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*Original article*

# The state of bioelements in the hair of free-ranging European bisons from Białowieża Primeval Forest

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## Abstract

Human or animal hair is considered to be a good indicator of the content of bioelements or toxic elements in human and animal organism. Its analysis is a good alternative for the arduous obtaining blood or biopsy samples. The aim of the performed research was the assessment of some chosen bioelements in the organism of European bison on the basis of their analysis in hair. The investigation material comprised hair samples obtained from 22 animals. All animals were divided into groups according to gender (males, females) and age (calves up to one year of age and animals older than 2 years). Samples were mineralized in nitric acid, under pressure in the microwave apparatus. The content of phosphorus, sulphur, magnesium, iron, titanium and vanadium were determined in hair samples. The content of these elements was determined using the ICP-AES method in accredited laboratory. The accuracy of determinations was tested using the standard reference material.

The mean content of phosphorus in hair amounted to 245.14 mg $\cdot$ kg$^{-1}$, SD 65.00, magnesium 97.32 mg $\cdot$ kg$^{-1}$, SD 33.16, iron 119.48 mg $\cdot$ kg$^{-1}$, SD 83.31 and titanium 2.368 mg $\cdot$ kg$^{-1}$, SD 2.097. In case of these elements, differences depending on gender and age were statistically insignificant. Mean content of sulphur in the European bison hair amounted to 3.41% with equalized content in the herd (SD 0.22%). Here also statistically insignificant differences depending on gender were observed. However, a statistically significant difference ($p \leq 0,05$) was demonstrated which depended on animal age. Mature European bisons had more sulphur in hair as compared to calves.

The concentration of vanadium (0.362 mg $\cdot$ kg$^{-1}$, SD 0.396 on the average) significantly differed in hair depending on the European bison age with much higher values in adult animals (calves 0.260 mg $\cdot$ kg$^{-1}$, animals older than 2 years 0.686 mg $\cdot$ kg$^{-1}$). Similar data were obtained while investigating hair of sea mammals.

**Key words**: European bison, hair, bioelements, age, gender

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## Introduction

A few generations of naturalists, foresters and scientists put a lot of work to reintroduce the European bison to the Białowieża Forest (Poland). Despite the success, European bisons are still treated as endangered species which results from a high hybridization, the lack of natural selection, the action of pathogens and parasites, worsening the habitat quality, including the density and limitation of feed resources also in the competition with deer and roe-deer (Głowaciński 2001).

European bisons, similarly as other free living ruminants which compete with them for feed, strongly depend on the conditions of life and the accessible fodder base. A high population density in Białowieża Forest (about 500 animals), limited area of occurrence, meadows running wild limit the amount of accessible fodder and thus cause the lack of meeting nutritional requirements (Krasińska and Krasiński 2004).

For many years the human or animal hair was considered to be a good indicator of the content of bioelements as well as toxic elements in the organism (Anke and Risch 1979, Bertram 1983, Apostoli 2002). Ikemoto et al. (2004) using the regression analysis found a positive correlation between the content of trace elements in the hair and in the liver, kidney or muscles. They stated that the correlation between the content of trace elements in various tissues allows the use of hair samples for monitoring the content of elements in the animal organism. Investigations performed in the last 20 years suggest that the analysis of human hair is an important alternative to arduous obtaining blood samples and biopsy in order to determine the content of bioelements (Radomska et al. 2005).

The lack of phosphorus leads to disorders of protein and carbohydrate synthesis. It is necessary for producing nucleic acids, its role cannot be overestimated in animal and human metabolism (Fan et al. 2009). Phosphorus deficit is easily recognized in animal breeding and causes losses which according to Underwood (1981) are very arduous economically as compared with the deficit of other mineral components in animals.

Sulphur is an important nutrient required for all living organisms. It is an essential constituent of cysteine, methionine, several co-enzymes (e.g., biotin, co-enzyme A, thiamine pyrophosphate and lipoic acid), thioredoxins, sulpholipids and all proteins which contain cysteine or methionine. Some of the most important functions of sulphur include its role in specialised peptides, such as glutathione and thioredoxins, in redox reactions, and the role of disulphide bond formation in the stabilization of protein structure (Zhao et al. 1999).

