used to measure this element. However, selenium monitoring in cervid muscles seems reasonable for two basic reasons. One is the need to determine the status of mineral supply in the local populations of deer, and another is to establish the levels of human exposure to selenium. As it was previously mentioned, venison has an opinion of a quality meat of high nutritional and dietary values. Its popularity is continuously growing, reaching out far beyond the hunters and their families. At present, venison is available in processed or unprocessed forms in many popular retail networks. The populations of game animals in Poland have increased over the last decade. According to the report by the GUS Statistics Poland (2016), the red deer population increased from 141,000 to 214,000 animals during the period of 2005-2015, while the population of roe deer rose from 692,000 to 867,000 individuals. Over the same period, an increase in the hunting harvest of both species has occurred. Over the last five hunting seasons, the total harvest of red deer has increased from 54,000 in the 2010/2011 season to 89,000 in 2015/2016. At the same time, the roe deer hunting harvest increased from 161,000 to 203,000. Meanwhile, cattle herds remained on a nearly unchanging level of 5.5 million heads.

The literature lacks information on an acceptable level of selenium in tissues of game animals. Jorhem et al. (1996) reported values for bovine muscles to range between 0.03 and 0.18 mg kg^{-1} of tissue wet weight. Similar data were shown by Pavlata et al. (2001). A level within the range of 0.01 to 0.05 could be a symptom of selenium deficiency in cattle, but free-living ruminants need less of this element.

Jarzyńska and Falandysz (2011), who analyzed red deer muscles, found an average selenium concentration of 0.043 mg kg^{-1} wet weight. Similar data have been reported by Dannenberger et al. (2013), in the *longissimus dorsi* muscle of the European roe deer in Mecklenburg – West Pomerania, and Lazarus et al. (2008), in the muscles of the red deer in Croatia, where the average selenium content was 0.048 mg kg^{-1} wet weight. Similar data were published by Kursa et al. (2010) for the roe deer in Czech Republic.

In addition, Dannenberger et al. (2013) found that selenium concentrations in roe deer muscles were not dependent on a region, age or gender.

Our results also indicate that age did not influence selenium concentrations in the muscles of the animals examined.

We found that selenium levels in the muscles of red deer and roe deer were similar to each other, although they were over 1.7 times higher in relation to another red deer population that inhabited the same region of Poland, as reported by Jarzyńska and Falandysz (2011). Results similar to ours were found by Brady et al. (1978) in the muscles of white-tailed deer in Michigan, USA (0.07 mg kg^{-1}wet weight), and by MacLachlan et al. (2016) in Australian sheep; the mean selenium content in muscles of the latter group of animals was 0.09 mg kg^{-1} wet weight, with significant variations found between regions: from 0.034, in Tasmania, to 0.12, in Queensland.

The selenium levels found in our studies were lower than those determined by Aastrup et al. (2000) in the caribou from Greenland (0.104 mg kg^{-1} wet weight). We also found a considerably higher selenium content in bovine muscles, 1.8 times higher than in the roe deer or red deer muscles, which was close to the upper limit of the reference range for this species. The observed differences between domestic cattle and cervids are due to the fact that cattle receive a properly balanced diet and mineral supplements, adjusted to this group of animals.

Under natural conditions, the mineral status of a given free-living animal is subject to fluctuations depending on many factors, such as the season, age, sex, species and level of oxidative stress (Flueck et al. 2012). Red deer and roe deer whose muscle tissues were analyzed in this study were shot during a hunting season, which in Poland is open for females from October 1st until January 15th of the following year. Pilarczyk et al. (2011) observed that the hepatic content of selenium in roe deer is subject to seasonal fluctuations, and is lower in winter. Selenium concentrations in soils on which the animals live is also important. The world average soil selenium content has been estimated at a level of 0.4 mg kg^{-1}, with some areas particularly high in selenium and some very low, the latter group widespread in Poland (Tan et al. 2016). The soils of Poland contain between 0.040 to 0.640 mg selenium in kg^{-1} dry weight (Tomza-Marciniak et al. 2010). Dębski et al. (2001) observed that a low, deficient environmental level of selenium in Poland affects more than 70% of the country's area. Appropriate manuring is applied in order to enrich the soil environment with selenium; however, only a small amount (less than 5% of the total content in a fertilizer) is used by plants (Li et al. 2016). It is so because the chemical form of selenium is more important for the plant's uptake than its total amount in the soil. Other factors involved in the selenium uptake by plants include a plant species, fertilizer application method and soil properties (Kikkert, Berkelaar 2013, Li et al. 2016). The selenium content in forage plants will be reflected in the ruminant's body. Chalabis-Mazurek and Wałkuska (2014) demonstrated that a low level of selenium in plants results in selenium deficiencies in sheep and roe deer.

The relatively high selenium content in the muscles of roe- and red deer, as compared to data published by Jarzyńska and Falandysz (2011), is a result of the presence of some individuals whose selenium tissue levels were close to the upper limit of the reference range for cattle. This high level in a fraction of the studied animals has probably two causes. One is the common use of salt licks. Manufacturers of these supplements recommend to make them available to cattle, but also to farmed game animals and those living at large. According to data declared by some producers, the selenium content in some slat licks for ruminants is 10 mg kg^{-1}.

Another cause may be deer foraging on crops growing on fertilized lands. A diet of deer is composed of wild plants, such as grass, herbs, tree buds, shrubs or leaves, but it can also include crop plants (Obidziński et al. 2013). Mineral supplementation and winter feeding of wild game animals improve their condition, so their populations have been increasing recently. The animals are hunted and their meat is then sold to consumers.

Determination of the daily human requirement of selenium is based on two basic markers: serum glutathione peroxidase (Gpx) activity and selenoprotein P (SEPP1) concentration. Depending on a country and the marker applied, the daily recommended human selenium intake varies from 30 to 75 μg (European Food Safety Authority 2014). According to the WHO (2004), a daily dose over 41 μg is sufficient to sustain an appropriate level of serum GPx activity in men weighing 60 kg for about 5 to 8 months. According to data by Nordic Council of Ministers (2014), SEPP1 concentration is a much better indicator, according to which the daily selenium requirement is 50 μg for women, and 60 μg for men. Given the content of selenium in the muscles of roe deer, red deer and cattle, it must be concluded that beef is a much better source of the nutrient than venison. An intake of about 100 g of beef from the studied cattle covers almost 30% of the daily requirement for selenium, while the meat or roe or red deer will provide from 13 to 16% of the daily requirement for this element.

CONCLUSIONS

In conclusion, the average muscle content of selenium in red deer and roe deer of north-eastern Poland, which is a region less abundant in this mineral, is higher than that reported by other authors. This demonstrates a good level of selenium supply in wild ruminants inhabiting the region, as the results from more than 80% of animals in both studied groups remained within the reference range. Nevertheless, the studied populations also comprised a few selenium-deficient individuals. Appropriate mineral supply translates into an increase in red deer as well as roe deer in this area, allowing the hunting of more individuals. Despite the high culinary

value of venison and its perception as more noble food, beef surpasses game meat when it comes to the selenium content. In addition, venison is highly variable in selenium levels.

Conflict of interest

The authors declare that they have no conflict of interest.

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