# Serum and tissue concentrations of selected biochemical and mineral compounds in relation to the incidence of *balanoposthitis* in European bison

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**In the blood serum of healthy and *balanoposthitis* affected European bison, levels of the following biochemical parameters: glucose, total protein, urea and albumins, and of Ca, inorganic P, Mg, Na, K, Fe, Cu, Zn, Mn and Se, were analysed. The concentrations of Ca, P, Mg, Na, K, Fe, Cu, Zn, Mn and Se in the liver and in kidneys and levels of Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn in the hair were also determined. The lower level of Fe in the blood serum of animals with *balanoposthitis* was the only significant difference amongst all the parameters examined. It should be noted that the level of glucose in the blood serum of both groups of European bison is considerably higher than that of domestic cattle.**

**KEY WORDS: *balanoposthitis*/ biochemical parameters/ European bison/ tissues**

The most frequent causes of mortality in the population of European bison found in the Białowieża Forest during the period from 1975 to 2002 included: trauma (16%), incidence of *balanoposthitis* in males (9%), parasite diseases, poaching and old age (8%) and drowning (3%) [8]. Pathological changes around the praeputium in males were observed for the first time in 1980 in the bison populations in Borecka and Białowieża Forests.

It is established that necrotic *balanoposthitis* in European bison is a polyethiological disease. There are many factors which may induce it, or may favour its development. One hypothesis suggests that a small genetic variation within the European bison population may be the cause of *balanoposthitis* [2, 3]. Other hypotheses include: a high degree of viral infections, such as infectious bovine rhinotracheitis (IBR) or bovine viral diarrhea (BVD), *Chlamydia*, mycoplasms and also conditionally pathogenic bacteria

*(Pseudomonas, Corynebacterium, Bacillus, Staphylococcus and Escherichia)* [9]. Kita et al. [6] also mentioned the role of the bacteria *Ureoplasma sp.* and *Streptococcus sp.* Wolf et al. [14] suggested that *Fusobacterium necrophorum* may play a key role in generating necrotic foci. The authors mentioned above found no viral infections in the cases studied. Osińska et al. [11] reported that physical damage, caused by ticks, might be the primary reason for inflammation of praeputium skin. Microfilaria *Onchocerca sp.* was also present in the skin of praeputium and labia pudenda of European bison. Due to secondary bacterial infections and environmental factors, necrotic and purulent changes took place.

Krasotchko et al. [9] mentioned that the following factors may favour the development of *balanoposthitis:* insufficient quantity of feed and the resulting nutritional deficiencies due to a high concentration of herbivorous animals within a relatively small area, also causing stress and lowering immunity. No data concerning the effect of deficiency of nutrients, including the shortage of mineral components on the incidence of *balanoposthitis* was found in the available literature. We undertook the studies with the aim of determining the relationship between the incidence *of balanoposthitis* disease and nutritional deficiencies as indicated by the levels of the selected biochemical parameters in blood serum and by the contents of the selected macro and micro minerals in certain tissues.

## Material and methods

The studies were conducted in the Białowieża Forest from 2000 to 2003. The tissue samples were collected *post mortem* during winter culling. The material included animal tissues: blood serum, liver, kidneys and hair, collected from 30 animals of both sexes.

The levels of glucose, total protein, urea and albumins in blood serum were determined by colorimetric method using a Vitros DT 60 II. The concentrations of Ca, inorganic P and Mg were determined by colorimetric method; the levels of Na and K were determined by ion-selective electrode, measuring the potential difference between a reference solution and the samples previously examined in Vitros DT 60 II. Blood serum samples, for the determination of micro minerals, were prepared by microwave mineralization in a closed system. The levels of minerals in blood serum were determined using the following procedures: Fe, Zn and Cu - by flame atomic absorption spectrometry (FAAS); Mn - by atomic absorption spectrometry with graphite cuvette (GF - AAS); and Se - by spectrofluorometry.

