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Hair Concentration of Selenium in European Bison in Relation to Sex and Age, with Regard to Liver and Kidney Se Levels

Tadeusz KOŚLA, Ewa Małgorzata SKIBNIEWSKA, Michał SKIBNIEWSKI, Marta Małgorzata KOŁNIERZAK, Iwona LASOCKA, and Hubert KMIEĆ

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Original article

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The average selenium levels measured in the analyzed European bison individuals (N = 22) were as follows: 0.137±0.041SD µg/g dry weight (DW), 0.175±0.032 and 1.004±0.239 µg/g wet weight (WW) in hair, liver and kidneys, respectively. Broken down by sex, the average hair selenium concentration was 0.140±0.046 and 0.134±0.037 µg/g DW in males and females, respectively. No significant differences in hair selenium content were found in relation to sex. As far as age is concerned, hair selenium content was 0.142±0.042 in calves; in the group of bison over 2 years of age, however, lower levels of selenium (0.126±0.040 µg/g DW) were recorded. There were no significant differences between the age groups in terms of selenium content. Based on our research and current literature data, we could presume selenium deficiency in the studied animals.

Key words: age, selenium, sex, hair, European bison.

Tadeusz KOŚLA, Ewa Małgorzata SKIBNIEWSKA, Marta Małgorzata KOŁNIERZAK✉, Iwona LASOCKA, Hubert KMIEĆ, Department of Animal Environment Biology SGGW, Ciszewskiego 8, 02-787 Warsaw, Poland.
E-mail: marta_kolnierzak@sggw.pl
Michał SKIBNIEWSKI, Department of Morphological Sciences SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland.

Selenium (Se) is an essential trace element for all living organisms. Although so important, selenium may be toxic to natural ecosystems due to its bioaccumulation potential (TAN *et al.* 2016). The mineral is found in nearly all rocks, soils, waters, as well as in the atmosphere. Its distribution in the Earth's crust, however, is uneven, with regions of selenium deficiency and areas showing locally excessive concentrations of its compounds (SCHRAUZER 2004). As an essential nutrient, selenium participates in a number of physiological processes including the regulation and functioning of the immune system.

Bound by selenoproteins, the element participates in the regulation of oxidative stress, redox mechanisms and other key cellular processes associated with innate and adaptive immune responses (DALGAARD *et al.* 2018). Selenium is an antioxidant whose activity is believed to be associated with many strong antioxidSeptember 30, 2019ative enzymes. It protects tissues against free radicals; hence, organisms with a deficiency of this trace element will be exposed to diseases ensuing oxidative stress, such as cancer, but also to heart diseases (OKOKO 2018).

Since selenium compounds have anti-carcinogenic properties, we need non-invasive techniques to monitor the levels of this element in the body. It has been suggested that hair and nails are appropriate biomarkers for Se status in the body (SALBE *et al.* 1993, JØRGENSEN 2000). CHRISTODOULOPOULOS *et al.* (2003) claim that the concentration of selenium in the coat hairs of cattle is a good indicator that may reveal either deficiencies or toxic levels of selenium in the body. Hair selenium level is a good indicator of body selenium level, especially in the long run.

oxidative cell damage. Glutathione peroxidase catalyzes the decomposition of produced hydrogen peroxide and fatty peroxides. Selenium plays an important role in cell-mediated immunity through the activity of glutathione peroxidase as a biologically active component of leukocytes. The element also directly affects large T lymphocytes by producing lymphokines. In terms of humoral immunity, selenium has an effect on the stimulation of specific antibacterial and antifungal antibodies (KONDRACKI & BEDNAREK 1996). The consequences of selenium deficiency in animals may involve muscle degeneration and diarrhea in young cattle, as well as an increase in disseminated hepatic necrosis in beef cattle. In cows, a deficiency of selenium may lead to fetal developmental abnormalities and death, retained placenta, and a general impairment of reproduction abilities. In addition, the mineral has detoxification properties in relation to heavy metals (KOŚLA 1999).

The European bison *Bison bonasus* (L., 1758) is a species saved by man from extinction in captivity and returned to its natural habitat. Although the restitution of the European bison has been successful (its population in Poland is growing and mortality remains low), the species needs attention (KRASIŃSKA & KRASIŃSKI 2004). Due to observed instability, including a lack of natural selection and a high level of hybridization, the IUCN (International Union for Conservation of Nature) classifies the European bison as an endangered species (POLISH RED DATA BOOK OF ANIMALS 2001).

