

Has the condition of European bison deteriorated over last twenty years?

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Twelve biochemical values of the serum (concentrations of sodium, potassium, calcium, glucose, urea nitrogen, creatinine, total protein, and enzyme activities of ALP, CPK, AspAT, AlAT, amylase) were determined for 102 bison *Bison bonasus* (Linnaeus, 1758) coming from the free-ranging population of the Białowieża Forest and from the breeding enclosure. The animals were culled in the winter periods of 2000/2001 and 2002/2003. In adult males only the activity of AlAT is significantly higher than in adult females. The remaining values do not differ significantly between the sexes in any of the three age groups (I – calves, II – youngsters, III – adults). Also, no significant differences in serum parameters were observed between healthy males and the ones exhibiting symptoms of *posthitis/balanoposthitis*. It was established that the following changes in the values of serum parameters are age-dependent: a decrease in potassium and glucose concentrations and the enzymatic activity of ALP, and an increase in AspAT activity. The results were compared to the corresponding data from the research carried out 20 years earlier (1980–1984) and it has been discovered that the values of seven parameters are significantly different as opposed to the previous results. The following differences were established: currently the concentrations of potassium and urea nitrogen are higher in all age groups; the concentration of glucose is higher in the groups of calves and youngsters; creatinine concentration is lower in adults; the activity of AspAT has increased in youngsters, only; the activity of AlAT has increased, and of amylase has decreased in all animals examined. The changes in the values of serum parameters were analysed in the context of data obtained from post-mortem examinations and we conclude that those changes reflect the growing number of pathomorphological changes in internal organs of the bison dissected. Changes localised mainly in the liver and lungs could be related to parasitical infestations; others were noted in kidneys and in the prepuce and penis area in the males with *posthitis/balanoposthitis* symptoms. In the discussion it is suggested that the deterioration of the physical condition of European bison inhabiting the Białowieża Forest may be connected to such environmental and populational factors as: winter supplementary feeding, winter aggregations, and a decline in immunity related to inbreeding.

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Introduction

Growing numbers of the world population of bison and its relative dispersion diminish the threat of extinction for the species. However, there exists a risk

originating from the history of the *Bison bonasus*, ie a scant genetic diversity of the world population (Olech 1987, 1989, 2003, Belousova 1999, Pucek *et al.* 2002). A high level of inbreeding may lead to the occurrence of inbreeding depression, frequent genetic defects, and large susceptibility to diseases. Free-ranging populations are also subject to various local factors which may adversely affect physical condition of their individuals. Numbers in free-ranging populations cannot rise unlimitedly, as surpassing of the numerical strength optimal for a given habitat endangers both the environment and the animals. The bison population of the Białowieża Forest is reduced on average by 11% every year, since 1971 (Krasieński *et al.* 1999). Materials taken from the animals eliminated in the process have been used to study many bison blood parameters (eg Wołk 1983, 1990, Wołk and Józefczak 1984, 1988, Gill 1989, 1992, 1999, Dziąba *et al.* 1991, Kita *et al.* 1995).

The data on biochemical parameters of the bison serum published by Wołk and Józefczak (1984, 1988) came from studies carried out on materials obtained in the winter seasons 1980–1984 from bison eliminated in the procedure of numbers reduction in the free-ranging population and in the herd living in the breeding enclosure in the Białowieża Forest. The beginning of the 1980s was the time when the first cases of *posthitis/balanoposthitis*, a disease of the urinogenital system in males, were identified (Krasieński 1994, Krasieński *et al.* 1994). In the years from 1980 to 2002 the symptoms of the disease were observed in between 1.5 and 14.8% of all males in the population, on average in 6.5% of males each year (Krasieńska and Krasieński 2004). In this period, post-mortem examinations revealed that pathomorphological changes in bison internal organs occurred with an increasing frequency. A large proportion of those changes were due to infestations by internal parasites, especially *Fasciola hepatica* and *Dyctiocaulus viviparus*. Other pathomorphological transformations and developmental defects were also noted (Piusiński *et al.* 1997, Matuszewska and Sysa 2002, Krasieńska and Krasieński 2004).