Magnesium belongs to basic macroelements. It is called the life element because of its role in metabolic processes in animals and man. The most important of these processes are maintaining the balance in proportions and transportation of cations and anions, and the impact on the permeability of cell membranes. Magnesium, similarly as phosphorus forms DNA and RNA structures, affects lipid metabolism, the level of catecholamines and ATP, regulates the processes of oxydoreduction,photosynthesis and activates many enzymes. Magnesium plays an important role in the prophylaxis and therapy of many diseases, prevents nervous hyperexcitability, depression and vegetative dystonia (Kabata-Pendias and Pendias 1999).

The main role of iron is its part in haemoglobin, whose level it stabilizes; however, iron also plays a very important role in immunological processes and metabolism of the organism. The organism possesses a system that maintains iron homeostasis, because its both excess and deficit are associated with cellular dysfunction. The regulation of iron absorption from the intestine and its salvage from old erythrocytes is of crucial importance for balancing the metabolism of that element. Hepcidin plays an important role in the communication with cells responsible for the absorption of iron and thus maintains iron homeostasis, functioning as a powerful negative regulator of the absorption and mobilization of this element (Kośla and Skibniewska 2010).

Titanium is considered to be a trace element which is present in human and animal tissues which, however, does not play any role in the metabolism and is indifferent for these organisms although for a long time it is considered to be necessary for plant development (Hruby et al. 2002). The results obtained by Pais and Jones (1997) suggest a favourable effect of titanium compounds, especially titanium ascorbate, dissolved in water on the health of man and animals. Still titanium is not as yet commonly considered as an element necessary for a proper development of animals (Kabata-Pendias and Pendias 1999).

The deficit of vanadium limits the growth of animals (in birds it also limits the growth of feathers), decreases the level of red blood cells, may cause an increase in the level of cholesterol and triglyceride in blood. The lack of vanadium may cause disturbances in the ossification process and it particularly affects the process of tooth mineralization (Kabata-Pendias and Pendias 1999). Its biological role is connected with the influence on the metabolic processes of lipids, phosphotransferases, sugars and monoamine oxidases. One cannot overestimate its part in the flow of ions through the cell membranes in animals and man in the, so called, sodium-potassium and calcium-magnesium pomp. It plays a key role in the phosphate metabolism (Kabata-Pendias and Pendias 1999). Vanadium is known from its bonds with

Table 1. Content of some chosen macroelements in the European bison hair.

| Parameter (n) | P (mg · kg$^{-1}$) | | S (%) | | Mg (mg · kg$^{-1}$) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Total (22) | 245.14 | 65.00 | 3.41 | 0.22 | 97.32 | 33.16 |
| Males (8) | 257.75 | 47.84 | 3.34 | 0.16 | 107.17 | 26.01 |
| Females (14) | 237.93 | 73.74 | 3.45 | 0.24 | 92.77 | 36.01 |
| Calves up to one year (16) | 250.88 | 68.08 | 3.35* | 0.20 | 107.29 | 31.08 |
| Older than two years (6) | 229.83 | 58,74 | 3.56* | 0.21 | 69.40 | 22.10 |

* – differences statistically significant at $p \leq 0{,}05$

Table 2. Content of some chosen trace elements in the European bison hair (mg · kg$^{-1}$).

| Parameter (n) | Fe | | Ti | | V | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Total (22) | 119.48 | 83.31 | 2.368 | 2.097 | 0.362 | 0.396 |
| Males (8) | 133.62 | 93.46 | 1.875 | 2.156 | 0.275 | 0.266 |
| Females (14) | 110.77 | 79.10 | 2.607 | 2.136 | 0.415 | 0.460 |
| Calves up to one year (16) | 127.00 | 96.33 | 2.438 | 2.354 | 0.260* | 0.253 |
| Older than two years | 100.67 | 34.45 | 2.183 | 1.336 | 0.686* | 0.524 |

* – differences statistically significant at $p \leq 0{,}05$

aminoacids (cysteine and histidine) (Rehder and Jantzen, 1998). However, in the experiment with radioactive vanadium ($^{48}V$) Sotogaku et al. (1999) did not obtain reliable results of including vanadium into the proteins of blood serum. With age the amount of vanadium in animal tissues increases which should be connected with a long period of half-life in the organism (Ikemoto et al. 2004).

