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SELENIUM CONTENT IN THE OVARIES OF FREE-LIWING *CERVIDAE* FROM NORDEASTERN POLAND

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Abstract. Selenium is counted among the trace elements necessary for the maintenance of metabolic processes occurring in the animal body, including the proper functioning of the reproductive system. The aim of the study conducted was to analyze the selenium content in the ovaries of red deer (*Cervus elaphus*) and European roe deer (*Capreolus capreolus*) in an attempt to establish a range of reference values for individuals of these species. Selenium concentrations in tissues tested were determined using spectrofluorometric method after wet mineralization in HNO_3 and $HClO_4$ mixture. Ovaries Se concentrations ranged from 0.034–0.338 mg·kg^{-1} wet weight and 0.015–0.285 mg·kg^{-1} wet weight in roe deer and red deer respectively. Based on the results, it was found that the average selenium content was higher in the ovaries of red deer and was 0.11 while in roe deer it was 0.09 mg·kg^{-1} wet weight. The gonads of European roe deer were characterized by higher variability of the analyzed parameter in relation to samples obtained from female red deer. The coefficient of variation in their case was almost 90.1, while the value of the coefficient of variation for selenium content in the parenchymal layer of ovaries in red deer was almost 67.6.

Key words: selenium, ovaries, ruminants, roe deer, red deer.

INTRODUCTION

Selenium (Se) is counted among the trace elements necessary for homeostasis in humans and animals. Its role in metabolic processes stems from being a component of selenoproteins that activate protective mechanisms against tumorigenesis, protect against cardiovascular disease, and exhibit anti-inflammatory properties (Pieczyńska and Grajeta 2015). The protective role of selenium is a result of its presence in glutathione peroxidase (GPx) and thioredoxin

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reductase (TrxRs) which are the main components of the enzymatic protection system against peroxidation of cellular structures (Mehdi and Dufrasne 2016; Rodríguez et al. 2018, 2020). Selenium also plays an important protective role against the effects of toxic metals such as cadmium, lead, mercury and arsenic (Kakela et al. 1999; Lazarus et al. 2011). This element is involved in the functioning of the reproductive system and the metabolism of thyroid hormones hence the phenomenon of deterioration of reproductive performance and productivity associated with deficiencies of this element is known in livestock (Rodríguez et al. 2018, 2020). Its relationship to reproduction was detailed many years back in male individuals. Initially, it was assumed that there were no differences in selenium metabolism between individuals of both sexes, since no gene encoding selenoproteins is located on sex chromosomes (Schomburg 2012). In males, it has been found to be essential for the proper development of the testes and their subsequent function in mature individuals (Behne et al. 1996). The testes are among the organs that are primarily supplied with selenium and capture the highest amounts of it of all mammalian tissues. It is admittedly captured with some delay but is then transported to the epididymides. The difference with the female gonads is that selenium is captured by the ovaries very quickly and then the levels drop (Schomburg 2012). Although, as already mentioned, the main biological role of selenium is manifested by its participation in selenoproteins it has been proven, using a number of mammalian and avian models, that it also has the ability to modulate genes (Kipp et al. 2009; Sunde and Reines 2011; Sunde 2016; Lammi and Qu 2018; Pappas et al. 2019; Seremelis et al. 2019; Qazi et al. 2021). Recent studies have observed that the use of sodium selenate can modulate mitochondrial dynamics in a way that improves mitochondrial function in polycystic ovary syndrome (PCOS) in rats (Atef et al. 2019). Selenium supplementation was also found to modulate genes related to fat metabolism and insulin in infertile women affected by PCOS and in women with gestational diabetes (Modarres et al. 2018; Karamali et al. 2020). From the available data, it appears that the role of Se in the functioning of the male gonads has been fairly well understood at the cellular and subcellular levels while in females, knowledge is scarce, especially since data on the role of Se in the modulation of the gene transcription cascade in ovarian tissue remains incomplete and requires further research for a comprehensive understanding of the effect of this element on the functioning of the female gonads. Paszkowski et al. (1995) found that a decrease in follicular fluid selenium content could be observed in patients with unexplained infertility. Moreover, in infertile women with premature ovarian dysfunction, elevated serum levels of antibodies to ovarian cells that are type 1 selenium-binding protein can be observed, indicating that it plays an important role in female gonadal function (Safiyeh et al. 2021). However, this knowledge remains fragmentary, and a specific starting point should be to determine the range of physiological Se concentrations in ovarian tissue in healthy individuals, thus establishing a range of reference values. The few data found in the available literature mainly concern humans and laboratory animals serving as experimental models. However, there are no data on the Se content of ovarian tissue of other species, especially free-living ruminants of the family *Cervidae*, which exhibit pronounced seasonality in reproduction. The aim of this study is to analyze the selenium content of the ovaries of red deer and European roe deer in an attempt to establish a range of reference values for individuals of these species.

