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Concentrations of zinc, cadmium and lead in the hoof horn of the European bison (Bison *bonasus bonasus*)

Zawartość cynku, kadmu i ołowiu w ścianie rogowej racicy żubra Europejskiego (Bison *bonasus bonasus*)

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Abstract
The aim of the study was the assessment of zinc, cadmium and lead concentrations in the hoof horn of the European bison free ranging in Białowieża Primeval Forest.
The investigation material comprised hoof samples collected from animals eliminated during annual selection. Animals were divided depending on gender (males and females). Metals content was determined using the inductively coupled plasma mass spectrometry method (ICP-MS). Mean metals concentrations in hoof were: 114.1, 0.15 and 0.45 mg·kg^{-1} dry matter for zinc, cadmium and lead, respectively. A significantly correlated dependence at $p \le 0.05$ was observed between the zinc and lead content in the material studied. No statistically significant differences in the metals content were observed depending on gender. It can be stated, that zinc, cadmium and lead concentrations in the hoof wall of the European bison from Białowieża primeval forest are in the reference values determined for the hair coat of other ungulates species.

Streszczenie
Celem pracy była ocena zawartości cynku, kadmu i ołowiu w ścianie rogu racicy żubra pochodzącego z Puszczy Białowieskiej. Materiałem badawczym były próby racic pobrane od zwierząt podczas corocznej eliminacji. Zwierzęta podzielono na grupy w zależności od płci (samce i samice). Zawartość metali określano za pomocą spektrometrii mas z jonizacją w plazmie indukcyjnie sprzężonej (ICP-MS). Średnie stężenia metali w ścianie racicy wynosiły: 114,1; 0,15 i 0,45 mg·kg^{-1} suchej masy odpowiednio dla cynku, kadmu i ołowiu. Stwierdzono statystycznie istotną zależność ($p \le 0,05$) między zawartością cynku i ołowiu w badanym materiale. Nie odnotowano różnic statystycznych w zawartości metali w zależności od płci zwierząt. Można stwierdzić, że zawartość cynku, kadmu i ołowiu w ścianie rogowej racicy żubrów z terenu Puszczy Białowieskiej znajduje się w zakresie wartości referencyjnych ustalonych dla okrywy włosowej innych gatunków zwierząt kopytnych

1. INTRODUCTION

The extant European bison is the largest undomesticated ruminant (*ruminantia*) in Polish forests. It belongs to the order *Artiodactyla* (even-toed ungulates), whose digital organ is covered by the integument derivative referred to as hoof. Phylogenetically, the hoof of ruminants is an intermediate structure between the claw in carnivores and the hoof in equines [König and Liebich 2007]. Hoof diseases in domestic ruminants are relatively frequent and problematic in terms of herd health maintenance [Muelling 2009, Dyce et al. 2010]. In undomesticated ruminants, a condition of the digital organ of an animal has virtually no importance to the herd; however, it is crucial for the survival ability of an individual. Pathological changes within the hoof impede migrations to new forage areas and, in consequence, the lame animal weakens and becomes exposed to possible predator attacks.
Each limb of a ruminant possesses two proper hooves, or claws – which cover the peripherals of the third and fourth digits – as well as two other toes, dewclaws, which never make contact with the ground and are carried by the vestigial second and fifth digits. Only the third and fourth digits bear the weight of the animal's body. Due to their small surface of contact with the ground, the organs are exposed to heavy loads, and any disease – including malformations of the keratin structures of the hoof – can cause a serious motion impairment [Muelling 2009]. Hoof diseases in free-living ruminants have been reported in many parts of the world and – if not caused by infections – many cases resulted from disturbances in the mineral metabolism [Flynn et al. 1977, Handeland and Virkøren 2005].
The quality of the hoof keratinised structures primarily depends on the availability of necessary minerals, such as calcium, zinc or copper [Muelling 2009]. The horny products of the skin also have the ability to bind heavy metals. So far, research has focused on hair, which proved to be a good diagnostic marker of long-term exposure to some metals, regardless of whether needed or toxic [Anke and Rish 1979, Kośla and Skibniewska 2010,

Skibniewska et al. 2011]. Unlike blood serum, whose concentrations of minerals change rapidly in response to changes in the physiological status, keratin structures, such as hair or hoof horn, respond considerably more slowly, allowing accurate assays for the periods within at least a few weeks prior to sampling [Jaśkowski et al. 1993, Stachurska et al. 2011]. The aim of this study was to determine the concentrations of zinc, cadmium and lead in the horny wall of the hoof in the European bison inhabiting the Białowieża Forest, with regard to the interaction between the metals and the differences between the sexes.