The aim of the present study was the evaluation of chosen bioelements in the European bison organism on the basis of analysis of mineral composition of hair.

## Materials and Methods

Hair samples were collected from 22 European bisons, eliminated within annual selection. Hair samples were collected from the animals backs and put into paper envelopes. Animals were divided into groups depending on their gender (males, females) and age (calves up to one year of age and animals older than 2 years). Hair samples were defatted in 70% ethyl alcohol in the apparatus for fat extraction, then they were rinsed with warm distilled water and three times in bidistilled water. Hair were breaking up and samples of 0.5 g were placed in the Teflon containers. Samples were mineralized in nitric acid (25 ml), under pressure in the microwave apparatus. The presence of phosphorus, sulphur, magnesium, iron, titanium and vanadium were determined in the samples. The contents of these elements were determined in the accredited laboratory using the method of atomic-emission spectrophotometry with inductively coupled plasma (ICP-AES). The accuracy of measurements was tested using the standard reference material.

The results obtained were analyzed statistically with the help of Statistica packet, Anova module and for the comparisons between the groups the least significant difference test was used (LSD).

## Results

The content of macroelements in the European bison hair is presented in Table 1. The mean phosphorus content amounted to 245.14 mg · kg$^{-1}$ with only slight standard deviation which points to the quite equalized group of animals. Also the differences between the group of males and females (the gender impact) are not large. Statistically insignificant were also the differences in that macroelement content depending on animal age.

The mean sulphur content in the hair of all European bisons amounted to 3.41%, with the equalized content in the herd (SD 0.22%). No statistically significant differences depending on gender were noted. However, a statistically significant difference ($p \leq 0{,}05$) was demonstrated depending on age. Mature European bisons had more sulphur in hair as compared to calves.

Magnesium content (mean value 97.32 mg · kg$^{-1}$, SD 33.16) did not differ in a statistically significant way depending on gender and age. However, if the magnesium content in the calves hair is estimated as 100%, in mature animals this value is 64.68% respectively. Still the lack of a bigger number of samples from mature animals does not allow the assessment whether that tendency is repeatable.

The content of trace elements in hair is presented in Table 2. The data concerning iron (the average 119.48 mg · $kg^{-1}$, SD 83.31) show a significant scatter inside the group of the investigated animals, no statistically significant differences were observed between males and females as well as young and older animals. They agree with the reference data for cattle hair (Puls 1998).

The content of titanium in hair was as follows: the average was 2.368 mg · $kg^{-1}$, SD 2.097; statistical test did not reveal any significant differences depending on gender and age, however, attention is called to the great scatter of results (high SD) in the group of males.

Vanadium which is considered to be an ultra-trace element (the average is 0.362 mg · $kg^{-1}$, SD 0.396) differed significantly in hair depending on the age of European bisons, mature animals had significantly more of this element (calves 0.260 mg · $kg^{-1}$, older than 2 years 0.686 mg · $kg^{-1}$).

## Discussion

Currently there are no well-documented data on the levels of some bioelements in tissues and hair of European bisons. The results obtained can be applied only to other mammalian species.

The level of phosphorus (Table 1) agreed with the reference data by Puls (1998). For cattle he reports from 200 to 300 mg · $kg^{-1}$ of phosphorus in hair. Similar data for cattle are reported by Kośla et al. (1985). However, the results obtained are clearly higher than those found in the European bison hair determined by Kośla et al. (1985), Kośla (1993a) and Dębska (2005). While analyzing the population of horses, Kośla (1988) did not observe the effect of gender (mares – geldings) in hair and in 8 investigated tissues. While analyzing the difference in phosphorus content in hair depending on gender, Anke and Risch (1979), similarly as in the present investigation, did not found any differences between phosphorus content in hair in both animal and human hair. However, Chojnacka et al. (2006) observed differences depending on gender: women -180 mg · $kg^{-1}$ and men -131 mg · $kg^{-1}$. Senofonte et al. (2000) have concluded, on the basis of analysis of their own results and the data from the literature, that it is difficult to establish the real impact of gender and age on the level of a certain element (19 elements were investigated). Here, the deciding element is the interaction of many factors. Analyzing the gender impact, they observed a statistically significant difference ($p \leq 0.05$), on the average in boys -201 mg · $kg^{-1}$ and in girls -188 mg · $kg^{-1}$. The effect of age in young people (3-13 years of age) was also statistically significant, with age the content of phosphorus in hair increased (groups: 3-6 years of age: 148; 6-10 years: 199; 10-13 years: 205, everywhere in mg · $kg^{-1}$ dry matter). These authors analyzed the results obtained depending simultaneously on gender and age. In case of phosphorus, they demonstrated the tendency of the content to increase with age in the group of boys (Senofonte et al. 2000).