MATERIAL AND METHODS

The study material consisted of ovary samples collected from 16 roe deer (*Capreolus capreolus*) and 17 red deer (*Cervus elaphus*). Age was determined by the dentition of the individuals. The research material was collected in the autumn of 2018 and 2019 in northeastern Poland on the border of the Mazowieckie and Warmińsko-Mazurskie Voivodships. All animals were

shot during the hunting season by hunters within the established hunting limits and were adult individuals.

Ovaries were collected in their entirety shortly after the shot during the evisceration of individuals by cutting off the ovarian ligament (*lig. ovarii proprium*) and its mesovarium, thereby separating the organ from the rest of the female reproductive system. They were then secured in polyethylene tubes, which were cooled and transferred to the laboratory for further processing. In preparation for selenium determination, organ preparation was carried out to separate samples from the parenchymatous layer of the organ (*zona parenchymatosa*), known as the ovarian cortex from its vascular layer (*zona vasculosa*) known as the ovarian medulla. Se content was determined only from tissue taken from the parenchymatous layer of the ovary. The study material was collected and frozen at −20°C until laboratory analysis. Selenium concentrations were determined by spectrofluorometric method (*spectrophotofluorometr* Shimadzu RF-5001 PC). Samples were digested in HNO_3 at 230°C for 180 minutes and in $HClO_4$ at 310°C for 20 minutes, followed by hydrolysis with 9% HCl. Selenium was derivatized with 2,3-diaminonaphthalene, with formation of a selenodiazole complex. The excitation wavelength was 376 nm; the fluorescence emission wavelength was 518 nm. A detailed method for the determination of selenium was presented in the work of Skibniewska et al. (2020). The accuracy of the analytical procedure was verified by measuring selenium levels in NCS ZC 71001 reference material (beef liver, n = 3; China National Analysis Center for Iron and Steel, Pekin, China).

The average recovery was 91.1% of the reference value. Selenium concentrations in the organs studied were expressed in $mg \cdot kg^{-1}$ wet weight of the tissues analyzed. Analyses were performed in triplicate. Statistical analysis was performed using Statistica Version 13.0 software (TIBCO Inc.™ StatSoft, Krakow, Poland). The normality of the distribution of the variables was tested using the *W* Shapiro-Wilk test. The data did not have a normal distribution, so the Mann-Whitney *U* test was used to compare differences between groups at a significance level of $p \leq 0.05$. No statistically significant differences were found.

RESULTS

Data on basic statistical parameters for selenium content of individuals of both species are presented in Table 1.

Table 1. Selenium content of tested tissues in European roe deer and red deer in $mg \cdot kg^{-1}$ wet weight

	N	Mean	Median	Min.	Max.	Q^{25}	Q^{75}	SD	Coeff. var.
Roe deer	16	0.0939	0.0578	0.0342	0.3382	0.0483	0.0972	0.0846	90.099
Red deer	17	0.1135	0.0874	0.0149	0.2850	0.0567	0.1528	0.0767	67.5845

Q_{25} – lower quartile, Q_{75} – upper quartile, SD – standard deviation, min. – minimum, max. – maximum, Coeff. var. – coefficient of variation.

There were no statistically significant differences between the females of the two species studied. Nevertheless, it can be noted that the greater variability was characterized by the gonads of European roe deer, in which the coefficient of variation was almost 90.1, while in red deer the value of the coefficient of variation for selenium content in the parenchymal layer of the ovaries was almost 67.6. Among the studied roe deer there were two individuals with extremely high selenium content of 0.3382 and 0.2551 $mg \cdot kg^{-1}$ tissue, respectively. In the case of red deer, a value above 0.25 was recorded in only one individual. The distribution of the data in individuals of both studied species is shown in Fig. 1.