Selenium has an especially meaningful impact on ruminant animals as selenoenzymes play an important role in reproductive processes and can affect population size. The aim of this study was to determine the selenium levels of selected tissues of the European bison and to relate them to the sex and age of the animals. Knowledge about selenium level in the hair and soft tissues of European bison is sparse. Further research is required to define the nutritional requirements of healthy bison.

Materials and Methods

The material consisted of samples collected from clinically healthy, free-ranging European bison harvested in the Białowieża Forest during the 2002/2003 winter season. Hair samples were taken from the backs of 22 individuals, 11 males (m) and 11 females (f), 14 of which were calves up to 1 year in age (m = 8; f = 6) and 8 individuals at age of 2 years or older (m = 3; f = 5). Soft tissue samples of the liver (N = 10) and kidneys (N = 11) were collected from parts of the same animals. There were no means to collect soft tissue samples from all subjects. The data on selenium content in the liver and kidneys were used only to compare the results to hair selenium levels, due to the small number of samples. Hair samples were cleaned from fat in 70% alcohol (a Soxhlet extractor), then washed in distilled water and rinsed three times in double-distilled water.

Selenium analysis of the bison tissue samples was conducted in 2015. Selenium measurements were carried out with fluorescence spectroscopy according to GRZEBUŁA and WITKOWSKI (1977). The concentration was measured at wavelength 518 nm, with excitation wavelength 376 nm, using the RF-5001 PC spectrofluorophotometer (Shimadzu). The accuracy of the measurement procedure was verified against the level of selenium in the reference material NCS ZC 71001 (China National Analysis Center). Selenium concentrations measured against the certified reference material are shown in Table 1. The outcomes of the analysis represented 92% of the reference value.

Statistical calculations were performed using Statistica 12.0^{TM} (StatSoft Inc) procedures. Given the Shapiro-Wilk's W test showed the sample population not to be normally distributed, non-parametric analyses were used. To determine any significant difference, the U Mann-Whitney test was performed ($P \leq 0.05$).

Results

Table 2 reveals a similar selenium content of all three types of bison tissues. The average selenium level in hair was 0.137µg/g DW, with an average of 0.140 µg/g DW and 0.134 µg/g DW in males and females, respectively, revealing no statistically significant difference in relation to sex (Table 3). The average selenium level in soft tissues

Table 1

Reference material

Reference material: Certificate of Certified Reference Material NCS ZC 71001 Beef liver			
Se	Certified concentration	Measured concentration	% of recovery
	0.56 µg/g DW	0.516 µg/g DW	92%

Table 2

Selenium in hair (μg/g DW), liver and kidneys (μg/g WW) of bison

	N	mean±SD	median	min	max	lower quartile	upper quartile
hair	22	0.137±0.041	0.133	0.077	0.245	0.109	0.162
liver	10	0.175±0.032	0.161	0.142	0.226	0.148	0.214
kidneys	11	1.004±0.239	0.967	0.600	1.371	0.809	0.809

Table 3

Selenium content in bison hair according to sex (μg/g DW)

Sex	N	mean±SD	median	min	max	lower quartile	upper quartile
male	11 (c=8; o=3)	0.140±0.046	0.132	0.091	0.245	0.109	0.162
female	11 (c=6; o=5)	0.134±0.037	0.138	0.077	0.191	0.103	0.169

c = calves (up to 1 year old); o = older (over 2 years).

was 0.175 and 1.004 μg/g WW for liver and kidneys respectively (Table 2).

The average selenium concentration in hair was 0.142 μg/g DW in calves (up to 1 year of age), and 0.126 μg/g DW in individuals over 2 years of age (Table 4). No significant difference was found between age groups.

Table 5 contains data collected from other studies to compare with our results.

