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SELENIUM CONTENT IN THE GONADS OF MALE AND FEMALE EURASIAN WILD BOAR (*SUS SCROFA*) FROM THE MAZOVIAN PROVINCE

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Abstract. Selenium is counted among the trace elements necessary to maintain metabolic processes occurring in the animal body, including reproductive processes. The aim of the study was to assess selenium content in testes and ovaries of wild boars (*Sus scrofa*) in an attempt to establish a range of reference values for individuals of this species. Selenium concentrations in tissues tested were determined using spectrofluorometric method after wet mineralization in HNO_3 and $HClO_4$ mixture. Based on the results, it was found that male wild boars accumulated significantly higher concentrations of Se than females. The average content of this element in the testes of wild boars was 0.32 mg · kg^{-1} while in the ovaries it was at the level of 0.14 mg · kg^{-1} wet weight. In addition, the gonads of males were characterized by a higher variability of the analyzed parameter in relation to samples obtained from female wild boars. The coefficient of variation in their case was almost 40.9, while the value of the coefficient of variation for selenium content in the parenchymal layer of the ovaries was 24.9. On the basis of statistical analysis, highly significant differences were found at $p \leq 0.01$ according to the sex of the individuals studied. Despite the fact that samples were taken from individuals living in areas with selenium deficiencies due to the biogeochemical background, it should be considered that the supply of Se is optimal, which is evident in the population growth dynamics of individuals of this species in the study area.

Key words: selenium, wild boar, ovaries, testicles.

INTRODUCTION

Selenium (Se), as an element essential for the normal function of the animal and human organism, has been on the spectrum of interest of many research teams involved in tissue

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and organ biochemistry, medicine, agricultural sciences and studies on element migration in the soil-plant-animal food chain (Mehdi and Dufrasne 2016; Modarres et al. 2018; Rodríguez et al. 2018; Karamali et al. 2020; Rodríiguez et al. 2020). With regard to its tissue and/or organ concentration, studies have been conducted mainly on laboratory animals and livestock, in which it determines productivity (Sarma et al. 2017; Schomburg and Arnér 2017; Atef et al. 2019). Against this background, data on Se content in the tissues of free-living animals can be considered sparse despite the fact that they, and especially game animals, are excellent indicators of the presence of selected substances in the environment due to their close relationship with the habitat, which is not subject to direct modification by humans (Pilarczyk et al. 2009; Nowakowska et al. 2016). Analysis of selenium content of their tissues can provide information on the biogeochemistry of a given region. Monitoring Se content in the organs of game animals from different habitats is important, among other reasons, because of differences in its distribution in the lithosphere. There are areas naturally rich in this element and regions with naturally low Se content, which include areas of central Europe, including most of Poland (Kabata-Pendias and Pendias 1999; Balicka-Ramisz et al. 2006; Pilarczyk et al. 2007, 2009). Selenium is essential for the proper functioning of animal and human organisms due to its participation in glutathione peroxidase (GPx) and thioredoxin 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). In addition, Se has protective effects against the toxic effects of heavy metals, which stimulate peroxidation at the cellular level (Hartikainen 2005; Lazarus et al. 2011). It also has protective properties against the toxic effects of aflatoxins that exhibit carcinogenic activity (Dębski et al. 2001; Pilarczyk et al. 2010). In addition to the aforementioned activities, Se participates, in a catalytic role, in the synthesis of thyroid hormones, is involved in the stimulation of the immune system and in the normal function of the central nervous system (Kuczyńska and Biziuk 2007). Among the numerous metabolic functions in which selenium plays an important role, its involvement in reproduction in the sense of ensuring male fertility by participating in testosterone synthesis, spermatogenesis and ensuring adequate sperm viability and motility, as well as the possibility of embryo implantation in females or maintaining placental function, should be emphasized. Se deficiency in animals has been found to negatively affect male and female reproduction (Kamada et al. 2014; Uematsu et al. 2016; Qazi et al. 2021; Titov et al. 2022).

