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BISONIANA XCVI

Concentrations of Creatine, Creatinine and Phosphorus in Skeletal Muscles of the European Bison

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A total of 66 bison were under study, both males and females, at ages ranging from 1.5 months to 21 years. These were animals eliminated from the population as a result of sanitary shots carried out in several breeding centres of Poland in 1982—1985. The concentration of creatine and its proportion in the pool of total nitrogen of muscles and variability of these traits were higher in bison than in cattle and in bison and cattle hybrids. No significant effect of sex or age on these traits was recorded. Some results showed a higher concentration of creatine and its higher proportion in the nitrogen pool of muscles in bison at the end of winter than at the end of spring. This suggests that the content of creatine in bison muscles is relatively stable, and changes of its concentration are of secondary character, resulting from the loss of labile muscle components in winter and their recovery in spring.

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1. INTRODUCTION

The earlier studies carried out on pigs (Witkowska & Grajewska, 1982) have shown that hybrids, as compared with pure races, are characterized by a higher concentration of creatine in muscles. Also in domestic cattle (Różyczka & Witkowska, 1984) the offspring of fathers with muscle hypertrophy and mothers of lowland black and white race had a higher concentration of creatine in muscles than the parents. In hybrids of male bison and domestic cows (F_1), the concentration of creatine in muscles was higher than in cattle (Witkowska & Grajewska, 1982; Witkowska 1985). This suggested that interspecies hybrids, like interrace hybrids, can have higher concentrations of creatine in muscles than the parental forms.

The purpose of the work has been to characterize concentrations of creatine, creatinine, and phosphorus, and also the pool of total nitrogen in muscles of bison. The results were compared with the earlier estimates for cattle and for bison and cattle hybrids (Witkowska, 1985; Witkowska

& Grajewska, 1982). Large differences in the age of the eliminated bison allowed an analysis of the effect of this factor on the muscle components under study.

2. MATERIAL AND METHODS

Bison were obtained as a result of sanitary shots carried out in Poland in 1982—1985 at Białowieża, Borki, Pszczyna, Niepołomice, Gołuchów, and Forest Inspectorate Wałcz. The age of these bison ranged from 1.5 months to 21 years. Samples were taken of *musculus longissimus dorsi (Ld)* from one embryon and 66 bison, including 36 males and 30 females, *musculus semimembranosus (Sm)* from 30 bison, and also *musculus semitendinosus (St)* and *rectus femoris (Rf)* from 20 bison. The bison shot were transported to forester's lodge, and then the skin and bowels were removed. The samples were taken from 1 to 2 hours after shooting. The samples were cooled and transported to the city of Bydgoszcz in thermoses with dry ice.

The concentration of creatine was estimated using the diacetyl method with alpha naphthol. The concentration of creatinine was estimated by the picrate method, that of inorganic phosphorus (Pi) and total phosphorus (Pt) by the molybdate method. Using the sum of the creatine and creatinine nitrogen, the nitrogen of total creatine (Nc) was calculated, and expressed as a percentage of the total nitrogen (Nt), determined by the Kjeldahl method.

3. RESULTS

Since the number of variable factors was large, their effects could not be analysed concurrently, thus successive comparisons were made. There were no sex-related differences in the components of the muscle *Ld*. Only the proportion of Nc in the pool of Nt was a little higher in males but statistically not significant (Table 1). Thus, for further comparisons, males and females were pooled.

The bison were grouped into 7 age classes (Table 2). The youngest bison, at an age of 1.5 to 7 months, had a higher concentration of Pt in the muscle *Ld* than the other groups. A higher concentration of creatine was observed at an age of 5—10 months, and a higher proportion of Nc in the pool of Nt occurred at the age of 8—10 months, suggesting an increase in the first period of life and a decrease in the final period of growth. These differences, however, were not statistically significant, thus the effect of age was not more important than the effect of other factors.

Mean results for bison from particular eliminations illustrate the effect of breeding centres and time of the year (Table 3). As the number of animals from particular eliminations was small and unequal, and since

Table 1

Concentration of creatine, creatinine, inorganic (Pi) and total (Pt) phosphorus and ratio of creatine nitrogen (Nc) to total nitrogen (Nt) in *longissimus (Ld)* muscle of European bison, dimestic cattle and their hybrids.