Discussion

SCHRAUZER (2004) cites a range of papers reporting a direct relationship between the selenium content in human hair and blood; hence, hair may be considered a good selenium level marker. The author (SCHRAUZER 2004) also reports that selenium measured in animal coat hair has been successfully used as an indicator of either deficiency or a toxic level of the element in animals. Similar relationships were reported by CHRISTODOULOPOULOS *et al.* (2003). In a controlled-regime feeding trial on goats, ANGELOW (1987) studied a control group (524 μg Se/kg of feed) and a treatment, Se-deficient group (below 38 μg Se/kg feed). The hair of the control-group goats contained, on average, 0.35 μg/g DW of selenium, whereas in the Se-deficient feeding group, the level of selenium was 0.15 μg Se/g DW (Table 5). The author observed significant differences in hair selenium levels at $P<0.001$. Hair selenium content in European bison from our results was similar to those mentioned in the goats on a Se-deficient diet. Two hypotheses can be put forward at this point. One, that the low content of Se is species-specific, or two, that we have a case of Se deficiency in the sampled

Table 4

Selenium content in bison hair according to age (μg/g DW)

Age	N	mean±SD	median	min	max	lower quartile	upper quartile
calves (up to 1 year of age)	14 (m=8; f=6)	0.142±0.042	0.134	0.091	0.245	0.110	0.169
older (over 2 years)	8 (m=3; f=5)	0.126±0.040	0.113	0.077	0.191	0.096	0.156

m = male; f = female.

Table 5

Selenium levels for wild and domestic ruminants (µg/g DW or WW)

Species	Locality	N	Concentration	Indications	Reference
Hair (µg/g DW)					
Cattle (dairy cow)	Greece	400	0.06-0.23 [a]	deficient	CHRISTODOULOPOULOS *et al.* 2003
			>0.25 [a]	healthy	
			0.232±0.155[c]	white hair	
			0.371±0.1735[c]	black hair	
Cattle	–	–	0.06-0.23 [a]	deficient	PULS 1994
			0.23-0.50 [a]	marginal	
			0.50-1.32 [a]	adequate	
			1.4-45.0 [a]	toxic	
Cattle (adult beef cow)	Canada	19	0.17±0.009 [b]	deficient	HIDIROGLOU *et al.* 1965
Cattle (1 month old beef calves)		26	0.19±0.063[b]		
Cattle (adults dams)		–	>0.25 [a]	dams giving birth to healthy calves	
			<0.12 [a]	dams giving birth to wmd calves	
Goat (adults)	Czech Republic	5	0.174±0.038[c]	control (healthy)	PAVLATA *et al.* 2011
	Germany (Jena)	37	0.35±0.02[c]	control (healthy)	ANGELOW 1987
		18	0.15±0.03[c]	deficient	
		9	0.353±0.042[c]	control (healthy); day 120 of the experiment	
		13	0.333±0.096[c]	control (healthy); day 210 of the experiment	
		15	0.377±0.114[c]	control (healthy); day 300 of the experiment	
		8	0.183±0.079[c]	deficient; day 120 of the experiment	
		5	0.129±0.046[c]	deficient; day 210 of the experiment	
		5	0.131±0.017[c]	deficient with treatment day 300 of the experiment	
Goat (newborn kids)	Germany (Jena)	4	0.331±0.037[c]	from healthy dams	ANGELOW 1987
		6	0.158±0.010[c]	from Se deficiency dams	
Sheep	–	–	0.03-0.30 [a]	deficient	PULS 1994
			0.06-0.20 [a]	marginal	
			0.20-4.00 [a]	adequate	
Rocky mountain elk	Washington, USA	8	0.110±0.62[c]	–	FIELDER 1986
Mule deer	Washington, USA	10	0.150±0.08[c]	–	FIELDER 1986
	California, USA	143	0.16 [f]	overall	ROUG *et al.* 2015
		33	0.21 [f]	male	
		108	0.15 [f]	female	
		36	0.15 [f]	≤1 year old	
		37	0.16 [f]	2-3 years	
		70	0.16 [f]	≥4 years	
Washington mountain goat	Washington, USA	6	0.070±0.021[c]	–	FIELDER 1986
Moose	Alaska, USA	5	0.9±0.38[c]WW	male	O'HARA *et al.* 2001
		23	0.50±0.20[c]WW	female	

Table 5 – cont.