2. MATERIAL AND METHODS

Samples of hoof were collected from 15 European bisons, eliminated during a winter culling in 2005 in the Polish part of Białowieża Forest. The slices of the hoof wall were burnt in muffle furnace (LM 212.11 VEB Elektro Bad Frankenhausen, Germany) at 450ºC and ash was transferred quantitatively to measuring flasks with a bidistilled water acidified to 2.5% HCl. The metal (Zn, Cd and Pb) concentrations in the examined samples were evaluated using inductively coupled plasma mass spectrometry (ICP-MS). The values were checked regularly against appropriate and certified reference materials (CRM – BCR 185 R). The percentages of recovery were 108%, 86% and 94% for Zn, Cd and Pb, respectively. The results are presented in $mg \cdot kg^{-1}$ of dry matter of the hoof sample. The outcome was statistically analysed using Statistica 10™ software (StatSoft, Inc.). Differences were considered as significant at the level $p \leq 0.05$. Relationships between the concentrations of zinc, cadmium and lead in the hoof were calculated using Spearman's correlation. The statistical significance of the correlation coefficients was tested at the level $p \leq 0.05$.

3. RESULTS AND DISCUSSION

The data on the content of zinc, cadmiu, and lead are presented in Table 1. The zinc content in the hoof wall varied within the relatively narrow limits. The highest value observed was 142.7, whereas the lowest was 93.5 $mg \cdot kg^{-1}$. In contrast, in the case of cadmium and lead their concentrations varied within the wide limits. The highest value observed was 29 and 21 times higher than the lowest ones in the case of cadmium and lead, respectively.

Considering the animal gender higher zinc and cadmium values were noted in females (Table 2). The highest values of lead were noted in the group of males. Statistical test did not reveal any significant differences depending on the sex of the animals.

A significantly correlated dependence was observed between the zinc and lead content in the material studied ($r = 0.62$, $p \leq 0.05$), Table 3.

Trace elements play an important role in the formation and maintenance of adequate biomechanical properties of the cutaneous keratin structures [Mülling et al. 1997]. An increase in the bioavailability of the minerals can significantly improve the integrity of the hoof horn [Ballantine et al. 2002]. Zinc is a component of over 300 enzymes, many of which are involved in the production of keratin of the horny wall and the sole of the hoof, which both serve as protection of the sensitive structures of the digital organ [Smart and Cymbaluk 1997, Mülling et al. 1997, Mülling and Budras 2002]. Zinc plays a key role in the process of keratin formation; it has a catalytic, structural and regulatory function [Cousins 1996]. The formation of integument keratin structures is a process of transformation of live epidermal cells into dead, cornified, structurally stable layers that do not show any metabolic activity [Tomlinson et al. 2004]. The catalytic function of zinc in the process of keratiaisation is performed with such enzymes as RNA polymerase, RNA transferase, alkaline phosphatase, carboxypeptidase, alcohol dehydrogenas, and carbonic anhydrase. These zinc-activated metalloenzymes play an important role in the differentiation of keratinocytes [Cousins 1996]. Interactions between zinc and toxic metals have been reported in various mammals, both maintained in farming conditions and kept in laboratories. This effect pertains particularly to the Z-–Cd relationship, since both metals have the ability to induce the formation of metallothioneins, and compete for the cation-binding metallothionein thiol group [Martelli et al. 2006]. Originally it was believed that the only function of metallothionein is to protect against the toxic effects of heavy metals (especially cadmium). Now it is clear that the proteins of this group act as regulators of zinc and copper metabolism, and protect cells against damage caused by alkylating agents, free radical, or ionizing radiation [Thirumoorthy et al. 2011].

The available literature lacks information on the concentrations of zinc, cadmiu, or lead in the hoof of the European bison. Our results can be compared only to those on other free-living ungulates or domestic ruminants. An interesting question is the relationship between metal concentrations in the hoof horn and the hairs of the coat. In our opinion, a comparison of data on hoof horn obtained from free-living animals with that sampled from livestock animals is not fully justified due to different conditions and environments both groups live in. The hoof horn of domestic cattle, which is housed in barns, being in permanent contact with often damp and manure-polluted bedding, can significantly vary in metal concentrations in relation to free-living animals, whose hooves are in contact with radically different substrates.

The results obtained in our study on the zinc content in the horny wall of the hoof capsule are similar to those observed in other ungulates. Sargentini et al. [2012], who analysed the content of zinc in the hoof capsule horn of a free-living, Tuscan endangered Amiata donkey, reported a value of 108.7 $mg \cdot kg^{-1}$dry matter.