The aim of this paper is to determine: (1) whether the values of biochemical parameters and activity levels of certain enzymes in the bison serum have changed since they were studied in the 1980s (Wołk and Józefczak 1984, 1988), and (2) whether the pathomorphological changes in internal organs observed during post-mortem examinations of culled European bison are reflected in those values.

Material and methods

Materials were obtained from 102 individuals, including 74 individuals (31 males and 43 females) from the free-ranging population and 28 individuals (9 males, 19 females) from the breeding enclosure in the Białowieża Forest. Because of the small number of bison, especially males, coming from captivity, the values of blood parameters in animals from the two categories are given jointly. The animals were divided into three age groups: calves (up to 1 year old), youngsters (between 1 and 4 years of age), and adults (over 4 years old). The individuals were culled in the process of reducing numbers of bison, effectuated by the Białowieża National Park in the winter periods (from December to March) of 2000/2001 and 2002/2003. The reasons for elimination varied and included: *posthitis/balanoposthitis* in males; poor physical condition; external deficiencies; various injuries, sometimes caused by poaching; aggressiveness towards humans (in case of bulls going out on farmlands); old age.

Except for the first of the reasons, the elimination criteria were the same in 1980s. The bodies were dissected and studied by a group of scientists made up of the National Park staff and other Polish and foreign academic institutions.

As most animals eliminated in the period under analysis turned out to possess pathomorphological changes of differing intensity in various internal organs, all the material was studied jointly, without differentiation into "healthy" and "sick" animals. The males were divided into two groups: exhibiting the symptoms of *prostatitis/balanoposthitis* (15 individuals) and the rest, diagnosed as free from the disease (25 individuals).

The data obtained were compared with the data on the same population accumulated 20 years earlier (1980–1984), in order to assess the changes in the state of health of the Białowieża bison population. Both sets of data were obtained from blood samples taken in the same time of the year, with the use of identical or analogical laboratory methods. The only exception is the values of ionised calcium concentration determined for this study, which cannot be directly related to the values of total calcium content determined earlier. Age groups were established along the same criteria. It all facilitates drawing conclusions and makes them more reliable.

Blood samples were taken from the cut jugular vein immediately after shooting the animal. The blood was instantly cooled, left to coagulate and centrifuged within a few hours. The serum was stored at a temperature of -20°C until the biochemical analyses were made, though no longer than a month.

Values of twelve biochemical parameters were determined. The concentrations of sodium, potassium, and calcium ions in the serum were assessed with the use of a AVL 988-4 ionoselective autoanalyser for electrolytes. The concentrations of glucose, urea nitrogen, creatinine, total protein, and the activities of alkaline phosphatase (ALP), creatinine kinase (CPK), aspartate aminotransferase (AspAT), alanine aminotransferase (AlAT) and amylase were measured by the use of a Humalyser, which is based on a highly sensitive photometric system. All the methods are recommended by the International Federation of Clinical Chemistry (IFCC).

The values of biochemical parameters in the three age groups and for each sex were compared with the use of analysis of variance (ANOVA) and Tukey's test. The Student's *t*-test was employed for analysing pairs of values determined for two groups of males: suffering from *prostatitis/balanoposthitis* and without the symptoms of it, and for analysing pairs of values obtained during the previous research in the 1980s and in the recent research of 2000–2003.

Results

There is just one serum biochemical parameter whose value is different in adult males and females: the activity of alanine aminotransferase (AlAT) (Table 1). The value is significantly higher in males ($F_{1,96} = 9.535$, $p < 0.01$). The serum of adult males also exhibits higher concentration of creatinine and higher activities of CPK and AspAT than the serum of adult females, but the differences are not statistically significant.