Species	Locality	N	Concentration	Indications	Reference
Alpacas	Czech Republic	54	0.48±0.24[c]	overall	Holasova *et al.* 2017
		18	0.47±0.21[c]	male	
		36	0.48±0.26[c]	female	
Llamas	Czech Republic	23	0.25±0.14[c]	overall	Holasova *et al.* 2017
		7	0.23±0.12[c]	male	
		16	0.25±0.15[c]	female	
Liver (µg/g WW)					
Cattle	–	–	0.02-0.17[a]	deficient	Puls 1994
			0.12-0.25[a]	marginal	
			0.25-0.50[a]	adequate	
			0.75-1.25[a]	high	
			1.25-7.00[a]	toxic	
Sheep	–	–	0.01-0.10[a]	deficient	Puls (1994)
			0.15-0.25[a]	marginal	
			0.25-1.5[a]	adequate	
			2.00-10.0[a]	high	
			15.0-30.0[a]	toxic	
	Nevada, USA	38	0.173±0.029[b]	high incidence of capture myopathy	Cox 2006
Deer	–	–	0.02-0.18 [a]	deficient	Puls 1994
			0.25-0.46 [a]	adequate	
			2 [a]	toxic	
Red deer	Norway	52	0.08±0.03[c]	calves	Vikøren *et al.* 2005
		80	0.10±0.05[c]	youth	
		112	0.13±0.11[c]	adults	
		245	0.11±0.09[c]	overall	
		5	0.20[d]	–	Frøslie *et al.* (1984)
	Poland	23	0.095±0.018[b]	overall	Pilarczyk *et al.* 2011a
		10	0.093±0.025[b]	male	
		13	0.097±0.027[b]	female	
		73	0.36±0.32[c] DW (0.091-1.671[a] DW)	–	Pilarczyk *et al.* 2009
		20	0.063±0.033[c]	healthy	Jarzyńska & Falandysz 2011
		177	0.084±0.005[b]	–	Nowakowska *et al.* 2015
	Croatia	52	0.241±0.053[b]	–	Lazarus *et al.* 2008
Mule deer	California, USA	54	0.15[f] (0.04-1.60[a])	overall	Roug *et al.* 2015
		27	0.22[f]	male	
		26	0.14[f]	female	
	Washington, USA	10	0.121±0.057[c]	–	Fielder 1986
	South Dakota, USA	38	0.64±0.05[b]	healthy	Zimmerman *et al.* 2008
Roe deer	Poland	11	0.07±0.02[c]	0.5-1 age (years)	Pilarczyk *et al.* 2011b
		21	0.06±0.03[c]	1.5-2 age (years)	
		24	0.07±0.03[c]	3 age (years)	
		18	0.06±0.02[c]	4-5 age (years)	
		74	0.06±0.03[c]	overall	
		96	0.62±0.57[c] DW (0.124-3.617[a] DW)	–	Pilarczyk *et al.* 2009
		23	0.06±0.03[c] (0.02-0.17[a])	–	Tomza-Marciniak *et al.* 2010
		164	0.088±0.002[b]	–	Nowakowska *et al.* 2014
	Germany	11	0.27±0.07[c]	–	Humann-Ziehank *et al.* 2008
	Norway	280	0.67±0.59[c] DW	–	Vikøren *et al.* 2011

Table 5 – cont.

