THE IRON CONTENT IN ORGANS OF FREE RANGING EUROPEAN BISON FROM THE BIAŁOWIEŻA HERD*

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Abstract

The aim of the study was to determine iron status in chosen organs of the European bison free ranging in Białowieża Primeval Forest. The material for analyses was obtained from animals eliminated during annual selection. Segments of liver, kidney, muscle, rib, and hoof were collected. Animals were divided depending on gender (males and females) and age (calves up to 1 year and animals older than 2 years). Mean iron concentration in liver was 263.59 mg · kg⁻¹ fresh tissue. The iron content was significantly higher in the group of animals older than 2 years ($P \leq 0.05$). The average content of iron in kidneys amounted to 156.70 mg · kg⁻¹ fresh tissue. The average iron content in muscles amounted to 79.95 mg · kg⁻¹ fresh tissue. Similarly to the liver samples a statistically significant difference ($P \leq 0.05$) was demonstrated depending on age. The average iron content in ribs and in the horn of the hoof wall of all European bison amounted to 38.90 mg · kg⁻¹ fresh tissue and 47.87 mg · kg⁻¹ dry matter, respectively. No statistically significant differences in the iron content were observed depending on gender.

Key words: European bison, iron status

Iron in humans and animals is considered as microelement indispensable for functioning of living cells and it is the component of all tissues and organs of mammals (Alsen et al., 2001; Jurczyk, 2000). Its main part, in an active form, is present in hemoglobin and myoglobin whereas reserve iron in a form of hemosiderin, transferrin and ferritin is stored in liver and spleen (Kośła, 1989; Kabata-Pendias and Pendias, 1999). Iron is a basic component of particles, transporting and storing oxygen, as well as of many enzymes which require energy generation; it also serves for pro-

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duction of intermediate metabolites and plays an important role in specific as well as non-specific immunological processes of organism (Andrews, 2002; Bednarek and Kondracki, 1997; Ganz, 2007). Iron is necessary for neutrophils in their bactericidal activity for production of hydroxide radicals (Bednarek and Kondracki, 1997; Kośła and Skibniewska, 2010); simultaneously, the toxic effect of free iron is ascribed to formation of reactive free radicals (Alsen *et al.*, 2001; Chung and Wessling-Resnick, 2003; Kleczkowski *et al.*, 2004). It was found that excess of iron predisposes to development of intracellular pathogens and blood pathogens and, even in the case of its small excess, the resistance of the host to diseases becomes lowered (Kośła, 1999; Collins, 2003; Ganz, 2003).

The organism has a system which maintains iron homeostasis because its excess as well as its deficiency is connected with cellular dysfunction. Regulation of iron absorption from intestines and recovery from old erythrocytes is of key importance for maintaining the equilibrium in iron management (Leong and Lönnerdal, 2004; Nemeth *et al.*, 2003; Nicolas *et al.*, 2001). Liver plays a fundamental role in maintaining iron homeostasis; it is the site for the storage of the overload of the discussed element (Zhang *et al.*, 2004). The liver is the place where hepcidin is produced in hepatocytes; it is a hormone which plays the main role in communication of iron reserves in organism with intestinal cells, responsible for its absorption and thus, it maintains iron homeostasis; it acts as a strong negative regulator of absorption and mobilization of the discussed element (Kushner *et al.*, 2001; Weiss, 2002; Andrews and Roy, 2003; Nemeth *et al.*, 2003; Ganz, 2003; Mazur *et al.*, 2003; Arnold *et al.*, 2008). On the grounds of the recent studies, it is known that hepcidin is a central hormone, regulating iron level and, at the same time, a mediator of innate immunity in humans and animals (Ganz, 2003; Nemeth *et al.*, 2003; Kośła and Skibniewska, 2010). Lack of hepcidin expression caused overloading with iron; the excess of the mentioned expression caused anemia connected with iron deficiency. In animal nutrition with iron-poor feed, the level of hepcidin was lowered and it increased in the case of iron-rich diet (Mazur *et al.*, 2003; Leong and Lönnerdal, 2004; Kośła and Skibniewska, 2010). It was revealed that in the case of iron-poor diet, its absorption in gastrointestinal system was increased; also, the concentration of iron transporters increased. The discussed increase was correlated with decline of liver hepcidin mRNA. In further experiments, it was shown that always in the case of a high level of hepcidin mRNA, the level of iron transporters was low and vice versa (Ganz, 2007).

The aim of the study was to determine the status of iron in the organs of the European bison, the greatest non-domesticated mammal of Europe, employing the analysis of selected organ samples collected from individuals of both genders at different age.