Mean values of four parameters differ significantly between age groups (Table 2). The concentration of potassium dwindles as the animals grow older ($F_{2,99} = 5.135$, the significant difference between age groups I and III: $p < 0.005$), and so does the content of glucose ($F_{2,99} = 11.552$, the significant differences between age groups I and III: $p < 0.000$ and II and III: $p = 0.005$). Alkaline phosphatase (ALP) activity level falls similarly ($F_{2,99} = 9.315$), and is the highest in the group of calves (the differences between mean values for age groups I and III: $p < 0.001$ and groups I and II: $p < 0.01$), unlike the activity of aspartate aminotransferase (AspAT), which reaches its maximum levels in the serum of adult animals ($F_{2,99} = 3.421$, significant difference between age groups II and III: $p < 0.05$).

Table 1. Comparison of mean serum biochemical parameters in adult males and females (age group III) of European bison from Białowieża Forest in the years 2000–2003. Significant difference was found for the value of ALAT (ANOVA-test: $p < 0.01$).

Parameter	Males ($n = 9$)			Females ($n = 20$)		
	Mean	SD	Range	Mean	SD	Range
Sodium (mmol/l)	133.27	17.17	99.5–150.9	144.04	21.98	86.4–197.2
Potassium (mmol/l)	7.50	2.76	4.9–12.9	7.77	2.09	3.9–13.1
Calcium (mmol/l)	0.78	0.14	0.5–1.0	0.92	0.37	0.1–0.4
Glucose (mmol/l)	2.91	0.72	1.3–3.8	3.80	3.10	0.1–13.6
Urea nitrogen (mmol/l)	10.86	6.65	3.9–26.1	10.45	4.85	2.5–20.3
Creatinine ($\mu\text{mol/l}$)	124.60	38.98	88.4–175.9	114.30	41.80	26.5–168.8
Total protein (g/l)	68.90	10.80	50.4–82.6	66.40	16.90	20.4–90.7
ALP (U/l)	56.99	30.12	21.2–80.2	57.06	52.26	21.2–229.0
CPK (U/l)	520.25	751.79	34.0–2298.0	274.22	283.96	81.0–1225.0
AspAT (U/l)	212.07	253.99	44.0–764.0	139.96	297.30	27.0–1395.0
ALAT (U/l)	51.30	28.99	30.0–125.0	30.87	17.35	8.7–82.0
Amylase (U/l)	23.17	22.10	5.0–32.9	17.43	25.70	4.0–109.6

The comparison between the values of serum parameters obtained in the 1980s and the corresponding values obtained recently reveals that values of seven parameters have significantly changed in individuals from the three age groups (Table 2, Fig. 1). Only the concentrations of sodium ions and total protein and the activities of ALP and CPK have remained on the same level, while all other parameters are different between two or more age groups, with varying significance (Table 2, Fig. 1). Calcium concentration has decreased and is now lower in all groups (but it represents only a part of total calcium content). The level of potassium is now, on the other hand, considerably higher in the three groups ($t_1 = 7.626$, $t_2 = 6.000$ and $t_3 = 4.737$, respectively, $p < 0.001$). Glucose content is higher in calves ($t = 6.441$, $p < 0.001$) and youngsters ($t = 3.007$, $p < 0.01$). The concentration of urea nitrogen has risen for all the groups ($t_1 = 5.725$, $p < 0.001$; $t_2 = 6.219$, $p < 0.001$; $t_3 = 3.337$, $p < 0.05$), while the concentration of creatinine decreased in adults ($t = 4.432$, $p < 0.001$). AspAT activity is higher now for all age groups, but only the increase in the second group is significant ($t = 4.429$, $p < 0.001$), given the high variability of this parameter. The activity of ALAT has increased in all age groups ($t_1 = 4.122$, $p < 0.001$; $t_2 = 3.994$, $p < 0.01$; $t_3 = 2.692$, $p < 0.01$), while the activity of amylase has declined in all the animals ($t_1 = 10.122$, $p < 0.001$; $t_2 = 4.977$, $p < 0.01$; $t_3 = 4.994$, $p < 0.001$) (Table 2, Fig. 1).