Item	Creatine mg/g			Creatinine mg/g		Nc, % Nt		Pi, mg/g		Pt, mg/g		
	n	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*
European bison, ♂♂	36	6.09	0.32	0.113	0.041	5.84	0.49	0.95	0.15	27	2.18	0.12
♀♀	30	5.96	0.40	0.111	0.036	5.57	0.38	0.94	0.16	24	2.13	0.14
Total, ♂♂+♀♀	66	6.03	0.36	0.112	0.039	5.72	0.46	0.95	0.16	51	2.16	0.13
Domestic cattle, ♂♂+♀♀	40	5.21	0.25	0.110	0.017	4.94	0.25	0.83	0.07	40	1.92	0.09
European bison and domestic cattle hybrids, ♂♂	10	5.56	0.13	0.110	0.007	5.39	0.17	0.97	0.26	—	—	—
♀♀	10	5.42	0.24	0.137	0.014	5.28	0.14	0.96	0.04	—	—	—
Total, ♂♂+♀♀	20	5.49	0.21	0.124	0.017	5.34	0.16	0.96	0.18	—	—	—

Table 2

Effect of age on concentration of creatine, creatinine, Pi, Pt and on Nc to Nt ratio in *Ld* muscle of European bison.

Group	Age	Number of animals			Creatine mg/g		Creatinine mg/g		Nc, % Nt		Pi, mg/g			Pt, mg/g	
		n	♂♂	♀♀	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*
0	Fetus (8—9 months)	1	—	1	3.58	—	0.034	—	5.35	—	0.56	—	1	1.44	—
1	1,5—4 months	8	5	3	6.02	0.30	0.143	0.010	5.56	0.20	1.05	0.13	5	2.26	0.06
2	5—7 months	10	7	3	6.12	0.30	0.116	0.040	5.74	0.48	0.95	0.16	8	2,26	0.10
3	8—10 months	11	4	7	6.10	0.34	0.109	0.038	5.92	0.54	0.93	0.21	10	2.19	0.10
4	12—18 months	7	5	2	5.83	0.32	0.081	0.046	5.62	0.54	0.98	0.12	6	2.14	0.12
5	24—30 months	10	5	5	5.99	0.53	0.107	0.038	5.88	0.53	0.95	0.15	6	2.11	0.16
6	3—7 years	13	9	4	6.07	0.35	0.114	0.038	5.64	0.41	0.87	0.11	11	2.08	0.09
7	10—21 years	7	—	7	5.89	0.39	0.116	0.037	5.47	0.35	0.97	0.15	5	2.04	0.14

Table 3

Concentration of creatine, creatinine, Pi, Pt and Nc to Nt ratio in *Ld* muscle of European bison from different breeding centres.

Breeding centre	Sampling date	Number of animals	Creatine mg/g		Creatinine mg/g		Nc, % Nt		Pi, mg/g		Pt, mg/g		
		n	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*	$\bar{x}$	*s*	*n*	$\bar{x}$	*s*
Wałcz	1983.12.27	1	6.05	—	0.136	—	5.84	—	1.00	—	—	—	—
Pszczyna	1982.06.17	3	5.65	0.04	0.076	0.008	5.14	0.29	0.85	0.01	3	1.90	0.06
Niepołomice	1982.11.04	5	6.01	0.31	0.057	0.027	5.37	0.14	0.91	0.12	5	2.17	0.10
Gołuchów	1984.04.11	6	6.24	0.22	0.117	0.017	5.98	0.20	1.02	0.07	6	2.16	0.07
Borki	1982.04.22	4	5.50	0.57	0.031	0.004	5.95	0.56	0.99	0.13	4	1.98	0.13
	1982.11.30	3	5.75	0.42	0.104	0.006	5.00	0.40	1.03	0.05	3	2.26	0.12
	1983.02.28	3	5.86	0.44	0.091	0.006	5.44	0.47	1.00	0.12	—	—	—
	1984.02.29	5	6.06	0.28	0.124	0.019	5.75	0.39	0.90	0.26	5	2.21	0.12
Total	—	15	5.81	0.44	0.089	0.037	5.59	0.54	0.97	0.16	12	2.15	0.16
Białowieża	1982.06.01	1	5.98	—	0.077	—	5.44	—	0.74	—	1	2.02	—
	1983.01.25	5	5.86	0.05	0.086	0.008	5.53	0.19	0.69	0.09	5	2.22	0.10
	1983.03.14	3	5.56	0.13	0.090	0.014	5.06	0.08	0.85	0.10	—	—	—
	1983.12.09	11	6.13	0.32	0.146	0.010	5.70	0.23	1.00	0.11	6	2.25	0.06
	1984.01.17	6	6.09	0.32	0.154	0.016	5.69	0.29	1.12	0.09	6	2.09	0.15
	1984.03.20	7	6.39	0.21	0.130	0.015	6.24	0.21	0.91	0.13	7	2.17	0.11
	1985.03.18	3	6.28	0.23	0.162	0.008	6.59	0.23	—	—	—	—	—
Total	—	36	6.09	0.34	0.130	0.030	5.72	0.39	0.94	0.17	25	2.17	0.12

many variable factors could interact, variance among them was not calculated. A lowest concentration of creatine in the muscle *Ld*, amounting to 5.65 ± 0.04 mg/g, was found in the bison from Pszczyna, shot on 17 June, 1982, and a highest concentration of 6.24 ± 0.22 mg/g occurred in the bison from Gołuchów, shot on 11 April, 1984. The difference in the season is only two months, but this is the period of greatest changes in nature in respect to food supply. Differences in the above results can be explained by the effect of the breeding centres compared, time of the year, year-to-year changes and, in addition, criteria used for the elimination of particular individuals from the herd.