Material and methods

The analysis included specimens of liver, kidneys, muscles, ribs and hooves from 20 bison aged from 5 months to 5 years, eliminated during annual winter selection.

The animals were classified in groups, according to gender (males [n 6] – females [n14] and according to age (calves up to 1 year of age [n15] – mature animals older 2 years [n5]). The samples were collected from the animals living in the Polish part of Białowieża Primeval Forest (52°35'–52°55'N, 23°30'–24°00'E). Segments of the liver, kidneys, muscles, ribs and hoof wall were collected into sterile plastic bags, cooled and stored at –20°C. Liver samples were collected from the left lobe. Renal samples were collected in such a way that they contained both the cortical and medullary part. In the case of muscles the collected samples comprised segments of the diaphragm crus. Rib samples were collected from dorsal part of the 13th or 14th rib. In the case of hoof the collected samples contained segments of the abaxial part of the horn wall. Tissue samples were prepared for the analyses by means of homogenization (except the samples of the ribs and hooves), collecting the weighed amount of 0.5–1.0 g into teflon containers. Mineralization of the material was performed in concentrated (67%) nitric acid (Merck, Darmstadt, Germany); the ratio of sample weight: acid weight was equal to 1:50, e.g. weight of the sample amounted to 0.8 g and it was treated with 40 g of acid; under pressure in the microwave apparatus (mineralization was conducted in microwave furnace, CEM-81 D, Matthews, NC, USA). A segment of 0.8–1.0 g was cut out from the rib, weighed and mineralized as above. The specimens of the horn of hoof wall were burnt in muffle furnace (LM 212.11 VEB Elektro Bad Frankenhausen, Germany) at 450°C and ash was transferred quantitatively to measuring flasks with a bidistilled water acidified to 2.5% HCl. In the case of liver, kidneys, muscles and rib their values were presented in relation to wet weight of the examined samples. Iron content in hooves was expressed in dry matter of the examined samples. In the mineralized samples, iron content was determined by the method of inductively coupled plasma atomic emission spectrometry (ICP-OES) and in the samples of hoof, it was performed using mass spectrometry (ICP-MS). The determinations were carried out in an accredited laboratory and the results obtained were compared using the reference material.

Statistical evaluation was performed with Statistica for Windows (StatSoft, Inc., Kraków, Poland). The data were submitted in the form of arithmetic means and standard deviation. In calculations, median was also employed and the upper and lower quartiles were determined. Statistical analysis of the results obtained was conducted based upon one-way analysis of variance, ANOVA module. The age of animals and their gender were used as a grouping variable. For statistical comparisons between averages in the particular groups, the Tukey's test of the honestly significant difference (HSD) was used, adopting the significance level for $P \leq 0.05$ and $P \leq 0.01$. The iron values were expressed as $\text{mg} \cdot \text{kg}^{-1}$ wet weight for liver, kidney, muscle and rib and as $\text{mg} \cdot \text{kg}^{-1}$ dry matter for hooves.

Results

Iron content in liver of the European bison according to gender and age is presented in Table 1. The mean iron level amounted to $263.59 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue. There were found significant differences ($P \leq 0.05$) depending on age. Iron concentra-

tion in liver significantly increased with age. Concentration of that element in the kidney (Table 2) averaged $156.70 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue. Statistical test did not reveal any significant differences depending on the sex and age of the animals. The level of iron in the muscles of the European bison (Table 3) averaged $79.95 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue. Statistically significant differences ($P \leq 0.05$) were found in muscles as well as in kidneys and liver (only trend) depending on age; higher level of iron was recorded in older animals. Mean iron contents in the ribs (Table 4) amounted to $38.90 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue. In the case of calves (up to 12 months of age), the arithmetic mean amounted to 41.53, while in older bisons (over 2 years of age) up to $31.00 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue. Similarly to the kidney samples, statistical test did not reveal any significant differences depending on the gender and age of the animals. The results of iron content in the horn of hoof wall are presented in Table 5. The mean concentration of iron in this tissue was $47.87 \text{ mg} \cdot \text{kg}^{-1}$ of dry matter. As can be seen from the table, no statistically significant differences in the content of that element depending on the animal sex and age were observed.

Table 1. The content of iron in the liver of European bison according to gender and age ($\text{mg} \cdot \text{kg}^{-1}$ fresh tissue)

Parameter	(n)	Average	SD	Q25	Median	Q75
Total number of animals	20	263.59	75.93	215.62	250.50	307.88
Males	6	246.88	72.77	225.75	247.50	315.00
Females	14	270.75	78.77	205.50	259.12	303.00
Calves up to 1 year of age	15	237.30 a	50.63	204.75	243.75	275.25
Animals older than 2 years	5	342.45 a	89.71	303.00	315.00	327.75

a – differences statistically significant at $P \leq 0.05$.