No statistically significant differences were discovered between the data on the serum of males suffering from *posthitis/balanoposthitis* and those on the animals free from it (Table 3). What is interesting, however, is an increase in creatinine level in the bulls with the symptoms of *posthitis/balanoposthitis*, and the higher activity of AspAT in the bulls not affected.

Table 2. Serum biochemical parameters (mean and SD) for European bison from Białowieża Forest in two periods: A = 1980–1984 (data after Wołk and Jóźefczak 1984, 1988) and B = 2000–2003. ANOVA and Tukey's HSD tests were used for comparing values of parameters in the three age groups of B-period. NS – non significant. For comparison of data from A- and B-periods see Fig. 1.

Parameter	Age group I (< 1 year old)				Age group II (1–4 years old)				Age group III (> 4 years old)				Significance of differences between age groups of B-period
	A (n = 33)		B (n = 46)		A (n = 15)		B (n = 27)		A (n = 28)		B (n = 29)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Sodium (mmol/l)	141.36	5.06	140.25	13.23	141.73	5.64	143.24	14.08	144.93	6.50	140.70	20.92	NS
Potassium (mmol/l)	6.68	1.26	9.42	1.93	5.21	0.76	8.65	2.81	5.40	1.23	7.68	2.27	I:III $p < 0.005$
Calcium (mmol/l)	2.14	0.27	0.97	0.26	2.12	0.23	1.04	0.26	2.04	0.44	0.88	0.32	NS
Glucose (mmol/l)	4.54	1.66	10.59	6.07	3.68	1.00	8.90	8.92	3.65	1.04	3.52	2.62	I:III $p < 0.000$; II:III $p < 0.005$
Urea nitrogen (mmol/l)	6.97	2.39	13.56	7.28	5.43	2.28	13.10	5.87	6.67	3.28	10.58	5.36	NS
Creatinine (μ mol/l)	109.62	35.36	105.20	29.17	128.18	30.06	134.37	32.71	155.58	38.90	117.57	40.66	NS
Total protein (g/l)	5.9.40	8.50	63.33	11.80	64.40	7.60	68.50	10.00	70.70	8.20	67.20	15.20	NS
ALP (U/l)	81.97	36.95	90.98	34.70	65.40	15.48	63.31	26.18	58.89	17.35	57.04	45.96	I:II $p < 0.01$; I:III $p < 0.001$
CPK (U/l)	455.30	711.46	666.77	872.25	435.20	420.58	349.78	434.28	425.68	697.36	352.95	482.00	NS
AspAT (U/l)	66.00	31.12	83.13	40.94	42.33	10.41	64.36	21.75	71.68	86.79	162.34	282.1	II:III $p < 0.05$
AlAT (U/l)	21.36	7.65	30.59	12.21	17.33	6.46	29.04	12.53	22.79	16.28	37.21	23.17	NS
Amylase (U/l)	69.70	31.98	12.22	7.61	61.60	37.19	13.07	8.79	72.85	51.54	19.21	24.39	NS