Serum levels of biochemical parameters and minerals found in European bison were compared to the reference values for cattle published by Winnicka [13] and Kuleta [10].

The concentrations of Ca, Na, K, Mg, Fe, Zn, and Cu in bison kidney and liver tissue were determined by flame atomic absorption spectrometry (FAAS); the levels of Mn, Cr and Co - by atomic absorption spectrometry with graphite cuvette (GF-AAS); inorganic P - by atomic emission spectrometry with plasma, activated by induction (ICP-OES) and Se - by spectrofluorometry.

Bison hair samples were screened for inorganic P, Ca, Fe, Mg, Zn and Mn using atomic emission spectrometry with plasma, activated by induction (ICP-OES). Cu was determined by atomic absorption spectrometry with graphite cuvette (GF-AAS); K and Na - by flame atomic absorption spectrometry (FAAS).

The levels of discussed components in liver, kidneys and hair of the European bison, were compared to the reference values for cattle according to Puls [12].

## Results and discussion

Differences in the serum concentrations of the selected biochemical parameters in bison males without disease and of those with *balanoposthitis*, were not statistically significant (Table 1).

**Table 1 - Tabela 1**
Levels of selected biochemical parameters in the blood serum of European bison with and without *balanoposthitis*
Poziom wybranych parametrów biochemicznych w surowicy krwi żubrów z *balanoposthitis* i bez *balanoposthitis*

| Biochemical parameter<br>Parametr biochemiczny | Group<br>Grupa | N | $\overline{X}$ | Sd | Reference values<br>Wartości referencyjne |
|---|---|---|---|---|---|
| Glucose (mmol/l)<br>Glukoza (mmol/l) | 1* | 10 | 8.03 | 4.61 | 2.22-3.33[1)]/2.2-4.5[2)] |
| | 2** | 16 | 9.29 | 5.67 | |
| Crude protein (g/l)<br>Białko ogólne (g/l) | 1 | 9 | 64.22 | 8.53 | 51-83[1)]/51-71[2)] |
| | 2 | 14 | 63.00 | 9.41 | |
| Albumins (g/l)<br>Albuminy (g/l) | 1 | 9 | 28.22 | 5.49 | 32-49[2)] |
| | 2 | 17 | 30.71 | 3.12 | |
| Urea (mmol/l)<br>Mocznik (mmol/l) | 1 | 9 | 6.19 | 1.31 | 3.3-6.7[1)]/1.66-7.47[2)] |
| | 2 | 17 | 5.46 | 1.79 | |

* - European bison with *balanoposthitis* - żubry z *balanoposthitis*
** - European bison without *balanoposthitis* - żubry bez *balanoposthitis*
[1)] - acc. to Kuleta [10] - wg Kuleta [10]; [2)] - acc. to Winnicka [13] - wg Winnicka [13]

The levels of glucose considerably exceeded the reference values whereas the levels of albumins were found to be below the reference values for cattle. Total protein and urea contents were within the range of the reference values for cattle.

No statistically significant differences were found between the contents of the analysed macro minerals in tissues of males with *balanoposthitis* and those without disease (Table 2). It should be noted that low levels of all the determined macro minerals were observed in the hair of the examined animals and they were found to be below the reference values for cattle, as given by Puls [12].

**Table 2 - Tabela 2**
Levels of macro minerals in blood serum, liver, kidneys and hair of European bison with and without *balanoposthitis*
Poziom makroelementów w surowicy krwi, wątrobie, nerkach i sierści żubrów z *balanoposthitis* i bez *balanoposthitis*