The lack of data concerning the content of sulphur in the European bison hair does not allow the comparison of the present results. However, the available data allows the statement that the content of sulphur in the European bison hair is similar to that in the hair of sheep (Anke and Risch 1979). While analyzing sulphur content in the hair of cats, Kośla et al. (2007) observed the mean value higher than in European bisons (40.99 g · $kg^{-1}$), the gender depended differences were non-significant (males 41.16, females 40.81 g · $kg^{-1}$), however, statistically significant differences ($p \leq 0.05$) were observed depending on age: young cats (up to 2 years of age) 40.07 g · $kg^{-1}$, older cats 41.90 g · $kg^{-1}$.

Magnesium contents in the European bison hair are slightly higher than those obtained by Dębska (2005) and similar to those by Kośla et al. (1985), however, lower than those obtained in another investigation by Kośla (1993a). Reference data for magnesium in cattle hair amount to 130-455 mg · $kg^{-1}$ in d.m. (Puls 1998). Anke at al. (2005a) have reported that in people the concentration of magnesium in hair (as well as in ribs and liver) depends on gender with women having more magnesium. With age the content of magnesium in the human organism decreases. The supplementation of diet for women with magnesium increased but statistically it does not affect the content of magnesium in hair: normal diet 270 mg · $kg^{-1}$d.m., diet with Mg supplementation: 318 mg · $kg^{-1}$ d.m. (Anke et al. 2006). In the horse hair, Kośla (1988) found insignificant differences depending on gender and age. The content of magnesium in mare hair was 618 and in mare mane hair 268, in gelding it was 780 and 211 mg · $kg^{-1}$ d.m., respectively. Depending on, age the mean values for hair in groups amounted to 567-737 mg · $kg^{-1}$ d.m. Comparing magnesium content in dog and cat hair, Skibniewski et al. (2006) determined in dogs 216.51 mg · $kg^{-1}$ (range from 50.16 to 400.36), and in cats 161.45 mg · $kg^{-1}$ s.m. (range from 60.00 to 302.00). Interesting results were obtained by Gray et al. (2008) while investigating the hair of seals. In the newly grown hair, they determined the content of magnesium as 585 mg · $kg^{-1}$ d.m., and in the falling out hair (during moulting) 894 mg · $kg^{-1}$ d.m., which shows that the method of sampling is important.

The content of trace elements is presented in Table 2. The data concerning iron in the hair of European bisons were higher than those obtained by Dębska (2005), and Kośla et al. (1985), while similar results were obtained in European bisons by Kośla (1993b). They are consistent with the reference data for the cattle hair (Puls 1998). Generally gender does not affect the accumulation of trace elements in mammals (O'Shea 1999), but Watanabe et al. (1998) observed in males a decrease with age of the content of heavy metals while in females no such dependence was observed. In horses, Kośla (1988) did not found any significant differences in hair depending on gender: in mares the content of iron amounted to 34 mg · kg$^{-1}$ d.m. and in geldings 31 mg · kg$^{-1}$ d.m. Also statistically insignificant differences were observed by this author in horses depending on age. In horses, the same content of iron was observed in the coat hair and mane hair.