Table 4

Concentration of creatine, Pi and Pt in several muscles of European bison

Items	n	*Longissimus dorsi*		*Semitendinosus*		*Semimembranosus*		*Rectus femoris*		F
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	
Creatine, mg/g	30	6.20	0.43	—	—	6.33	0.45	—	—	1.32
Pi, mg/g	30	0.93	0.14	—	—	0.99	0.16	—	—	2.68
Pt, mg/g	20	2.15	0.12	2.16	0.11	2.26	0.08	2.20	0.09	4.39 [1]

[1] $p<0.05$.

No significant differences in the concentration of creatine and Pi were noted between the muscles *Ld* and *Sm* (Table 4). When the concentration of Pt was compared in 4 muscles, the highest valueas were obtained in the muscle *Sm* (Table 4). The lowest concentration of Pt in the muscle *Ld* was observed in bison from Pszczyna, shot on 17 June, 1982, and the highest concentration in bison from Borki, shot on 30 November, 1983, and from Białowieża, shot on 9 December, 1983 (Table 3). The interpretation of these results is difficult since the interaction of all variable factors may be involved here.

4. DISCUSSION

In vivo about 80% of creatine occurs in the form of phosphocreatine (CP), and the end product of its conversion, creatinine, is quickly excreted with urine so that its concetration in muscles is very low (Beard, 1943; Bursuk & Dubnoff, 1947). *Post mortem*, CP is quickly converted into creatine (Van Pilsum & Hiller, 1959; Bendal, 1979), and creatine is very slowly converted into creatinine (Witkowska, 1981). In the bison under study, the mean concentration of creatinine in the muscle *Ld* was

0.112 mg/g, and it is similar to those in cattle and their hybrids (Table 1), and in pigs (Witkowska, 1981, 1982). The concentration of creatinine ranged from 0.028 to 0.170 mg/g, thus the fluctuations were much higher than in other species. This could have been an effect of less standard conditions with respect to meat sampling, it preservation and transportation (Dahl, 1962; Witkowska, 1981).

No sex-related differences were found in the content of the components under study for bison, like for bison and cattle hybrids, domestic cattle (Różyczka & Witkowska, 1984) and pigs (Witkowska & Grajewska, 1974; Witkowska & Kotik, 1984). In 66 study bison, the mean concentration of creatine in the muscle *Ld* was 6.03 mg/g and ranged from 4.88 to 6.66 mg/g, whereas the mean proportion of Nc in the pool of Nt was 5.72%, and it ranged between 4.62 and 6.85%. The earlier studies on several species have shown that the proportion of Nc in the pool of Nt is more constant than the concentration of creatine in muscles (Witkowska & Grajewska, 1982). The present results show that the variation in the proportion of Nc in the pool of Nt in bison is similar to the variation in the concentration of creatine (Table 1).

Both the concentration of creatine and its proportion in the nitrogen pool of muscles are higher in bison than in cattle and their hybrids, and this does not confirm the suggestion that hybrids (between species and between races) are characterized by a higher concentration of creatine and a higher proportion of Nc in the pool of Nt than their parents.

The high concentration of creatine in muscles of bison may be species-specific as a result of a high concentration of protein and a low concentration of fat (Schulc *et al.*, 1971; Kotik, unpubl. data). Among the animals examined so far, a higher concentration of creatine was recorded only for Weddell's seals (6.36 ± 0.25 mg/g). Meat of these seals was characterized by a high concentration of protein, and due to this the proportion of Nc in the pool of Nt was at the same level as in cattle, pigs, and lambs (Witkowska & Grajewska, 1982).

So far, the study on the effect of age mostly concerned the period of growth, and they show that the concentration of creatine in muscles increases for a longer or shorter period after birth (Fisher *et al.*, 1956; Marechal & Plaghki, 1979). This can be explained by a reduction in the content of water (Bailey, 1969). Also a decrease in the concentration of creatine in muscles of cattle between months 4 and 12 of their lives was observed (Witkowska, 1985), and in muscles of pigs between weeks 18 and 30 of their lives (Witkowska & Grajewska, 1984). This can be explained by an intense system of raising, resulting in a "dilution" of creatine by labile components of muscles in the later period of growth. The most frequent reason for elimination of the youngest bisons was

their poor condition and presumably dehydration, which could lead to the increased concentration of creatine in group 1 (Table 2). The rearing of bisons, although they are supplied with additional food in winter, is rather extensive, and their growth is lower than in beef cattle. As a result, the concentration of creatine was not significantly changed in the final period of growth (Table 2). Although the material analysed was not homogeneous, the present results suggest that the concentration of creatine in muscles of bisons is relatively stable over their life span.