| Tissue | Parameter | Ca | | P | | Na | | K | | Mg | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tkanka | Parametr | 1* | 2** | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Blood serum (mmol/1) | N | 9 | 17 | 9 | 17 | 9 | 16 | 6 | 15 | 9 | 17 |
| Surowica krwi (mmol/1) | $\overline{X}$ | 2.25 | 2.34 | 2.68 | 2.48 | 139.67 | 138.81 | 8.22 | 7.99 | 0.96 | 0.95 |
| | Sd | 0.48 | 0.14 | 0.57 | 0.60 | 3.77 | 5.50 | 1.03 | 1.81 | 0.13 | 0.16 |
| | reference values | 2.25-2.99[1)] | | 1.45-2.10[1)] | | 126.1-163.1[1)] | | 3.84-6.39[1)] | | 0.74-1.32[1)] | |
| | wartości referencyjne | 2.25-3.03[2)] | | 1.00-2.71[2)] | | 134.8-156.5[2)] | | 3.8-5. l[2)] | | 0.78- 1.23[2)] | |
| Liver | N | 7 | 5 | 7 | 5 | 7 | 5 | 7 | 5 | 7 | 5 |
| (ppm in fresh tissue) | $\overline{X}$ | 29.71 | 33.64 | 3388.29 | 3296.80 | 1041.71 | 965.60 | 3074.57 | 3047.00 | 197.29 | 200.60 |
| Wątroba | Sd | 5.43 | 8.36 | 324.05 | 320.07 | 237.35 | 204.98 | 322.63 | 414.04 | 14.47 | 17.18 |
| (ppm w świeżej tkance) | reference values | 30-200[3)/ 4)] | | 2 000-4 400[3)] | | 530-3 450[3)] | | 1 400-3 950[3)] | | 100-250[3)/ 4)] | |
| | wartości referencyjne | | | | | | | | | | |
| Kidneys | N | 7 | 5 | 7 | 5 | 7 | 5 | 7 | 5 | 7 | 5 |
| (ppm in fresh tissue) | $\overline{X}$ | 53.44 | 62.18 | 2421.14 | 2328.80 | 2348.00 | 2589.00 | 2643.71 | 2437.40 | 179.14 | 184.40 |
| Nerki | Sd | 9.22 | 13.34 | 166.01 | 323.15 | 325.70 | 281.73 | 491.61 | 422.43 | 6.34 | 14.45 |
| (ppm w świeżej tkance) | reference values | 45-200[3)/ 4)] | | 2 600[3)] | | 1 450-1 700[3)] | | 1 800-2 600[3)] | | 50-200[3)] 50-100[4)] | |
| | wartości referencyjne | | | | | | | | | | |
| Hair | N | 13 | 8 | 13 | 8 | 12 | 8 | 12 | 8 | 13 | 8 |
| (ppm in dry matter) | $\overline{X}$ | 640.15 | 732.63 | 144.31 | 147.50 | 112.27 | 148.99 | 301.74 | 394.75 | 101.38 | 97.45 |
| Sierść | Sd | 159.03 | 412.64 | 34.83 | 69.25 | 103.15 | 169.35 | 210.65 | 308.59 | 37.19 | 80.54 |
| (ppm w suchej masie) | reference values | 1 000-25 000[3)] | | 200-300[3)] | | 430[3)] | | 2 000-8 000[3)] | | 130-455[3)] | |
| | wartości referencyjne | | | | | | | | | | |

* - European bison with *balanoposthitis* - żubry z *balanoposthitis;* ** - European bison without *balanoposthitis* - żubry bez *balanoposthitis*
[1)] -acc. to Kuleta [10] -wg Kuleta [10]; [2)] - acc. to Winnicka [B] - wg Winnicka [13]; [3)] - acc. to Puls [12] for cattle - wg Puls [12] dla bydła; [4)] - acc. to Puls [12] for bison - wg Puls [12] dla bizona