The content of titanium due to the lack of data cannot be directly compared, however, the levels obtained are lower than mean values for human hair reported by Kabata-Pendias and Pendias (1999). In accordance to these data, Chojnacka et al. (2006) determined in the hair of men 3.56 mg · kg$^{-1}$ and 4.30 mg · kg$^{-1}$ in the hair of women while Senofonte et al. (2000) for a large group of young people (n=396) found 0.79 mg · kg$^{-1}$ without significant difference depending on gender (boys 0.79, girls 0.80). However, these values are lower than those obtained in the present investigation in the hair of European bison. Senofonte et al. (2000) analyzed age dependency of the content of titanium. A highly significant dependence ($p \leq 0.001$) between all three groups (group 3-6 years of age: 1.18 ± 0.93; group 6-10 years: 0.52 ± 0.36; and group 10-13 years: 0.87 ± 1.62 mg · kg$^{-1}$). With a detailed analysis of dependence of simultaneously age and gender it has been concluded that there is no clear gender – and age – dependence of the concentration of titanium in human hair (Senofonte et al. 2000).

Vanadium, considered to be an ultra-trace element, differed significantly in the hair depending on the age of European bisons. Mature animals had significantly more vanadium. Watanabe et al. (1998) observed in sea mammals the increase of heavy metals with age. Similarly, an increase with age of the content of vanadium in the liver of various sea mammals was observed by Saeki et al. (1999). Ikemoto et al. (2004) did not observe such dependence in the hair of seals. These authors using the Spearman;s rank correlation coefficient determined positive correlations of vanadium content with increasing age in the liver, rib and muscle, however, in hair correlation coefficients were low and in some populations – negative. Vanadium content in the hair of seals was higher than that found in European bisons. In the hair of three species of seals, Ikemoto et al. (2004) determined 1.0 ± 0.8; 0.71 ± 0.39 and 3.1 ± 1.0 mg · kg$^{-1}$ of vanadium respectively. The authors observed age dependence in case of vanadium only in the liver and gender dependence in the liver and kidney. In Antarctic seals, Gray et al. (2008) found 1.82 mg · kg$^{-1}$ vanadium in old hair (moulting) and 0.91 mg · kg$^{-1}$ in newly growing hair. The investigation of hair in young people (n=156) revealed the mean vanadium content amounting to 1.22 ± 1.43 mg · kg$^{-1}$, with the lack of statistically significant differences depending on gender, no statistically significant differences were observed depending on age (Senofonte et al. 2000). Chojnacka et al. (2006) determined in human hair 0.143 ± 0.107 mg · kg$^{-1}$ vanadium, presenting the reference range as 0.36-0.80 mg · kg$^{-1}$, with the difference depending on gender: women 0.059, men 0.193 mg · kg$^{-1}$. According to Anke et al. (2005b), the differences depending on gender arise from high consumption by men of beer very rich in vanadium (28 μg/L).

## Conclusion

Phosphorus content in the European bison hair matched the reference values for cattle hair and at the same time was higher than levels obtained by the majority of authors investigating the European bison hair. Based on data found in the literature one can assume that there exists species variability of phosphorus concentration in hair. However, there is no basis for the statement concerning the differences in phosphorus content depending on gender and age.

The present results dealing with sulphur concentration in hair point to their great variability depending on species, but there are no significant differences depending on gender. In older animals the content of sulphur in hair is higher.

Magnesium contents in the hair of European bisons were lower than those found in cattle hair, on the other hand, they did not differ much from the data for human hair. In the European bison hair, similarly as in literature data for other species, there are no significant differences in magnesium content depending on gender and age. The data concerning the impact of gender and age are presented in relation to human hair.

While analyzing trace elements in the European bison hair and comparing the results with the literature data it can be stated that the level of iron corroborate the reference data for cattle hair. In this case there are also the differences between species. European bison is characterized by a higher iron content as compared to other animal species and also to human hair. Considering age, no significant differences in the iron level were observed in European bison, although they were observed in humans. Considering gender, statistically significant difference was observed in dogs, females had their hair more saturated with iron. In humans the situation is reversed, i.e. the hair of man contains more iron.

The information concerning the content of titanium in human or animal hair are rarely published in literature. However, it could be assumed that the obtained values are similar to the data concerning human hair.

The concentration of vanadium in the European bison hair differed depending on age; similar results were obtained while investigating sea mammals. Statistically significant differences concerning vanadium content in hair depending on gender were also observed in adult people, however, they resulted from nutritional preferences.

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