The Eurasian wild boar (*Sus scrofa*) is a game animal of great economic importance due to the fact that it is a carrier of the African swine fever virus (ASFV). Due to the need to reduce the possibility of transmission of the infection to breeding pigs, a decision was made to depopulate this species. The number of wild boars in Poland in 2010 was estimated at about 249,900, while in 2022, after mass shooting, it was estimated at about 55,500. In the 2021/2022 hunting season, a total of 143.8 thousand of these animals were shot (CSO 2022). The systematic shooting carried out does not have the desired effect, as specialists in the biology of this species believe that the annual reproduction rate of wild boar can reach a value of 300% (Probst et al. 2017; Flis 2019). Therefore, it seems reasonable to study all the factors that affect the reproduction of these animals, including not only the availability of food, climatic changes, anthropogenic pressure, but also their mineral economy with particular emphasis on elements that have a significant impact on the function of the gonads determining the efficiency of reproduction. The aim of this study is to determine the content of Se in the gonads of Eurasian wild boar females and males from the areas of Mazovian Province in order to establish a range of values for individuals of both sexes, which could in

the future serve as one of the elements used to create a database of reference values for this species in Poland.

MATERIAL AND METHODS

The material for the study consisted of gonads collected from 47 wild boars (*Sus scrofa*). These were ovaries (n = 20) and testes (n = 27), which were obtained in the fall of 2018 and 2019 in the Mazovian province. All animals were shot during the hunting season by hunters within established hunting boundaries. The boars were fully mature individuals. Ovaries were taken in their entirety immediately after shooting during the evisceration of the animals by cutting off the ovary's own ligament (*lig. ovarii proprium*) and its mesentery (*mesovarium*), thereby separating the organ from other parts of the female reproductive system. The testes were also collected shortly after the wild boar was shot. The gonads were then packed into polyethylene transport containers, which were cooled and transferred to the laboratory for further processing. In preparation for selenium determination, organ preparation was performed 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 core. Se content was determined only in tissue taken from the parenchymatous layer of the ovary. When testicular tissue was analyzed, a preparation was performed, during which the epididymis was separated, the white membrane (*tunica albuginea*) and the central part of the organ in the form of the mediastinum testis were removed, as it contains the testicular network that constitutes only the sperm exit pathways. Representative parenchymal samples were then taken from the central part of the testicular lobes.

The test material was stored at −20°C until laboratory analyses. Selenium content was determined by fluorescence spectroscopy using a Shimadzu RF-5001 PC spectrophotofluorometer. The measurement was carried out at a fluorescence emission wavelength of 518 nm and an excitation wavelength of 376 nm. The samples were digested in concentrated nitric acid (HNO_3) at 230°C for 180 minutes and in perchloric acid ($HClO_4$) at 310°C for 20 minutes. The sample was then hydrolyzed in 9% hydrochloric acid (HCl) to reduce Se^{6+} to Se^{4+}. Selenium was derivatized with 2,3-diaminaphthalene to form a selenediazole complex. A detailed method for the determination of selenium is presented in Skibniewska et al. (2020). The accuracy of the analytical method was based on the reference material NCS ZC 71001 (bovine liver, n = 5; China National Analysis Center for Iron and Steel, Beijing, China). The selenium concentration in the studied gonads was 91% of the reference value. The selenium content of the organs studied was expressed in mg · kg^{-1} fresh weight. Analyses were carried out in triplicate. Statistical calculations were performed using the Statistica statistical package version 13.0 (TIBCO Inc.™ StatSoft, Kraków, Polska). The normality of the distribution of variables was tested using the Shapiro-Wilk W 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 $p \leq 0.05$ and $p \leq 0.01$. Highly statistically significant ($p \leq 0.01$) differences were found in the content of this element in the gonads depending on the sex of the individuals studied.

RESULTS

Data on basic statistical parameters for selenium content in male and female individuals are shown in Table 1.

Table 1. Selenium content in the gonads of individuals studied (in mg · kg^{-1} wet weight)

Group	Mean	SD	n	median	Min.	Max.	Q25	Q75	GEM	CV
Males	0,316**	0.129	27	0,365	0.134	0.518	0.211	0.451	0.288	40,889
Females	0.142**	0.035	20	0.136	0.104	0.214	0.116	0.151	0.138	24.983
All animals	0.242	0.132	47	0.211	0.104	0.518	0.136	0.371	0.210	54.795

** Statistically significant differences at $p \leq 0.01$. Q_{25} – lower quartile; Q_{75} – upper quartile; SD – standard deviation; GEM – geometric median; CV – coefficient of variation.

Highly statistically significant differences were found between individuals of both sexes. The mean value registered in males was more than 2.2 times higher than in females. Nevertheless, it can be noted that the gonads of males were characterized by a much higher variability of the observed values than in the case of females. In the case of males, the coefficient of variation was 40.88, while in the case of females, its value was 24.98. The variability of the observed values is presented in Fig. 1, where it can be seen that there is a group of male individuals whose testicular selenium contents are within the range recorded in female gonads.