Table 3 - Tabela 3
Levels of micro minerals in blood serum, liver, kidneys and hair of European bison with and without *balanoposthitis*
Poziom mikroelementów w surowicy krwi, wątrobie, nerkach i sierści żubrów z *balanoposthitis* i bez *balanoposthitis*

| Tissue<br>Tkanka | Parameter<br>Parametr | Cu | | Fe | | Zn | | Mn | | Se | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1* | 2** | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Blood serum (umol/1)<br>Surowica krwi (umol/1) | N | 8 | 15 | 8 | 15 | 8 | 15 | 8 | 14 | 10 | 14 |
| | $\overline{X}$ | 11.59 | 10.52 | 35.89$^{B}$ | 56.37$^{A}$ | 10.54 | 13.18 | 0.12 | 0.12 | 0.32 | 0.25 |
| | Sd | 3.53 | 3.85 | 8.14 | 18.13 | 3.94 | 2.79 | 0.03 | 0.03 | 0.07 | 0.11 |
| | reference values<br>wartości referencyjne | 12.2-18.9[1)] | | 17.9-35.8[1)]<br>21.5-35.8[2)] | | 12.2-45.9[1)] | | 0.36-1.82[1)] | | 1.27-2.54[1)] | |
| Liver<br>(ppm in fresh tissue)<br>Wątroba<br>(ppm w świeżej tkance) | N | 12 | 11 | 7 | 5 | 13 | 13 | 13 | 14 | 13 | 14 |
| | $\overline{X}$ | 5.38 | 5.37 | 104.93 | 78.06 | 63.36 | 45.24 | 3.56 | 3.60 | 0.15 | 0.17 |
| | Sd | 1.73 | 2.73 | 38.83 | 35.48 | 31.61 | 18.25 | 0.66 | 0.75 | 0.02 | 0.03 |
| | reference values<br>wartości referencyjne | 25-100[3)/ 4)] | | 45-300[3)/ 4)] | | 25-100[3)/ 4)] | | 2-6[3)] | | 0.25-0.50[3)] | |
| Kidneys<br>(ppm in fresh tissue)<br>Nerki<br>(ppm w świeżej tkance) | N | 13 | 14 | 7 | 5 | 13 | 14 | 13 | 14 | 11 | 9 |
| | $\overline{X}$ | 6.57 | 7.21 | 88.54 | 81.80 | 17.16 | 20.36 | 1.04 | 1.07 | 1.06 | 1.29 |
| | Sd | 0.99 | 0.84 | 27.82 | 31.87 | 1.95 | 6.26 | 0.21 | 0.15 | 0.32 | 0.39 |
| | reference values<br>wartości referencyjne | 4-6[3)/ 4)] | | 30-160[3)/ 4)] | | 18-25[3)/ 4)] | | 1-2[3)] | | 1.0-1.5[3)] | |
| Hair<br>(ppm in dry matter)<br>Sierść<br>(ppm w suchej masie) | N | 13 | 8 | 13 | 8 | 13 | 8 | 13 | 8 | _ | _ |
| | $\overline{X}$ | 4.39 | 5.48 | 25.64 | 34.60 | 99.65 | 107.65 | 15.39 | 12.09 | - | - |
| | Sd | 1.17 | 1.86 | 22.84 | 22.29 | 8.71 | 10.30 | 8.27 | 10.48 | - | - |
| | reference values<br>wartości referencyjne | 6.7-32[3)] | | 59-200[3)] | | 100-150[3)] | | 0.5-70[3)] | | - | |

* - European bison with *balanoposthitis* - żubry z *balanoposthitis;* ** - European bison without *balanoposthitis* - żubry bez *balanoposthitis*
[1)] -acc. to Kuleta [10] -wg Kuleta [10]; [2)] - acc. to Winnicka [13] - wg Winnicka [13]; [3)] - acc. to Puls [12] for cattle - wg Puls [12] dla bydła; [4)] - acc. to Puls [12] for bison - wg Puls [12] dla bizona
A, B - at P≤0.001; A, B - przy P≤0.001

The concentration of Ca in the liver of bulls with *balanoposthitis* was at the low end of the reference values for cattle. The level of inorganic P in blood serum in both groups of bison exceeded the reference values [10]. However in the kidneys, these levels were found to be below the reference values [12]. The level of Na in the kidneys of both groups of European bison considerably exceeded the respective value for cattle. This also applies to the concentrations of K in blood serum in both groups and K in the kidneys of bison with *balanoposthitis.*

