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Tadeusz Kośla*, Michał Skibniewski**, Ewa M. Skibniewska*

The effect of gender and age on the sulphur tissue content in free living European bisons

Wpływ płci i wieku na zawartość siarki w tkankach żubra wolno żyjącego

* *Prof. dr hab. Tadeusz Kośla, dr hab. Ewa M. Skibniewska, Department of Biology of Animal Environment, Warsaw University of Life Sciences, Ciszewskiego 8 St., 02-786 Warsaw; e-mail: tadeusz_kosla@sggw.pl*

** *Dr Michał Skibniewski, Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159 St., 02-776 Warsaw*

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Abstract
The presented investigations aimed at determining sulphur status in the European bison free ranging in Białowieża primeval forest. The sulphur concentration in the liver, kidneys, muscle and rib was determined. The material for analyses was obtained from animals eliminated within annual selection. They were grouped according to gender (males, females) and age (calves up to one year of age, animals aged over two years). The sulphur concentrations were determined using the inductively coupled plasma mass spectrometry method in the accredited laboratory. Statistical analysis was carried out using Statistica™ 10 packet. No statistically significant differences in the sulphur content were observed with respect to the sex and age of animals. The mean content of the discussed element in the liver of European bisons amounted to 6.75 g•kg^{-1} of the fresh tissue, 6.82 g•kg^{-1} in kidneys, 6.66 g•kg^{-1} in muscles and 0.44 g•kg^{-1} in ribs.

Streszczenie
Przedstawione badania miały na celu określenie zawartości siarki u żubra wolno żyjącego w Puszczy Białowieskiej. Określono zawartość siarki w wątrobie, nerkach, mięśniach i żebrach. Materiał do analiz uzyskano od zwierząt eliminowanych w ramach corocznej selekcji. Zwierzęta pogrupowano w zależności od płci (samce, samice) i wieku (cielęta do jednego roku życia, zwierzęta w wieku powyżej dwóch lat). Stężenie siarki oznaczano za pomocą metody ICP-MS w akredytowanym laboratorium. Analizę statystyczną przeprowadzono przy użyciu Statistica™ 10. Nie stwierdzono istotnych statystycznie różnic w zawartości siarki w zależności od płci i wieku zwierząt. Średnia zawartość tego pierwiastka wynosiła: w wątrobie żubrów 6,75 g•kg^{-1} w świeżej tkance, w nerkach 6,82 g•kg^{-1}, w mięśniach 6,66 g•kg^{-1} i w żebrach 0,44 g•kg^{-1}.

1. INTRODUCTION

Sulphur is one of the elements widely distributed in nature. It appears in the pure form and in numerous inorganic and organic compounds, e.g. in all living organisms [Rejman-Czajkowska 1980, Zhao et al. 1999]. In organic compounds, sulphur mainly appears in the bivalent form; these compounds may be treated as alkyl or aryl derivatives of hydrogen sulphide [Rejman-Czajkowska 1980]. Sulphur, as an element essential for life, is present in living organisms as an element that is able to appear at various degrees of oxidation as it takes part in numerous oxidation–reduction reactions. In the course of synthesis, plants produce thioamino acids essential for the structure of proteins and also a number of biochemically active organic sulphur compounds [Rejman-Czajkowska 1980, Zhao et al. 1999]. In the last years, resulting from the pro-ecological action aiming at limiting the sulphur supply to the soil, some signals appeared reporting the deficit of this element in the soils of many countries. The problem of sulphur fertilisation especially in the cultivation of cruciferous plants became important for agriculture, including in north-Europe [Kaczor and Zuzańska 2009]. It was noted that sulphur fertilisation of soils that were initially poor in this element results in the increase of crop yield as well as its quality [Hallmark and Brown 1994; Kaczor and Zuzańska 2009]. In another research, the authors observed the inducing of sulphur deficit in plants due to a high light intensity [Resurreccion et al. 2002].