Species	Locality	N	Concentration	Indications	Reference
White-tailed deer	Michigan, USA	8	0.24±0.02[b]	–	BRADY *et al*. 1978
	South Dakota, USA	42	0.81±0.05[b]	healthy	ZIMMERMAN *et al.* 2008
	Nova Scotia	54	0.6-4.0[a] DW	–	POLLOCK 2005
Rocky Mountain Elk	Washington, USA	9	0.070±0.066[c]	–	FIELDER 1986
Moose	Sweden	2080	0.250±0.288[c]	–	GALGAN & FRANK 1995
	Norway	118	<DL-10.9[a] DW	calves	VIKØREN *et al.* 2011
		131	<DL-16.1[a] DW	youth	
		164	<DL-13.3[a] DW	adults	
		422	1.87±2.75[c] DW	–	
		–	0.42[d]	–	FRØSLIE *et al.* 1984
	Nova Scotia	48	0.73[e] DW (0.29-3.57[a] DW)	–	POLLOCK 2005
Reindeer	Norway	73	0.87±0.26[c] DW	–	VIKØREN *et al.* 2011
Washington mountains goat	Washington, USA	10	0.022±0.020[c]	–	FIELDER 1986
European bison	Poland	19	0.17±0.02[c]	closed breeding	DĘBSKA 2005
		61	0.15±0.03[c]	open breeding	
		43	0.146±0.026[c]	calves	
		15	0.166±0.015[c]	youth	
		22	0.175±0.030[c]	adults	
		27	0.161±0.029[c]	male	
		53	0.155±0.028[c]	female	
		17	0.035[f]	individuals kept in the enclosure	DURKALEC *et al.* 2018
		13	0.027[f]	free-ranging herd	
		18	0.031[f]	male	
		12	0.037[f]	female	
	Białowieża, Poland	25	0.042[f] (<0.001-0.113[a])	both sexes, closed and open breeding	
	Smardzewice, Poland	5	0.023[f] (0.013-0.026[a])	both sexes, closed and open breeding	
Kidney (µg/g WW)					
Cattle	–	–	0.18-0.40[a]	deficient	PULS 1994
			0.40-1.00[a]	marginal	
			1.00-1.50[a]	adequate	
			2.00-2.50[a]	high	
			2.50-5.00[a]	toxic	
Sheep	–	–	0.046-0.6[a]	deficient	PULS 1994
			0.7-1.1[a]	marginal	
			0.9-3.0[a]	adequate	
			4.0-6.0[a]	high	
			6.0-15.0[a]	toxic	
Deer	–	–	<0.8[a]	deficient	PULS 1994
			0.6-1.10[a]	adequate	
Red deer	Poland	177	0.621±0.029[b] (0.123-3.027[a])	–	NOWAKOWSKA 2013
		73	2.72±0.88[c] DW	–	PILARCZYK *et al.* 2009
Roe deer	Poland	74	0.41±0.19[c]	–	PILARCZYK *et al.* 2011b
		96	2.99±1.78[c] DW	–	PILARCZYK *et al.* 2009
		23	0.33±0.13[c] (0.14-0.61[a])	–	TOMZA-MARCINIAK *et al*. 2010
		164	0.503±0.015[b]	–	NOWAKOWSKA *et al*. 2014

Species	Locality	N	Concentration	Indications	Reference
Moose	Nova Scotia	21	2.9^{e} DW ($1.8\text{-}5.3^{a}$ DW)	–	POLLOCK 2005
European bison	Poland	17	1.43 ± 0.37^{c}	closed breeding	DĘBSKA 2005
		46	1.07 ± 0.32^{c}	open breeding	
		29	0.96 ± 0.28^{c}	calves	
		13	1.26 ± 0.31^{c}	youth	
		21	1.39 ± 0.36^{c}	adults	
		20	1.16 ± 0.36^{c}	male	
		43	1.17 ± 0.37^{c}	female	

WW – wet weight; DW – dry weight; DL – detection limit; a – range; b – $\bar{x}\pm$SEM; c – $\bar{x}\pm$SD; d – arithmetic mean; e – geometric mean; f – median; „–” – no data

free-ranging bison. There may be an analytic factor, too, as ANGELOW (1987) used the hydration method of selenium determination, whereas we used fluorometry. CHRISTODOULOPOULOS *et al.* (2003) report that the selenium level of bovine hair from a healthy population, ranges above 0.25 μg/kg DW, while values ranging between 0.06 and 0.23 μg/kg DW show deficiency. The reference data of PULS (1994) show clear species differences for cattle, i.e. a species similar to bison, and in relation to these data our values indicate a selenium deficit (Table 5).

PAVLATA *et al*. (2011) reported that in the hair of clinically healthy goats, selenium levels were 0.174 μg/g DW, whereas HIDIROGLOU *et al.* (1965) found that 0.17 μg/g DW of selenium in beef cow's hair indicated a deficiency. These studies highlight the differences related to selenium demand between species. Under deficiencies resulting from Keshan disease (congestive cardiomyopathy caused by i.e. a dietary deficiency of selenium), hair selenium concentrations are lower than 0.12 μg/g DW (LEWANDER 1986), i.e. below those measured in our study (Table 2).