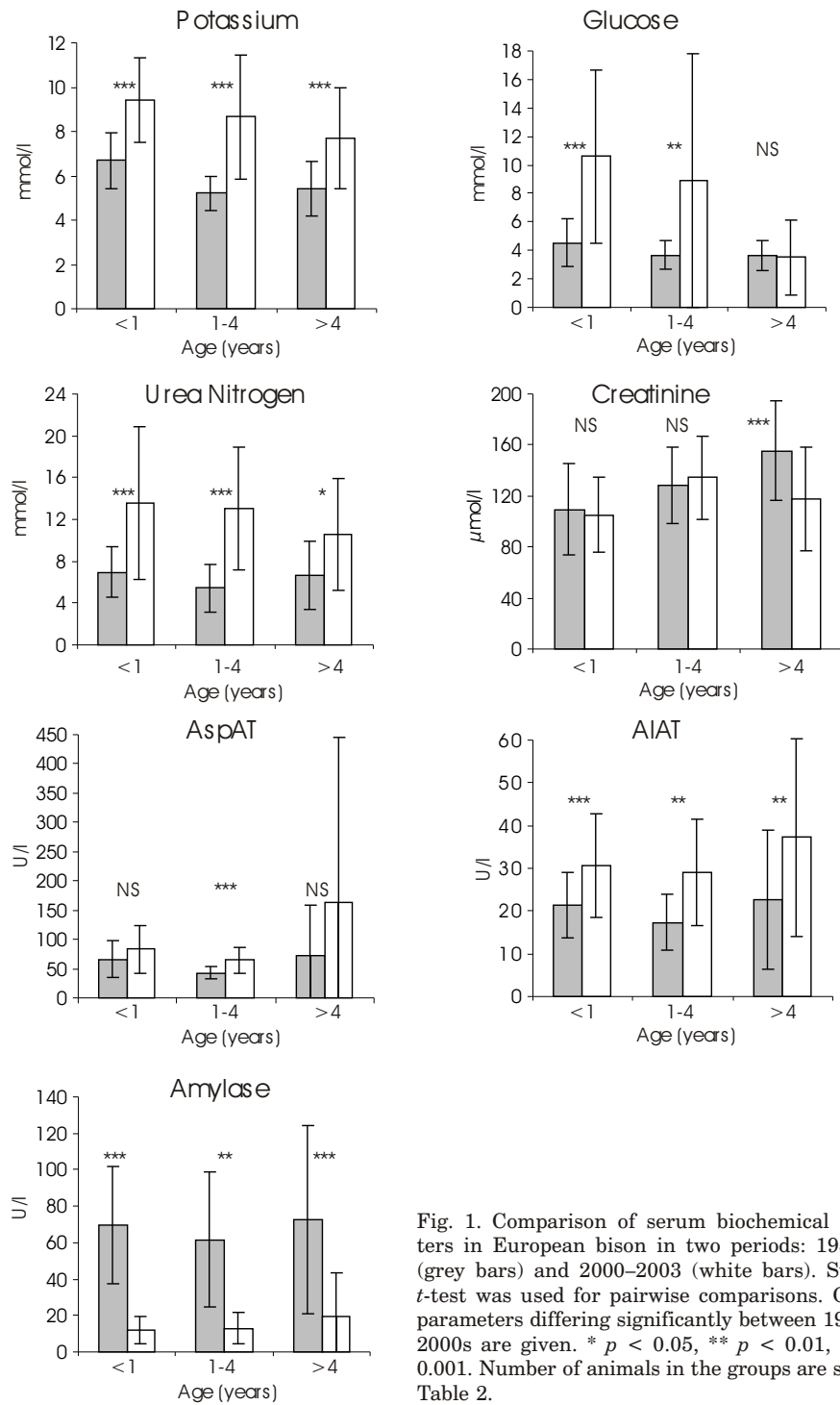


Fig. 1. Comparison of serum biochemical parameters in European bison in two periods: 1980–1984 (grey bars) and 2000–2003 (white bars). Student's *t*-test was used for pairwise comparisons. Only the parameters differing significantly between 1980s and 2000s are given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Number of animals in the groups are shown in Table 2.

Table 3. Serum biochemical parameters (mean and SD) in male European bison without symptoms of *posthitis* and suffering from *posthitis*. There were no significant differences when Student's *t*-test was used.