The concentrations of micro minerals in tissues of animals with and without *balanoposthitis* were similar, but a significantly higher serum concentration of Fe was found in animals without the disease (Table 3). Low levels of micro minerals were present in the hair of the animals. The contents of Zn in the bison without *balanoposthitis* and Mn in both groups of animals were in agreement with the reference values. The levels of Cu and Fe in the hair of both groups and of Zn in the animals with *balanoposthitis* were found to be below the reference values. In both groups, the levels of Fe in blood serum exceeded the reference values; Mn serum concentration was found to be below these values. Levels of Cu and Se in blood serum and in liver were also lower than in cattle. The concentration of Zn in blood serum and kidneys of males with *balanoposthitis* was found to be below the reference values. The level of Cu in the kidneys in both groups of the European bison exceeded reference values.

The differences in the concentrations of biochemical parameters measured in the tissues of animals with and without *balanoposthitis* were not statistically significant. In contrast to our findings, Gill |2] observed a lower level of serum glucose in diseased animals. Kita et al. [6] reported higher levels of urea in blood serum of animals with *balanoposthitis.*

In our study (Table 1), high levels of serum glucose were found. They were up to four times higher than the reference values for cattle and also considerably higher than levels of glucose in blood serum of bison reported by Hawley & Peden [4]. In earlier studies, conducted on the same population, Dziąba et al. [1], Wołk & Józefczak [15] and Gill [2] did not find glucose levels higher than the reference values. The earlier study of Wołk & Krasińska [16] reported levels of glucose in blood serum similar to our findings, with a simultaneous considerable decrease in amylase activity which would indicate a disorder in carbohydrate metabolism in the European bison. Low tissue levels of Mn, Zn and Cu (see Table 3), minerals which participate in glucose metabolism, may be implicated in altering the carbohydrate transformation processes. Mn is an activator of glycosinotransferase, kinase and decarboxylase - enzymes regulating metabolism of glucose and other carbohydrates. Zn is found in glucagon and insulin. Cu has an influence on the regulation of glucose metabolism [5, 7].

The high level of glucose in animal blood serum may also be caused by inflammation of the kidneys [12], as observed in numerous cases in European bison from the Białowieża Forest. In agreement with our studies, Wołk & Krasińska [16] did not find any differences in the glucose content in blood serum in European bison with *balanoposthitis* and without this disease. Also Dziąba et al. [1] did not observe such differences.

Low levels of serum albumins reported in our studies, suggesting insufficient supply of dietary nitrogen in European bison, did not correspond with the findings of Dziąba et al. [1] and Wołk & Krasińska [16]. Despite there being no significant differences in the levels of biochemical parameters in the European bison, with or without the disease, it should be noted that a higher level of urea and total protein but lower concentration of glucose and albumins in blood serum in bison with *balanoposthitis* was observed.

The only significant difference between the two groups of animals, with respect to micro and macro minerals, is a higher level of Fe ($P \leq 0.001$) in the male European bison without *balanoposthitis,* exceeding the upper reference values for cattle [10, 13]. In the diseased group, the level of Fe was found to be on the border of the upper reference values for cattle. In the studies of Dziąba et al. [1], who compared Fe concentrations in blood serum of healthy European bison to those in bulls with *balanoposthitis* or with other diseases, the levels of Fe in all three groups were within the range of the reference values for cattle. Those authors did not find significant correlation between the level of serum Fe and the occurrence of *balanoposthitis.*

The concentration of Fe in body tissues can influence susceptibility of the organism to infections. Fe plays a role in the growth of microorganisms. It is also involved in the functioning of the immunological system through its presence in transferrin receptors; granulocytes contain and release lactoferrin, macrophages and lymphocytes release transferrin and contain ferritin. Fe is necessary in the production of $H_2O_2$ and hydroxide radicals which determine the bactericidal properties of neutrophils. Fe activates the generation of antibodies and thus participates in the defense mechanism of animals [7]. A significantly lower serum level of Fe found in the European bison suffering with *balanoposthitis* may be considered as an indication of infection.