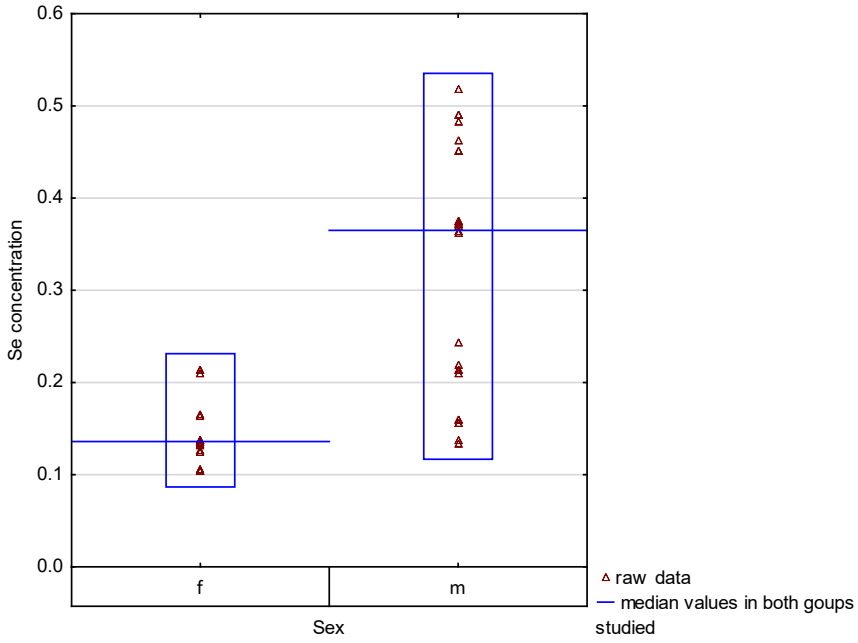

Fig. 1. Graph of Se content variation in individuals of both sexes (in mg · kg^{-1} wet weight)

DISCUSSION

The steadily increasing wild boar population in Poland, which was observed until 2015, has become problematic for several reasons. First and foremost is ASF, the first case of which was detected in 2014. The disease poses a threat to swine, and its reservoir invariably remains wild boar. In the following years, ASF virus infections increased rapidly, and the number of outbreaks in wild boars in Poland from the registration of the first infection until September 2021 was estimated at 12071 (Woźniakowski et al. 2016; Flis 2019; Pejsak and Woźniakowski 2021). Accordingly, an action has been taken to reduce the population of these

animals, estimated at about 67,900 in 2021. Nonetheless, ASF outbreaks are constantly being detected, and from January to August 2023 their number reached 30 (GVI 2023). Further reasons why the wild boar has become a problem animal is that part of the population of these animals has become synanthropic individuals colonizing urban areas causing damage from foraging in recreational areas and residential areas. These animals also cause traffic collisions and occasionally attack people and pets. In addition, wild boar cause numerous damages to agricultural crops (Zalewski et al. 2020). Most of the problems described are a direct consequence of the high reproduction rate of this species, which has become part of the environmental changes observed in recent decades. The wild boar is characterized by a significant potential for population growth dynamics, which has been revealed as a result of a number of complementary environmental factors. The first is undoubtedly the change in crop structure. Poland's agricultural landscape is currently dominated by corn, rich in readily available, high-energy nutrients. In addition, zearalenone present in decomposing corn grain has a significant effect on the gonadal function of these animals, as it acts as an agonist and partial agonist for estradiol, which in domestic pigs causes hyperestrogenism (Bielas et al. 2017). In the case of wild boar, zearalenone induces earlier sexual maturation of females, which are now capable of breeding as early as the end of the first year of life, while the biology of this species under temperate climate conditions allowed females to reach maturity at the turn of the second and third year of life (Nicpoń and Hulewicz 2015; Zalewski et al. 2020). The dynamics of the growth of the wild boar population is also a resultant of climate change causing, among others, an increase in the frequency of oak and beech seed years, the fruits of which provide an excellent food base for these animals (Pejsak and Woźniakowski 2021). Along with the availability of high-energy food, reproductive processes are also affected by the state of the body's mineral supply. Selenium is an element that has a special effect on animal reproduction, since in males it is necessary for the proper development of the testes and their subsequent function in mature individuals, as well as for maintaining adequate semen quality in terms of sperm viability (Behne et al. 1996). Therefore, the testes have the highest demand for this element of all organs of the body. Analyzing the pharmacokinetics of Se-containing compounds, it was found that there is a significant difference between the levels of selenium in the male and female gonads, as well as a difference in the distribution time of this element, which is taken up by the ovaries quickly, but does not reach the level recorded in the testicular tissue. In the case of the ovaries, the level of absorbed Se decreases steadily along the timeline while its uptake by the testes occurs with some delay and consequently reaches a much higher content than recorded in the female gonads (Schomburg 2012). Deposited in the testes, selenium protects the sperm mitochondria from oxidative processes. Selenium, in the form of selenocysteine, is the catalytic centre of the antioxidant glutathione peroxidase, hence there is a higher concentration of selenium in the gonads in males than in females (Roveri et al. 2001).