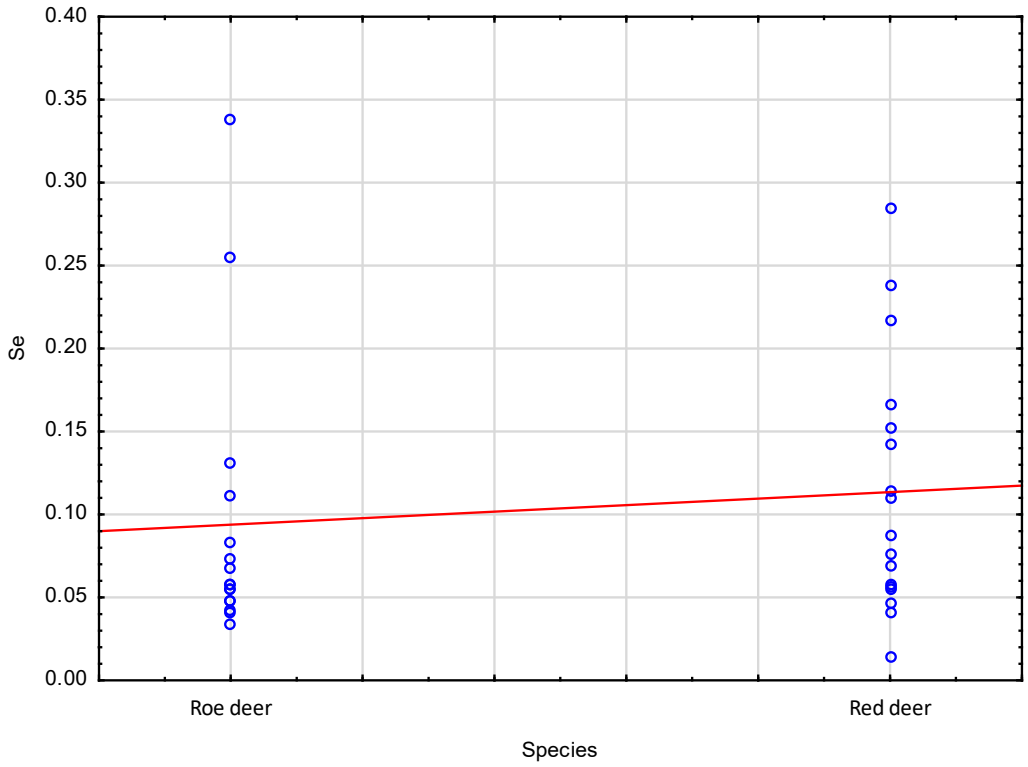

Fig. 1. Distribution of values obtained in all individuals tested (Se in mg·kg^{-1} wet weight)

DISCUSSION

As mentioned at the beginning of the paper, the role of selenium in the reproductive processes of female individuals has not been fully understood, and no reference values for its content in the ovaries have been developed so far, which could serve as a reference for determining the adequate state of supply of the said organ with this element. On the basis of studies conducted on laboratory animals using selenium transport markers, it was found that selenium is accumulated in individual organs and cells to different degrees, reaching extremely different values. In laboratory rats fed a diet containing 300 µg·kg^{-1} of selenium, it was observed that its concentration in kidney tissue was 5.3 µg·kg^{-1} dry weight of the organ, while in the testis a value of 6.1 was registered, while in the epididymis as much as 11.2. In the latter two of these organs, the high concentration of selenium is due to its extremely high content in sperm, which is estimated at about 25 µg·kg^{-1}. At the other end of the scale is CNS tissue, where values below 0.5 µg·kg^{-1} are registered (Behne et al. 2000). Thus, in the opinion of the authors of this paper, a universal range of reference values for selenium content for the whole organism cannot be established, and each tissue or organ should have a separate scale that allows comparisons within species and the determination of interspecies differences in its concentration in analogous organs.

Until the early 1980s, selenium was thought to play its biological role mainly as a result of its participation in the activity of glutathione peroxidase responsible for protection against peroxidation of cellular structures (Sarma et al. 2017). However, soon after the initial research on the biological role of this element, it was established that its deficiency is accompanied by impaired function of immune responses, which can also affect reproductive efficiency. Among others, selenium deficiency in cattle has been found to be accompanied by a number of symptoms associated with abnormalities of the female reproductive system, such as placental retention, abortions, stillbirths, irregular estrous cycles, early embryo mortality, cystic ovaries, silent estrus, and

low conception rates (Corah and Ives 1991; Randhawa and Randhawa 1994; Kamada et al. 2014; Uematsu et al. 2016; Sarma et al. 2017). It was noted that infertility in cows was observed particularly frequently in individuals kept in selenium-deficient areas, and after supplementation of this element in the diet, reproductive rates improved, indicating that this element is essential for the proper function of both the male and female reproductive systems (Aréchiga et al. 1998). Initially, it was assumed that selenium deficiencies caused inadequate levels of progesterone necessary to maintain pregnancy and myocardial failure in the developing fetus (Underwood and Suttle 2004; Kamada et al. 2014).