Parameter	Males without symptoms of <i>posthitis</i> (<i>n</i> = 25)		Males suffering from <i>posthitis</i> (<i>n</i> = 15)	
	Mean	SD	Mean	SD
Sodium (mmol/l)	138.33	14.35	141.70	16.54
Potassium (mmol/l)	8.38	2.49	9.02	2.60
Calcium (mmol/l)	0.96	0.20	0.92	0.32
Glucose (mmol/l)	7.17	6.34	7.62	7.83
Urea nitrogen (mmol/l)	12.71	6.98	12.54	5.51
Creatinine (μ mol/l)	116.69	35.71	126.59	34.39
Total protein (g/l)	66.80	8.90	67.44	13.23
ALP (U/l)	76.01	36.07	63.62	28.53
CPK (U/l)	490.78	744.42	519.75	573.95
AspAT (U/l)	129.57	164.91	75.47	34.89
AlAT (U/l)	36.78	23.53	35.56	12.82
Amylase (U/l)	14.24	14.82	12.05	6.33

Discussion

It is vital to assess the physical condition of free-ranging bison in the Białowieża Forest and the possible risks to their health, as further rational and efficient strategies of protection of the species need to be developed. The studied population, no longer reduced in numbers by large predators, inhabits the Białowieża Forest, a small remnant of the European primeval forest. The bison are fed with hay in wintertime, which leads to their gathering into extensive herds during that season, and this kind of prolonged aggregation in small areas may undermine their health.

It is commonly acknowledged in human and veterinary medicine that values of some biochemical serum parameters are correlated to physiological efficiency, immunity, and the state of nourishment. Undue increase or decrease in any of those values may constitute an important diagnostic indicator suggestive of physical disorders. The following part of the paper discusses the diagnostic significance of those biochemical serum parameters in bison whose values are considerably different than in the earlier research.

The main difficulty in making a correct interpretation of the values of biochemical serum parameters in European bison is the lack of reference values established for the species. The corresponding data on domestic cattle cannot be treated as applicable, although they may be used as a background reference. Peinado *et al.* (1999) quotes mean values of seven biochemical indices of the blood from twenty bison, but the animals lived in a zoological garden. That is why it

seems crucial to compare the biochemical parameters determined twenty years ago in free-ranging bison with the data obtained in this research. The results may constitute a basis for the assessment of the physical condition of European bison living in the Białowieża Forest at the present time. Data coming from researches carried out in Białowieża in other periods of the year than the winter and often with the use of different laboratory methods or without dividing animals into age groups (Dziąba *et al.* 1991, Kośła 1993, Kita *et al.* 1995, Kozło *et al.* 1997, Gill 1999) provide information which may serve to establish the range of variability of the studied parameters.

There has been a noticeable increase in the level of potassium in bison examined in Białowieża of all age groups over the last two decades (Table 2, Fig. 1). This level also considerably exceeds the values quoted for European bison in Dziąba *et al.* (1991) and Peinado *et al.* (1999) and the reference values for domestic cattle (Table 4). A heightened concentration of potassium in the serum (hyperkalemia) may be associated with kidney disorders, pneumonia, and pleurisy or/and acute pancreatitis. The kidneys of animals dissected often had cysts (in 18 individuals from 102 total), and many bison examined were diagnosed as having pulmonary helminthosis (nematodes) or pneumonia and bronchitis (26 individuals), usually attributable to parasitical infestation. The concentration of potassium in the animals recently examined in Białowieża decreases significantly with the increasing age of bison, but still remains high.

The level of calcium is strikingly low in opposition to this value in domestic cattle and to the American bison (Table 4), and is remarkably lower for all age groups than it used to be twenty years ago. It needs to be stressed, however, that the current data represent only the level of ionised calcium, which invalidates any statistical comparison with the results of the previous research when total calcium level was determined, inclusive of calcium chemically bound, especially with albumin. If it is assumed that the current results are reduced approx. by half (ionised calcium makes up about 46% of total calcium), then the concentration of calcium determined in this research would be still lower than the values quoted by Marler (1975) for American bison and by Winnicka (2002) for domestic cattle (Table 4).

Hypocalcemia may be symptomatic of nephrosis or renal insufficiency in the phase of urine forming, acute pancreatitis, or vitamin D deficiency. Dziąba *et al.* (1991) discovered a shortage of this element in the hay fed to bison in wintertime and concluded that this factor must be responsible for the deficiency of calcium observed in the animals' blood serum and hair. Blood samples examined in our research were taken in winter, when bison are fed with hay in large quantities. Kośła (1993), who also quotes low calcium levels in the bison serum, does not, however, confirm the occurrence of calcium deficit in the bison fodder.