SD – Standard Deviation, Q25 – Lower Quartile, Q75 – Upper Quartile.

Table 2. The content of iron in the kidney of the European bison according to gender and age ($\text{mg} \cdot \text{kg}^{-1}$ fresh tissue)

Parameter	(n)	Average	SD	Q25	Median	Q75
Total number of animals	20	156.70	43.07	136.25	154.50	174.75
Males	6	146.00	36.65	138.00	152.75	154.50
Females	14	161.29	46.03	134.50	158.00	179.50
Calves up to 1 year of age	15	150.93	36.32	134.50	151.00	168.50
Animals older than 2 years	5	174.00	60.77	154.50	170.00	201.50

SD – Standard Deviation, Q25 – Lower Quartile, Q75 – Upper Quartile.

Table 3. The content of iron in the muscles of the European bison according to gender and age ($\text{mg} \cdot \text{kg}^{-1}$ fresh tissue)

Parameter	(n)	Average	SD	Q25	Median	Q75
Total number of animals	20	79.95	33.21	55.50	73.12	104.62
Males	6	61.50	25.93	45.75	55.50	75.00
Females	14	87.86	33.61	63.00	79.12	105.75
Calves up to 1 year of age	15	69.55a	25.11	52.50	63.00	96.00
Animals older than 2 years	5	111.15 a	37.54	77.25	105.75	136.50

a – differences statistically significant at $P \leq 0.05$.

SD – Standard Deviation, Q25 – Lower Quartile, Q75 – Upper Quartile.

Table 4. The content of iron in the rib of the European bison according to gender and age (mg · kg⁻¹ fresh tissue)

Parameter	(n)	Average	SD	Q25	Median	Q75
Total number of animals	20	38.90	30.51	19.00	29.50	45.50
Males	6	35.67	30.59	19.00	24.50	39.00
Females	14	40.29	31.53	22.00	32.00	49.00
Calves up to 1 year of age	15	41.53	33.90	19.00	30.00	49.00
Animals older than 2 years	5	31.00	17.20	18.00	29.00	42.00

SD – Standard Deviation, Q25 – Lower Quartile, Q75 – Upper Quartile.

Table 5. The content of iron in the hoof of the European bison according to gender and age (mg · kg⁻¹ dry matter)

Parameter	(n)	Average	SD	Q25	Median	Q75
Total number of animals	20	47.87	38.90	23.00	40.00	48.00
Males	6	70.33	54.91	37.00	47.00	126.00
Females	14	32.89	11.50	23.00	30.00	41.00
Calves up to 1 year of age	15	54.90	45.89	23.00	38.50	54.00
Animals older than 2 years	5	33.80	13.65	26.00	40.00	41.00

SD – Standard Deviation, Q25 – Lower Quartile, Q75 – Upper Quartile.

Discussion

To estimate trace element status of animals, reference values need to be determined. However, a wide variety of thresholds are published by different authors. There is little published information on essential metal concentrations in European bison to compare with the data obtained in this study. In the research of Dębska (2005) and Dymnicka et al. (2009), the content of iron in the liver of healthy European bison amounted to 78.06±35.48 mg · kg⁻¹ fresh tissue, i.e. considerably less than in the present experiment; however, Puls (1998) reported the range of iron content in liver of cattle as well as of bison to be 45–300 mg · kg⁻¹ fresh tissue. In the liver of big game animals (roe deer, stag and wild boar) hunted in the northern part of Poland Falandysz (1994) found 40–54 mg · kg⁻¹ wet weight depending on species. The values obtained in the above mentioned research are similar to those observed in the tissues of other species of large ruminants, i.e. domestic cattle in which mean iron liver concentration was 44 mg · kg⁻¹ wet weight (Falandysz, 1993). The results obtained here for the European bison may be considered as high values. Similar data for iron concentrations in the liver were obtained by Miranda et al. (2006) in cattle from northern region of Spain in which average iron concentrations were 96.2 mg · kg⁻¹ wet weight. Evidently higher values were observed in livers of fallow deer from Slovenia. In this case mean iron concentrations were 141 mg · kg⁻¹ (Vengušt and Vengušt, 2004). Domesticated mammals that typically receive feed of local origin have homogenous dietary composition with relatively constant mineral status. The diet of European bison is very heterogenous, so it can be assumed that their feeds vary markedly in iron contents. Neither Dębska (2005) in the bison, nor Vengušt and

Venguřt (2004) in the fallow deer have found differences in iron content in the liver depending on age, unlike the presented results.