Among ruminants, studies on the effects of selenium on female reproduction have been done in cattle, and the majority of them concerned problems related to placental retention, the prevalence of which in the U.S. is determined to be 10%, but in selenium-deficient areas it occurs in almost 50% of cows. Selenium injections of 2.3 to 23 mg Se per animal were found to significantly reduce the incidence of placental retention. Ultimately, it was determined that low doses ranging from 2.3 to 4.6 mg Se per cow were effective, and there was no need for high doses of selenium preparations (Coufalik 1985; Eger et al. 1985; Hansen and Deguchi 1996). Similar observations have also been made in other areas of the world, such as Denmark and Norway (Ropstad et al. 1987; Hansen and Deguchi 1996). Consequently, it has been established that animals with glutathione peroxidase levels in whole venous blood below 70 units/g hemoglobin should be given selenium preparations by injection or by the oral route, which is as effective as parenteral administration (Tasker et al. 1987). The studies described here focused on analyzing selenium levels in various biological matrices, primarily in whole venous blood, and on studying GPX activity. In fact, the mechanism of action of selenium in ovarian tissue in the mature female and in the body of the developing fetus is much more complex. It is known that selenium and selenoproteins, due to their involvement in the regulation and modulation of antioxidant balance, are needed for optimal reproduction in females.

Selenium supplementation also helps alleviate placental oxidative stress. In animals and humans, about 35 selenoproteins have been identified, containing selenocysteine in their active sites (Arthur 2000; Kryukov et al. 2003; Raymond and Ralston 2004). Selenoproteins synthesis is important especially during early mammalian development (Serdaru et al. 2004). Selenoproteins participate in antioxidant defense by eliminating reactive oxygen species, including hydrogen peroxide and toxic products of aerobic respiration (Shchedrina et al. 2010). Reactive oxygen species reacting with unsaturated lipids cause degradation of cell membranes. Thioredoxin reductase and four glutathione peroxidases (GPX) as well as other selenoproteins act as scavengers of oxidants in tissues. When considering the metabolic role of selenium in individuals of both sexes, it should be noted that there are significant differences (Vindry et al. 2018). Male gonads indeed have a high demand for selenium, as the glutathione peroxidase 4 protein GPX4 is involved in spermatogenesis, and its absence leads to male infertility (Marchlewicz et al. 2016). In females, selenium is concentrated in the ovaries to a much lesser degree, and capturing the relationship between low levels and infertility is not as spectacularly evident as in males. Sex differences in selenium concentration are also evident in the blood serum, liver, kidneys and pituitary gland (Schomburg and Arner 2017).

These are mainly due to the expression of selenoproteins, especially in selenium-deficient diets. To date, it has not been clearly confirmed whether this occurs at the transcript level or at the translational level. A study on the association of gonadectomy to determine the effect of steroid sex hormones on selenium metabolism in individuals undergoing surgery found a decrease in the expression of selenoproteins such as SELENOP, DIO1, and GPX3, demonstrating the association of sex hormones with selenium metabolism, particularly the regulation of the levels of the aforementioned selenoproteins (Vindry et al. 2018). In addition to this pathway of selenium effects on the body, in studies conducted on a number of mammalian and avian models,

selenium has also been shown to modulate the transcriptional cascade of genes, demonstrating that its biological role is not limited to its participation in selenoproteins (Kipp et al. 2009; Sunde and Raines 2011; Sunde 2016; Lammi and Qu 2018; Pappas et al. 2019; Seremelis et al. 2019; Qazi et al. 2021). Current studies on the effects of selenium on ovarian tissue are mainly aimed at investigating its effects on gene regulation and association with polycystic ovary syndrome (PCOS) in women because both selenium and its nanocomplexes have been shown to improve insulin resistance in women suffering from PCOS (Shabani et al. 2018; Butt et al. 2020; Abdallach et al. 2023). The authors of this paper are not aware of studies showing selenium levels in the ovarian tissue of free-living ruminants, including wild *Cervidae*. Therefore, it is not possible to compare the results of our study with literature data.