Early studies using labeled $_{75}Se$ administered in the form of H_2SeO_3 found that approximately 3 weeks after injection of the preparation, the testis and epididymis concentrated about 41.8% of the total content of the administered isotope, while the highest values recorded in ovarian tissue were only 0.3% (Brown and Burk 1973). Observations, initially conducted mainly in laboratory animals, have generated considerable interest in the involvement of Se in male gonadal function, which has been well understood and documented at both the cellular and subcellular levels, while knowledge of its detailed activity in ovarian tissue is fragmentary and requires the additions necessary for a comprehensive understanding of the relationship of Se to female gonadal function (Schomburg 2012). Evidence for the important role of Se in ovar-

ian function was provided by the study of Grazul-Bilska et al. (2009). The authors observed that in sheep, Se absorbed with food by mother can play a role in early folliculogenesis and ovarian follicle cell proliferation and is also involved in angiogenesis and proliferation of ovarian stromal tissue in the fetus. Hence, it can be inferred that it plays an important role in the formation of female gonads during the early stages of individual development (Mirone et al. 2013). The results of studies conducted in recent years indicate a strong association between Se deficiency and perinatal complications. Oxidative stress, exacerbated by deficiencies of this element, has been found to cause a variety of disorders, such as spontaneous miscarriages and intrauterine growth arrest in the fetus (Agarwal et al. 2012). Se deficiencies are also in close association with premature births (Kocak et al. 1999; Tara et al. 2010). In the available literature, there are no studies containing data on the Se content of the gonads of male and female wild boars. An additional factor hindering the creation of a reference database is the habitat of the animals and the associated food base constituting the source of supply of this element, since it should be expected that different levels of Se may be registered in specimens from urban areas while others in the gonads of wild boars that do not live permanently in urban environments. The results obtained in our study indicate that there is relatively little variation in the values recorded in females relative to males. The median values in the studied groups differ significantly, and according to the literature, the values registered in males are significantly higher than those observed in the female group by almost 2.7 times. However, in the group of males, a clear separation into two subgroups can be observed, whose clear boundary is the value of 0.3. Nevertheless, among the male individuals, the lowest observed values exceed the median value observed in the group of females. It is likely that these are young individuals, not yet participating in reproduction, while the group of males with high Se content in testicular tissue are older, fully sexually active boars. Data on the reproductive biology of wild boar indicate that males can reach sexual maturity around 10 months of age having reached a body weight of about 30 kg (Mauget and Boissit 1987; Mauget and Pepin 1991; Kozdrowski and Dubiel 2004; Flis 2019). These are only indicative data, as climatic conditions and population structure are important in the process of sexual maturation, as young males may start sexual activity earlier in the absence of older individuals competing for females. In a study conducted in France, it was found that in individuals at the age of 10 months, spermatozoa appear in the epididymides, indicating the ongoing process of spermatogenesis. This is accompanied by an increase in testicular weight and testosterone levels (Mauget and Boissit 1987; Mauget and Pepin 1991). Based on the results of our study, it can be observed that this is also associated with an increase in Se content in the testicular tissue. All males were classified as sexually mature on the basis of gonad morphology; however, some of them were juveniles. As already mentioned, the Se content of gonad tissue depends on many factors, such as species, habitat and biogeochemical background. Although most of Poland's regions are Se-poor areas, where its content in soils ranges from 0.04 to 0.64 $\mu g \cdot g^{-1}$ DM, it is introduced into the environment through various routes (Piotrowska 1984; Dębski et al. 2001; Gupta and Gupta 2017). Nowadays, liming of soils with Se preparations has become popular. Foliar supplementation of Se in plants is also used, which consequently increases its bioavailability to animals.