The concentration of glucose is significantly lower in the group of adult animals than in other groups, and it is also lower than the values established by Peinado *et al.* (1999) in European bison and by Hawley and Peden (1982) in American bison,

but still falls within the scope of reference values for cattle (Winnicka 2002) (Table 4). The blood of suckling calves, which are technically not completely ruminant, normally contains a high amount of glucose, which lessens as they grow older and develop fermentation processes in the rumen, where monosaccharides from the diet are reduced to volatile fatty acids. However, a considerably higher level of glucose nowadays in the groups of calves and youngsters in comparison to the data from twenty years ago, along with a large variability of this parameter, are suggestive of carbohydrates metabolism disorders. A conspicuous decline in amylase activity in opposition to the data from the previous research points to the occurrence of such disorders as well; it may also be connected with necrosis of the pancreas, which was not, however, confirmed by autopsy results. The activity of amylase in European bison is currently lower than the corresponding reference value for domestic cattle (Table 4). This decline in amylase activity may be also ascribable to heavy metal poisoning (Winnicka 2002). High levels of lead, cadmium, chromium, and strontium were found in testicles of bison culled in the Belarussian part of the Białowieża Forest (Kozlo *et al.* 1997). However, findings published by Dmowski (1998) demonstrate that the concentrations of heavy metals in tissues from 13 European bison (from the Polish part of the Białowieża Forest) were generally low, and only kidneys of aged animals contained an increased amount of cadmium.

The concentration of urea nitrogen is currently higher in all age groups, and exceeds the values established in American bison and the corresponding reference values for cattle (Table 4). It is most likely associated with kidney disorders, or else with disorders of the cardiovascular system. In the results of the previous research, the level of urea nitrogen was commensurate with the corresponding reference value for domestic cattle (Wołk and Józefczak 1988). Recently observed increase in the concentration of urea nitrogen concurs with the occurrence of numerous pathomorphological changes in kidneys and myocardium noted during autopsies of the bison examined for this study.

The serum level of creatinine in bison remains stable throughout their life. All mean values of creatinine concentration are within the scope of the corresponding reference value for cattle.

The higher activity of alkaline phosphatase (ALP) in the serum of calves is an effect of increased osteoblastic activity in growing young animals. Analogically, in American bison the activity of ALP is higher in young animals than in adults (Hawley and Peden 1982). The value of this parameter has not changed since the 1980s, and is comparable to the corresponding reference value for cattle (Tables 2 and 4).

The activity of aspartate aminotransferase (AspAT) has risen in the group of youngsters from 1 to 4 years old, as compared to the value determined twenty years ago. In the group of adults it exhibits a great variability and possesses a high mean value, significantly exceeding the value established for youngsters and the corresponding reference value for cattle, although it is lower than the value for American bison (Table 4). This indicates to disorders of the liver and/or to

inflammatory infections of the pancreas. An increase in the activity of alanine aminotransferase (AlAT), of the same aetiology, occurs in all age groups, and is larger in males than in females, which goes along with the results of post-mortems revealing pathomorphological changes of the liver. The majority of those transformations may be attributed to infestation with intestinal parasites. Changes in the liver due to the infestation with *Fasciola hepatica* were observed in 26% of all the animals examined, while in 42% of the bison infested the activity of AlAT was higher than average and the activity of ALP (also indicative of the state of the liver) was higher than average by 27%. The levels of AspAT, AlAT, and ALP activity determined in this study are higher than the levels quoted by Gill (1992), although comparable to the levels established for American bison (Table 4).