Determination of selenium content in other tissues of red deer and European roe deer was conducted by Pilarczyk et al. (2009), who included in their study the effect of seasonality on fluctuations in its level in tissues. The average selenium content in the liver and kidneys of red deer and European roe deer was 0.36, 2.72, 0.57 and 2.99 $\mu g \cdot kg^{-1}$ dry weight of tissue, respectively. Based on an analysis of selenium content in liver tissue, the authors showed that selenium deficiencies were found in 100% of animals in winter, 94% in summer and 75% in the autumn season. Tomza-Marciniak et al. (2010) in a study of tissues of roe deer from the Greater Poland region also confirmed deficiencies of this element. This is of interest because in the case of free-living animals, mineral deficiencies can impinge on their overall condition, limiting the body's ability to avoid predator attacks. The state of supply of free-living animals mainly depends on the biogeochemical background, i.e., on the abundance of soils in a given element. In the case of Se, its soil reserves are taken up by plants that are then food for animals.

In Poland, more than 70% of the land is selenium-deficient areas, where selenium content in soils ranges from 0.04 to 0.64 $\mu g \cdot g^{-1}$ DM (Piotrowska 1984; Dębski et al. 2001). The sampling regions for our study are also areas of low selenium content, so the results above 0.2 mg recorded in two roe deer and three doe may seem intriguing. Particularly in the case of roe deer, two individuals with extremely high selenium contents of 0.3382 and 0.2551 $mg \cdot kg^{-1}$ wet tissue were recorded. In the case of red deer, a value above 0.25 occurred in only one doe. These results are clearly an outlier for the remaining individuals. At the same time, it should be noted that the median selenium content in the ovaries of red deer took on a higher value than in roe deer. The explanation for the observed phenomenon of the incidental occurrence of high selenium content in ovarian tissue can only be the fact of selenium supplementation in free-living animals. Hunting clubs administering game in their districts commonly use salt licks, which, in the opinion of hunters, significantly improve the condition of deer, thus projecting the quality of trophies obtained. Data on the most popular preparations indicate that they contain selenium at 10 mg/kg of preparation.

The observed high levels of selenium in the ovaries of selected individuals are likely due to its recent intake as a supplement. In studies conducted on laboratory animals using selenium isotopes, it was found that ovarian tissue captures up to 75% of the administered dose at a time much faster than is the case with the male gonads, and then a steady decline can be observed in the timeline, also observed in liver and blood serum (Schomburg 2012). It can be concluded that the recorded high levels of selenium in some individuals represent a peak that occurs soon after its oral intake while the relatively even values observed in other females reflect the actual selenium content of ovarian tissue.

CONCLUSIONS

On the basis of the study, it can be concluded that the selenium content of the ovaries of European roe deer and red deer from northeastern Poland ranges from 0.148 to 0.342 $mg \cdot kg^{-1}$. The

analyzed organs of roe deer were characterized by greater variability compared to the material taken from female red deer. Despite the fact that samples were taken from individuals living in areas with selenium deficiencies due to the biogeochemical background, high values were registered in selected individuals, probably due to supplementation used by hunters to maintain the good condition of animals located in particular hunting districts.

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ZAWARTOŚĆ SELENU W JAJNIKACH WOLNO ŻYJĄCYCH *CERVIDAE* Z TERENU PÓŁNOCNO-WSCHODNIEJ POLSKI

Streszczenie. Selen zaliczany jest do pierwiastków śladowych niezbędnych do zachowania procesów metabolicznych zachodzących w organizmie zwierząt, m.in. do prawidłowego funkcjonowania układu rozrodczego. Celem prowadzonych badań była analiza zawartości selenu w jajnikach jelenia szlachetnego (*Cervus elaphus*) oraz sarny europejskiej (*Capreolus capreolus*), stanowiąca próbę ustanowienia zakresu wartości referencyjnych dla osobników tych gatunków. Selen w badanych tkankach oznaczono metodą spektrofluorometryczną po wcześniejszej mineralizacji w mieszaninie HNO_3 i $HClO_4$. Zawartość selenu mieściła się w zakresie 0,034–0,338 mg·kg^{-1} i 0,015–0,285 mg·kg^{-1} mokrej masy odpowiednio u saren i jeleni. Na podstawie uzyskanych wyników stwierdzono, że średnia zawartość selenu była wyższa w jajnikach jeleni i wynosiła 0,11, podczas gdy u saren była to wartość 0,09 mg·kg^{-1} mokrej masy. Gonady sarny europejskiej cechowały się większą zmiennością, analizowanego parametru w stosunku prób pozyskanych od samic jelenia szlachetnego. Współczynnik zmienności wynosił w ich przypadku niemal 90,1, natomiast wartość współczynnika zmienności dla zawartości selenu w warstwie miąższowej jajników u jelenia szlachetnego wynosiła niemal 67,6.

Słowa kluczowe: selen, jajniki, przeżuwacze, sarna, jeleń szlachetny.