Animals, which are incapable of carrying out the reduction of sulphates and amino acid synthesis obtain the life essential thioamino acids and vitamins which are synthesised by plants. Animals perform the transformation of these compounds. The excess of the organic sulphur compounds is eliminated from the animal organism in the form of sulphates [Rejman-Czajkowska 1980]. In the enzymatic proteins, sulphur amino acids determine the biological activity of the protein particle [Zhao et al. 1999]. Due to the sulphur bridges, enzymatic protein is kept in the proper spatial arrangement. The cleavage of these bridges leads to the enzyme denaturation [Nowacki 1980]. The increase of the sulphur content in feed causes the growth of productivity in ruminants [Wang et al. 2002]. On the basis of the results of an experiment, Oduguwa et al. [2001] suggest that the inorganic sulphur salts may partly replace methionine in the feed ration for broilers. While investigating the copper contamination of the meadow cover, Frøslie and Norheim [1983] observed that the normal sulphur level in plants (0.15%–0.24% d.m.) did not affect copper metabolism, and that sulphur level had no essential effect on the decrease of copper assimilation in sheep. It was noted that the addition of 10 g S/kg d.m. of feed caused the increase of cadmium concentration in the liver and kidneys of cattle and swine, although swine gathered more cadmium in these organs [Anke et al. 1989]. On the other hand, the addition of sulphur decreased the copper content in the

liver and kidneys of cattle and swine. In the opinion of the authors, the decrease of copper concentration in the organs of swine with the increased sulphur dose was incomprehensible. Bremner et al. [1987] observed an antagonistic effect of the sulphur content on the assimilability and metabolism of copper. In another research, Boila and Wittenberg [1990] observed in growing bulls a drastic decrease (by half) of the copper content in the liver in the group of a high sulphur level in feed as compared to a group with a low sulphur level. A high sulphur level in feed also caused an increase of the zinc content in the liver. These authors [Wittenberg and Boila 1988] also observed lower gains in bulls fed a high sulphur level ration. In the investigations *in vivo* and *in vitro* in sheep, the elimination of molybdenum with the addition of sulphur was observed and vice versa – sulphur was eliminated when the molybdenium was added [Ryan et al. 1987]. The addition of sulphur or molybdenum to the feed ration caused the decrease of the copper content in the organism with an increased elimination of copper with urine [Weber et al. 1983].

The aim of the present investigation was the analysis of the effect of gender and age of European bisons on the tissue sulphur content in the living conditions in Białowieża forest.

2. MATERIAL AND METHODS

The analysis included samples of the liver, kidneys, muscle and rib of 20 European bisons obtained during winter selective cull. Samples were collected to sterile plastic bags and stored at a temperature of -20°C until the time of analyses. Animals were divided into groups depending on gender (males [n = 6] and females [n = 14]) and age (calves up to 1 year of age [n = 15] and mature animals of over 2 years [n = 5]). Samples were prepared for analyses by their homogenisation (except the rib) and then they were mineralised in the nitric acid under pressure in the microwave apparatus. In the mineralised samples, sulphur was determined by the inductively coupled plasma mass spectrometry. The determinations were performed in the accredited laboratory and the obtained results were compared using the reference material. Statistical analysis was carried out using the Statistica 10.0 packet. Prior to the analysis, the distribution of variables was checked using the Shapiro–Wilk *W* test. The data did not have the normal distribution, and so in order to state the differences between the investigated groups the nonparametric Kruskal–Wallis test was applied. The significance of differences was tested at the level $P \leq 0.05$.

3. RESULTS AND DISCUSSION

The mean sulphur content in the liver of European bisons amounted to 6.75 g • kg^{-1} of the fresh tissue, 6.82 g • kg^{-1} of the fresh tissue in kidneys, 6.66 g • kg^{-1} of the fresh tissue in muscles and 0.44 g • kg^{-1} of the fresh tissue in the rib. The effect of gender on the sulphur content in the tissues and organs of European bisons is presented in Table 1. The sulphur content was equalised in the groups which is confirmed by low values of standard deviation and also by small differences between the quartile 25 and quartile 75. The analysed sulphur values in the groups of females and males did not differ in a statistically significant way in any tissue.