ANGELOW (1987) carried out a long-term study of selenium levels in the coat hair of goats. On day 120 of the experiment he found 0.353 μg/g DW of selenium in the control group, and 0.183 μg/g DW, $P<0.001$, in goats with a selenium deficiency. On day 210 of the experiment, the author measured 0.333 μg/g DW of selenium in the control group and 0.129 μg/g DW of selenium in the deficient group ($P<0.001$). After 300 days of the experiment, the selenium content in the control goats' hair was 0.337 μg/g DW, and in goats with Se deficiency with introduced treatment, was 0.131 μg/g DW ($P<0.001$). Studies show that with adequate supplementation (90 days), selenium levels can be seen to increase in hair. ANGELOW (1987) also studied the content of selenium in hair in newborn goat kids – the offspring of the control group and the offspring of Se-deficient group dams. The results of Se content were 0.331 and 0.158 μg/g DW ($P<0.001$) for the control and deficient group, respectively. The content of Se in the hair of young goats was also examined before 56 days of age. At 56 days of life, the average selenium content was 0.353 and 0.124 μg/g DW in the control group and the deficient group, respectively ($P<0.001$). The kids from the deficient group were born with a Se deficiency of about half of the content in relation to its content in healthy kids' hair. Over the first 56 days of life, the content of Se in hair decreased to 1/3 of the content in the healthy (control group) young goats (ANGELOW 1987).

In a study by HOLASOVA *et al*. (2017) on two South American camelids (llamas and alpacas), the mean hair selenium content in llamas was 0.25 μg/g DW, and 0.48 μg/g DW in alpacas. In this study, significant differences were found in relation to age or sex for both species.

On the basis of the obtained results (Table 2) it can be concluded that the content of selenium in the examined tissues decreases as follows: kidneys > liver > hair. ANGELOW (1987) achieved similar relationships in his studies.

In the analysis of BENEMARIYA *et al.* (1993), selenium liver content in goats was 0.427 μg/g WW, whereas ZIMMERMAN *et al.* (2008) found 0.81 μg/g WW in deer liver (Table 5). These were much higher values than those in our research (Table 2). DĘBSKA (2005) obtained the same selenium values in the liver of adult European bison as we did in our research, however their values for calves were lower (Table 5). The obtained values were lower in comparison with the standards for cattle assembled by PULS (1994), these values were in the range of clinical deficiency for cattle and red deer (PULS 1994) (Table 5).

Nowakowska (2013) investigated the content of selenium in the liver of wild animals in Poland. An average 0.088 μg/g WW and 0.084 μg/g WW was found in roe deer and red deer, respectively. In each case, the reported values were lower than our results (Table 2). Nowakowska *et al.* (2015) reported, that there are deficits of selenium in the environment of Poland. Flueck *et al*. (2012), who compared a large number of individuals, reported the following data range on the selenium levels in the liver of wild animals: from 0.05 μg/g WW to 0.62 μg/g WW. Our data remain within the lower part of the range (Table 2).

The selenium levels of kidneys (Table 2) in our study's population were similar to those found in Dębska's study of European bison (2005), which remained in the lower range of the values considered by Puls (1994) as a normal level for cattle (1.0-1.5 μg/g WW of kidney) (Table 5). Słupczyńska *et al.* (2009) measured 1.59 μg/g WW of selenium in sheep kidneys which is 58% higher than our results. Nowakowska (2013) also studied kidney selenium levels and reported 0.503 μg/g WW and 0.621 μg/g WW in the kidneys of, respectively, roe deer and red deer, which are lower in relation to our results for European bison kidneys. Contrary to data presented by Nowakowska (2013), we found no correlation in the selenium concentration of the liver and kidneys of the studied animals. Pilarczyk *et al.* (2011b) emphasizes the lack of a soil selenium map of Poland, nonetheless there are results of several studies which indicate selenium deficiency in Poland.

Considering the small number of samples, clear statements are difficult to make, however based on our results and with comparison to literature data, selenium deficiency of the analyzed bisons can be implied.

Conclusions

1. The content of selenium in the hair of European bison was similar to those in the hair of other wild species.

2. The content of selenium in the liver was lower compared to reference data, remaining in the range of clinical deficiency for cattle and red deer (Puls 1994). The resulting European bison liver selenium levels were lower compared to the literature data for wild, livestock, and laboratory animals.

3. Kidney selenium levels were similar to those reported in the literature on European bison and remained within the reference range for cattle, being however lower compared to those reported by other authors both for wild and experimental animals.

4. The selenium concentrations in European bison, if compared to literature data, reveal clear inter-specific variability.

5. Based on our research and current literature data we can presume that the studied animals were selenium deficient.

Author Contributions

Research concept and design: T.K.; Collection and/or assembly of data: M.S., H.K.; Data analysis and interpretation: T.K., E.M.S., I.L., H.K.; Writing the article: T.K., M.M.K.; Critical revision of the article: E.M.S., M.S.; Final approval of article: M.M.K.

Conflict of Interest

The authors declare no conflict of interest.

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