The data given above evince a growing extent of parasitological infestations and the violation of balance between parasite and host, still observed in the 1980s (Wołk 1990). Parasitologic researches carried out in recent years demonstrate an increasing extensiveness and intensity of parasitological infestations in the bison population of the Białowieża Forest. Currently, the collection of helminths identified in the bison from the free-ranging population of the Polish part of the Białowieża Forest is made up of 33 species, including 21 species of gastro-intestinal nematodes (Drózdź 2000, Drózdź *et al.* 2002). In comparison to the animals living in captivity, helminthofauna of free-ranging bison is larger by 11 species characteristic of the deer family (Drózdź 2000). Two dangerous helminths are common in European bison: *Fasciola hepatica* (infests 44% of the population) and *Dictyocaulus viviparus* (58% of the population) (Drózdź *et al.* 2002).

Biochemical parameters of the serum in bulls with the symptoms of *posthitis/balanoposthitis* constitute a separate matter. The disease is limited to organs of the urinogenital system, and most of the males exhibiting the symptoms were in a good general condition (Kraśniński 1994, Kita *et al.* 1995, Gill 1999). No significant differences between the values of blood parameters in affected and healthy males were observed. There is a statistically insignificant difference: the males free from the symptoms of *posthitis/balanoposthitis* had a higher activity of AspAT in comparison to the males with the disease. This difference in AspAT activity may be connected to the fact that more males without *posthitis* had pathomorphological changes in liver or lungs due to parasitic infestations.

Comprehensive research on *posthitis/balanoposthitis* in male European bison in both Polish and Belarussian populations of the Białowieża Forest (Kita *et al.* 1995, Krasochko *et al.* 1997, Piusiński *et al.* 1997, Jakob *et al.* 2000, Borchers *et al.* 2002, Thiede *et al.* 2002) has failed to determine the primary aetiology of the disease, but the factors responsible for the development and spreading of *posthitis/balanoposthitis* are now relatively well known. An increase in the activities of AspAT and AlAT and heightened levels of α - and γ -globulin in males affected with *posthitis/balanoposthitis* were observed in the Belarussian bison population (Krasochko *et al.* 1997). Wolf *et al.* (2000) stated that the aetiology of the disease is potentially connected to winter aggregations of bison in small areas in the forest and to their

immunodeficiency, especially given the genetic bottleneck effect occurring in the species. Possibly, arthropods inflict micro-lesions in the prepuce and in many cases transmit pathogenic agents and subsequently infections with various bacteria prompt the development of the disease.

By way of general assessment of the state of health of the bison whose blood was examined it has to be stressed that only 7 individuals out of 102 total did not exhibit pathomorphological changes in internal organs. In the remaining animals such changes were usually located in lungs, kidneys, and the liver, and, in the males afflicted with *posthitis/balanoposthitis*, in the area of prepuce and penis. Frequently, one individual had such changes in several organs.

A growing number of pathomorphological changes, usually caused by parasitical infestations (lung and gastro-intestinal nematodes), in internal organs of European bison examined lately may be related to the phenomenon of bison winter aggregations around the feeding points, occurring each winter over a period of many years. Prolonged, lasting about 4-5 months gatherings of the animals in small areas may be conducive to the transmission of pathogens and parasites. Numerous attempts to disperse such aggregations made by the Białowieża National Park have so far been unsuccessful. However, the deterioration of bison's condition manifest in recent years creates an urgent need for an effective method of doing it.

On the other hand, the deterioration of bison's state of health in the Białowieża Forest may be brought about by intensive inbreeding (Olech 1987, 1989, 2003, Pucek *et al.* 2002) and the resulting inbreeding depression, leading to an impairment of the immune response in the animals. The mean inbreeding coefficient in lowland European bison rose from 0.399 in 1980s to 0.480 in 1996–2002 (Olech 2003).

In conclusion, it needs to be stated that values of biochemical serum parameters constitute a reliable indicator of bison's physical condition and may be used for this purpose in comparative studies in the future. The deterioration of the state of health of European bison inhabiting the Białowieża Forest is possibly related to such environmental and populational factors as winter foddering and the resulting winter aggregations and continuous inbreeding and the resulting decline in the animals' resistance to diseases.

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