The results of the analyses of the dependence of the sulphur content on age are presented in Table 2. While analysing the sulphur content in four tissues, no age effect on the content of this element was observed, as it was in the case of analysis of the gender effect. While assessing the sulphur content it can be stated that similar contents of the bioelement are in the organs (liver and kidneys) and in the muscle tissue; however, the sulphur content in the rib is several times lower. Difference in the sulphur content in particular tissues and organs is connected with the fact that this element performs various functions in them. In their research on human hair, Lehn et al. [2011] noted from 43 to 52 mg • g^{-1} S. Some small differences were observed in the sulphur content in time which depended on the amino acids built into the hair, mainly cystein. Sulphur compounds may be the catalyst of biochemical reactions, the carriers of oxygen and electrons. Sulphur amino acids form crosswise bonds between the polypeptide chains conditioning the establishing of the secondary protein structure [Seidler and Maziarka 1980]. The experimental addition of sulphur and molybdenium to the feed ration resulted in a high copper level in the sheep blood serum which was released from the tissues of the organism; such an effect was not observed when sulphur alone was added to the feed [Lamand et al. 1980]. In ruminants, the proper sulphur level in the feed allows for the sulphur amino acid synthesis by the rumen bacteria [Ryan et al. 1987, Lamand 1989]. Most of the feed rations for cattle and sheep contain proper amounts of sulphur [Ammerman and Henry 1985]. It may also be noted in the feed intake by European bisons free living in the forest. Due to the lack of published data, it is impossible to compare of the obtained results concerning the sulphur content in the soft tissues of European bison. Medvedev [1995] investigated the sulphur content, among others, in the bones of the reindeer living in Karelia, in north-west Russia. He observed, like the authors of the present research, the lack of significant differences between the investigated groups. The obtained results concerning the sulphur content in the bones amounted to 0.52 ±0.14 g • kg^{-1} d.m., which means that they were comparable with those obtained in European bisons. The authors did not find any significant effect of gender and age on the sulphur content in bones. A statistically insignificant trend was noted that with the reindeer age the sulphur content in the bones

Table 1. The sulphur content in the tissues and organs of European bisons depending on gender [g • kg^{-1} fresh tissue]

	Liver		Kidneys		Muscle		Rib	
	♂	♀	♂	♀	♂	♀	♂	♀
N	6	14	6	14	5	7	6	14
Mean	6.73	6.76	7.03	6.73	6.93	6.47	0.58	0.38
SD	0.34	0.41	0.56	0.78	0.20	0.70	0.27	0.16
Median	6.80	6.88	7.21	7.18	6.88	6.49	0.47	0.37
Min.	6.24	6.03	6.00	5.31	6.74	5.11	0.34	0.16
Max.	7.23	7.35	7.52	7.48	7.26	7.27	1.02	0.71
Q_{25}	6.48	6.41	6.87	6.18	6.83	6.26	0.40	0.20
Q_{75}	6.85	7.06	7.40	7.33	6.95	6.98	0.76	0.48

Table 2. The sulphur content in the tissues and organs of European bisons depending on age [g • kg^{-1} fresh tissue]

	Liver		Kidneys		Muscle		Rib	
	≤1 year	2≥ years	≤1 year	≥ 2 years	≤1 year	≥ 2 years	≤1 year	≥ 2 years
N	15	5	15	5	10	2	15	5
Mean	6.64	7.09	6.78	6.94	6.57	7.12	0.50	0.25
SD	0.36	0.23	0.76	0.65	0.59	0.19	0.20	0.10
Median	6.68	7.09	7.11	7.33	6.77	7.12	0.48	0.20
Min.	6.03	6.85	5.31	6.00	5.11	6.98	0.19	0.16
Max.	7.23	7.35	7.52	7.48	7.27	7.26	1.03	0.40
Q_{25}	6.24	6.87	6.18	6.51	6.38	6.98	0.37	0.19
Q_{75}	6.92	7.29	7.33	7.36	6.88	7.26	0.54	0.31

decreased. A similar trend can be observed (Table 2) in the investigated European bison rib. Out of the four tissues investigated in the present research, the trend of decreasing the sulphur content with age was only observed in the rib; in the liver, kidneys and muscles the tendency was reverse, as in the coat of European bisons where a significantly higher sulphur content was observed in European bison aged over 2 years [Kośla et al. 2011]. Similar results were obtained in the investigated cat's hair by Kośla et al. [2007] – the hair of cats older than 2 years contained significantly more ($p \leq 0.05$) sulphur than the hair of younger cats.

4. CONCLUSIONS

The mean sulphur content in the liver of European bisons amounted to 6.75 g • kg^{-1} of fresh tissue, in kidneys 6.82 g • kg^{-1} of fresh tissue, in muscles 6.66 g • kg^{-1} of fresh tissue and in the ribs 0.44 g • kg^{-1} of fresh tissue. The sulphur content was equalised in the groups which is confirmed by low values of standard deviation and also small differences between the quartile 25 and quartile 75. In conclusion, while analysing the sulphur content in four tissues, no effect of gender or age on the content of this macroelement was observed.

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