Dębska (2005) and Dymnicka et al. (2009) found in kidney $81.80 \pm 27.82 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue in healthy European bison. Puls (1998) gives the reference values between 30 and $160 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue for kidneys of bison as well as for cattle. It may be assumed that the values obtained are in the upper range for the discussed data. The present study showed that iron concentrations in the kidneys of European bison were higher than those reported by Falandysz (1994) in cattle. Falandysz (1993) found that iron concentration in the liver of big game animals ranged from 67 to $83 \text{ mg} \cdot \text{kg}^{-1}$ wet weight. Much higher iron levels were found by Miranda et al. (2006) in cattle from northern Spain. Mean iron concentrations in kidneys of these animals were $105 \text{ mg} \cdot \text{kg}^{-1}$ wet weight.

In the case of muscle, values obtained in the present study are much higher than those reported by other authors. In the research of Falandysz (1994) the concentrations of iron in muscles of big game animals (roe deer, stag and wild boar) hunted in the northern part of Poland ranged from 24 to $31 \text{ mg} \cdot \text{kg}^{-1}$ wet weight. In cattle from the same region of Poland mean iron concentrations in muscles were $23 \text{ mg} \cdot \text{kg}^{-1}$ wet weight (Falandysz, 1993). Slightly higher values for cattle were obtained by Miranda et al. (2006). In the muscles of those animals mean iron concentrations were $56 \text{ mg} \cdot \text{kg}^{-1}$ wet weight. The mean iron concentration observed in bones in this study was in general similar to results published by other authors. In fact, published data on iron levels in wild ruminants is sparse, so it is difficult to evaluate present results with respect to other studies. If we compare the mentioned data with the values obtained by Kořla (1989) in metatarsal bone of the horse, i.e. $52\text{--}75 \pm 58 \text{ mg} \cdot \text{kg}^{-1}$ of dry matter when calculated into fresh tissue (40–45% of water in the bone; Smith and Andrews, 1964), we will receive $28.6\text{--}45.0 \text{ mg} \cdot \text{kg}^{-1}$ fresh tissue; therefore, we may assume that the discussed data are comparable. Similar data were obtained Doyle (1979) for cattle. The mean iron concentration observed in metacarpal and metatarsal bones of those animals was $28 \text{ mg} \cdot \text{kg}^{-1}$ wet weight.

The level of microelements in animal hooves, in spite of the basic importance for adaptation of the animals to the changed ground foundation has not been habitually analysed (Drożdż, 1981). In the studies of Drożdż (1981) the content of iron in hooves of Lowland Black-and-White cows was equal to $33.3 \text{ mg} \cdot \text{kg}^{-1}$ DM, i.e. lower than the mean value for European bison but similar to that for females and mature animals.

In conclusion, we may state that the iron concentrations in selected organs of European bison are higher than those observed in other species of herbivorous animals. Given that no information is available about the concentration of iron in European bison from the Białowieża herd, further research is necessary to determine reference values. This will be helpful for future studies on the physiology of this species.

The experiment was based on the tissues collected postmortem and did not require the acceptance of the ethical committee according to the law in force.

The authors declare that the experiment complies with the current laws of Poland and declare that they have no financial conflict of interest.

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Zawartość żelaza w tkankach żubra ze stada białowieskiego

STRESZCZENIE

Celem badań było oznaczenie zawartości żelaza w wybranych narządach żubrów żyjących wolno w Puszczy Białowieskiej. Materiał do badań pozyskano od zwierząt eliminowanych podczas dorocznej selekcji. Pobrano wycinki wątroby, nerek, mięśni, żeber i racic. Osobniki podzielono w zależności od płci (samce i samice) oraz wieku (cielęta do 1 roku i zwierzęta powyżej 2 lat). Średnia zawartość żelaza w wątrobie wynosiła 263,59 mg · kg⁻¹ świeżej masy narządu i była ona istotnie wyższa w grupie zwierząt starszych. Średnia zawartość żelaza w nerkach wynosiła 156,70 mg · kg⁻¹, zaś w mięśniach 79,95 mg · kg⁻¹ świeżej masy. Podobnie jak w przypadku wątroby zaobserwowano tu istotne statystycznie różnice ($P \leq 0,05$) pomiędzy grupami wiekowymi. Średnia zawartość żelaza w żebrze oraz rogu ściany racicy wynosiła odpowiednio 38,90 mg · kg⁻¹ świeżej masy oraz 47,87 mg · kg⁻¹ suchej masy. Nie zaobserwowano istotnych statystycznie różnic w zależności od płci badanych zwierząt.