In livestock, Se requirements range from 0.05 to 0.10 $mg \cdot kg^{-1}$ dry weight of feed while Se toxicity occurs at 2–5 $mg \cdot kg^{-1}$ dry weight of feed (Wu et al. 2015). Taking into account the behavior of wild boar involving rutting in soils, it should be noted that part of the Se load enters its digestive tract directly from the soil hence it should be assumed that this species is resistant to deficiencies of this element. Nevertheless, its main source is plant food. As Se exhibits

similar chemical properties to sulfur, it is taken up by plants through pathways responsible for the absorption of the latter element. Transport within plant tissues also takes place using sulfur transporters (Sors et al. 2005; Dumont et al. 2006). Based on the Se content of their tissues, plants are divided into so-called Se hyperaccumulators, secondary Se accumulators, and species that do not accumulate the element. The first group is limited to plants growing in regions exceptionally rich in Se, in the tissues of which its level is above 1,000 mg · kg^{-1} dry weight and these are plants not found in our geographic zone. In contrast, there are plants that do not accumulate Se, the level of which in their tissues does not exceed 100 mg · kg^{-1} dry weight (Galeas et al. 2007; Bodnar et al. 2012). However, it should be noted that plants fertilized with fertilizers containing this element actively defend themselves against its excess in tissues by sequestering Se in the vacuoles of mesophyll cells in leaves. The aforementioned group includes, among others, cereals (Mazej et al. 2008). Selenium deposited in this form is taken up by animals foraging in crop fields, including wild boars. Korol et al. (1992) found that the Se content of cereal grains from Poland is less than 0.1 mg · kg^{-1} while Wesołowski (2006) stated that its content in corn can reach up to 1 mg · kg^{-1}. Given that corn crops in many areas of the country are dominant, wild boars using this type of food cover their metabolic needs for Se, which, combined with zearalenone present in corn, causes an acceleration of the rate of sexual maturation of animals and an increase in reproductive rates. The results obtained in our own study of 0.365 and 0.136 should be considered as reference values of normal Se content in the tissues of male and female wild boars, respectively, from the Mazovian region.

CONCLUSIONS

Based on the study, it can be concluded that the selenium content in the ovaries of Eurasian wild boar females from the Mazovian region of Poland ranges from 0.104 to 0.214 mg · kg^{-1}, while in male specimens it ranges from 0.134 to 0.518 mg · kg^{-1} wet tissue weight. Males are characterized by much greater variation related to sexual activity. Despite the fact that samples were taken from individuals living in areas with selenium deficiencies due to the biogeochemical background, it should be considered that the supply of Se is optimal, which is evident in the population growth dynamics of individuals of this species in the study area. This is related to the increasing acreage of corn cultivation which is a much better source of Se than traditionally grown cereals. In addition, the status of Se supply is also likely to be influenced by the widespread use of mineral fertilizers with Se additives, as well as foliar fertilization of crops with this element.

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ZAWARTOŚĆ SELENU W GONADACH SAMCÓW I SAMIC DZIKA EUROAZJATYCKIEGO (*SUS SCROFA*) Z WOJEWÓDZTWA MAZOWIECKIEGO

Streszczenie. Selen zaliczany jest do pierwiastków śladowych niezbędnych do zachowania procesów metabolicznych zachodzących w organizmie zwierząt, w tym procesów rozrodczych. Celem prowadzonych badań była ocena zawartości selenu w jądrach i jajnikach dzików (*Sus scrofa*), będąca próbą ustanowienia zakresu wartości referencyjnych dla osobników tego gatunku. Stężenie selenu w badanych tkankach oznaczono metodą spektrofluorometryczną po mineralizacji na mokro w mieszaninie HNO_3 i $HClO_4$. Na podstawie uzyskanych wyników stwierdzono, że samce dzików kumulowały istotnie wyższe stężenia Se niż samice. Średnia zawartość tego pierwiastka w jądrach dzików wynosiła 0,32 mg · kg^{-1}, podczas gdy w jajnikach była to wartość na poziomie 0,14 mg · kg^{-1} mokrej masy. Ponadto gonady samców cechowały się większą zmiennością analizowanego parametru w stosunku do prób pozyskanych od samic dzika. Współczynnik zmienności wynosił w ich przypadku niemal 40,9, natomiast wartość współczynnika zmienności dla zawartości selenu w warstwie miąższowej jajników wynosiła 24,9. Na podstawie analizy statystycznej stwierdzono wysoko istotne różnice przy $p \leq 0,01$ w zależności od płci badanych osobników. Mimo że próby pobrano od osobników bytujących w rejonach, na których występują niedobory selenu wynikające z tła biogeochemicznego, należy uznać, że zaopatrzenie w Se jest optymalne, co uwidacznia się w dynamice wzrostu liczebności populacji osobników tego gatunku w badanym rejonie.

Słowa kluczowe: selen, dziki, jajniki, jądra.