It should be noted that the contents of all elements analysed in bison hair, were found to be below the reference values for cattle reported by Puls [12]. The levels of all these elements, with the exception of Mg, were lower in healthy animals than in those with *balanoposthitis;* however the differences were not statistically significant. The Ca concentration in the blood serum of bulls with *balanoposthitis* was found to be on the lower border of the reference values; in the liver it was below these values. The level of K found in kidneys was above the reference values (Table 2).

In the present study, the level of Ca in blood serum of both groups of animals was higher as compared to the studies of Wołk & Krasińska [16] on the Białowieża bison, where it was found to be below the reference values for cattle. Dziąba et al. [1] suggested that the serum level of Ca may be dependent on the stage of plant vegetation.

The contents of Cu and Fe in the hair of both groups of the European bison and the Zn level in animals with *balanoposthitis* were found to be below the reference values.

Most of the concentrations of micro minerals in blood serum in animals studied were found to be below the reference values for cattle [10, 13]. This refers to the serum levels of Cu, Mn and Se in both groups of bulls examined (Table 3) and also to the level of Zn in animals with *balanoposthitis.* In earlier studies, conducted by Dziąba et al. [1], the levels of Zn and Cu in blood serum of healthy and sick males were found to be within the normal range for cattle. In addition, the contents of Cu and Se in the

liver and the level of Zn in the kidneys of the diseased bison were below the reference values for cattle.

Very low contents of Se and Zn in the tissues, probably resulting from a deficiency of these elements in the trophic chain, have an unfavourable effect on the immune system of the European bison.

As Se has an influence on glutathione peroxidase activity, its deficiency may result in peroxidative damage to the cells. Additionally, Se plays a significant role in the immune system since glutathione peroxidase is a biologically active component of leucocytes. Se directly affects T lymphocytes through lymphokine production. In respect to humoral resistance, Se has an influence on the stimulation of specific antibacterial *(E. coli)* and antifungal *(Aspergillus flavus)* antibodies [7, 11].

Also Zn plays a role in the immunological system, for example in stimulating biochemical processes leading to an increase in the serum levels of carotenes and vitamin A, which participate in the synthesis of antibodies. It is also responsible for protecting the epithelium against penetration by pathogenic microorganisms.

To increase animal resistance to infections from a nutritional point of view, it is advisable to introduce supplements of micro components such as Se, Zn and Cu into the diet, preferably in the form of salt licks.

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## Zawartość wybranych wskaźników biochemicznych i mineralnych w surowicy krwi i tkankach żubrów ze schorzeniem *balanoposthitis*

S t r e s z c z e n i e

W surowicy krwi żubrów ze schorzeniem i bez schorzenia *balanoposthitis* określono następujące wskaźniki biochemiczne: glukozę, białko całkowite, mocznik i albuminy oraz Ca, P nieorg.,Mg. Na. K. Fe, Cu, Zn, Mn i Se. W wątrobie i nerkach tych zwierząt analizowano zawartość - Ca, P, Mg, Na, K, Fe, Cu, Zn. Mn i Se, we włosach - Ca. P. Mg. Na. K, Fe. Cu. Zn i Mn. Różnice istotne statystycznie między żubrami ze schorzeniem i bez schorzenia *balanoposthitis* w zawartości wyżej wymienionych wskaźników wystąpiły jedynie w niższym poziomie Fe w surowicy krwi żubrów z *balanoposthitis.* Na uwagę zasługuje znacznie wyższy poziom glukozy w surowicy krwi zwierząt obydwu grup, znacznie przekraczający wartości referencyjne dla bydła.