Zinc concentrations in bison hooves were similar to those observed in the coat of the cattle. According to Puls [1994], the proper level of Zn in cattle coat ranges between 100 and 150 $mg \cdot kg^{-1}$. Cadmium bovine coat hair concentrations considered normal remain in the range of 0.009 to 0.6 $mg \cdot kg^{-1}$ dry weight. A Cd concentration in the range of 0.67 to 16.0 is considered elevated, whereas cadmium poisoning means that its content in bovine hair grows to extreme values, from 40 to as much as 100 $mg \cdot kg^{-1}$ dry weight. Stachurska et al. [2011] during the analysis of samples collected from Polish Konik horses found that the cadmium concentrations in the wall of the hoof capsule were below the detection threshold.

Lead is one of the most toxic heavy metals. Its toxic activity leads to the dysfunction of the liver, kidney, bone marro, and the central

Table 1. Content of some chosen metals in the European bison hoof (mg·kg^{-1} of dry matter)

Elements	n	Arithmetic mean	Standard deviation	Median	Range	Lower quartile (25%)	Upper quartile (75%)
Zn	15	114.1	15.15	118.5	93.5-142.7	99.4	142.7
Cd	15	0.15	0.23	0.04	0.03-0.87	0.04	0.18
Pb	15	0.45	0.55	0.26	0.11-2.35	0.24	0.34

Table 2. Zinc, cadmium and lead content in the roof of European bison depending on their gender (mg·kg^{-1} of dry matter)

Elements	Females			Males		
	n	Range	Mean ± SD	n	Range	Mean ± SD
Zn	9	98.5-140.2	116.1 ± 13.43	6	93.9-142.7	110.8 ± 18.24
Cd	9	0.03 - 0.87	0.21 ± 0.29	6	0.04 - 0.18	0.06 ± 0.05
Pb	9	0.11 - 0.88	0.35 ± 0.22	6	0.13 - 2.35	0.59 ± 0.86

Table 3. Correlation coefficients between zinc, cadmium and lead in the hooves

Elements	Zinc	Cadmium
Cadmium	0,04	
Lead	0,62*	0,05

*Differences statistically significant at $p \leq 0.05$.

nervous system. Metabolic disorders caused by lead poisoning result from an impaired activity of enzymes, protein structural abnormalitie, or from changes in the mineral metabolism of the body (Kośla 1999). Information on the concentrations of lead and other heavy metals in the body come from an analysis of blood, milk, urine, bon, and the integumentary system appendages, mainly hair. There is an opinion that the latter structures have enough metal bioaccumulation ability to serve as a good material for metal content analysis [Kośla et al. 2004, Rashed and Soltan 2005, Kośla et al. 2011a,b, Skibniewska et al. 2011, Curi et al. 2012]. It has been demonstrated that concentrations of toxic heavy metals in the hair of animals are positively correlated with their levels in parenchymal organs such as the liver, kidne, and skeletal muscles [Rashed and Soltan 2005, Curi et al. 2012]. Lead content observed in our study was much lower than those reported by the Italian authors, who found lead in the same structure of the hoof at the level 3.6 mg·kg^{-1}. Considerably lower levels were found in the corneous sole of the hoof, where the average value was 0.9 mg·kg^{-1} of dry matter.

Stachurska et al. [2011] carried out an analysis of the samples collected from Polish Konik horses and found the average lead concentration in the wall of the hoof capsule at a level 0.39 mg·kg^{-1}. A normal level of lead found in the hair of cattle remains between 0.5 and 5 mg·kg^{-1}. High concentrations of lead in bovine coat range from 3.5 to 90 mg·kg^{-1}, whereas toxic levels of lead range between 10 and 100 mg·kg^{-1} dry weight. The results of our analysis correspond with the normal concentrations of cadmium and lead in the bovine coat. Stachurska et al. [2011], who analysed the coat of the Polish Konik horse, measured cadmium and lead at the levels 0.01 mg·kg^{-1} and 0.22 mg·kg^{-1}, respectively. These data are also similar to our findings.

4. CONCLUSIONS

In conclusion it can be stated that zic, concentration in the horny wall of the hoof in the European bison inhabiting the Białowieża Forest remains within the reference ranges established for the hair coat of other ungulates. Low cadmium and lead concentrations in the keratzeised hoof structures reflect low environmental concentrations of both metals. Given that no information is available about the concentration of essential and heavy metals in European bison from the Białowieża herd, further research is necessary to set the reference values which will be helpful for studies concerning the physiology of these animals.

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