A high concentration of creatine can be observed in the cases of protein deficiency in the diet since growth rate is reduced and the deposition of labile components in muscles is inhibited (Witkowska & Smulikowska, 1985). Also in starved animals the concentration of creatine is high because labile components of the muscles are used at a higher rate (Kim *et al.*, 1983). The increased concentration of creatine observed in bison from Gołuchów in April, 1984, and from Białowieża in March, 1984 and 1985 can be explained by difficult conditions in winter, deficiency of proteins and other components of the diet, or emaciation caused by pathological factors.

A low concentration of creatine was recorded in bison from Pszczyna killed in June, from Borki in April, and from Białowieża in March (Table 3). The bison killed in June could have been in a better condition because they were feeding on young vegetation, rich in readily assimilated components, and this could account for an increase in the level of labile components of muscles and the "dilution" of creatine. The low content of creatine in bison killed in April and March could have been caused by pathological factors. In bison from Borki killed in April, the concentration of proteins in muscles *Ld* was less than 19%. This low concentration of protein suggests that these animals were more weakened than the others, and this accounted for the loss not only of labile components from muscles but also a part of reserves of solid nitrogen compounds, including creatine.

The concentration of Pt, like the concentration of Nt in muscles *post mortem* are relatively stable, at least for several days of keeping samples in good conditions. The decomposition of CP and other transformations of phosphorous compounds occurring in muscles shortly before slaughter, upon slaughter, and for 48 hours after slaughter lead to an increase in the concentration of Pi and a concurrent reduction in the concentration of organic phosphorus (Vogel *et al.*, 1985). In pigs killed directly after their transportation (tired) the concentration of Pi in the muscle *Ld* is about 40% higher than in pigs killed after a rest, and 48 hours after the slaugher by about 20% higher than 5 and 45 min after the slaughter (Witkowska, 1982). When different species are compared, the proportion

of Pi ranges from 40 to 66% of Pt (Witkowska & Grajewska, 1982). The concentration of Pi in the muscle *Ld* in bison is similar to that observed in bison and cattle hybrids and a little higher than in cattle (Table 1). The differences in the concentrations of Pi between successive eliminations could also result from variable conditions of transportation and preserving the samples and also from the stress due to shooting (Witkowska, 1982).

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STĘŻENIE KREATYNY, KREATYNINY I FOSFORU W MIĘŚNIACH SZKIELETOWYCH ŻUBRÓW

Streszczenie

Zbadano 66 żubrów, w tym 30 samic i 36 samców, pochodzących z odstrzałów sanitarnych przeprowadzonych w latach 1982—1985 w kilku ośrodkach hodowlanych Polski. Wiek żubrów wahał się od 1,5 miesiąca do 21 lat. Średnie stężenie kreatyny w mięśniu *longissimus dorsi (Ld)* żubrów wynosi 6.03±0.36 mg/g. Średni udział azotu kreatyny całkowitej (Nc) w puli azotu całkowitego (Nt) 5.72±0.73%, a stężenie fosforu całkowitego (Pt) 2.16±0.13 mg/g. Stężenie kreatyny i udział Nc w puli Nt w mięśniu *Ld* badanych żubrów było wyższe niż uprzednio wykazywano u bydła i żubroni (Tabela 1), jak również u kilku innych gatunków zwierząt, z wyjątkiem fok (Witkowska i Grajewska, 1982). Średnie stężenie kreatyniny i fosforu nieorganicznego (Pi) było natomiast zbliżone do obserwowanego u bydła i żubroni, jedynie standardowe odchylenie od średniej było wyższe u żubrów, na skutek większej zmienności pod względem czasu i temperatury przechowywania prób od odstrzału do analiz.

Nie wykazano istotnego wpływu płci (Tabela 1) i wieku (Tabela 2) na badane cechy, obserwowano jednak pewne tendencje do wyższych wartości u zwierząt młodszych. Różnice w stężeniu badanych składników mięśni obserwowane między poszczególnymi ośrodkami (Tabela 3) mogły być skutkiem wpływu pory roku. Porównanie żubrów z 7 eliminacji przeprowadzonych w Białowieży (Tabela 3) sugeruje, że zawartość kreatyny w mięśniach jest stosunkowo stała a wyższe stężenie kreatyny w mięśniach zwierząt pod koniec zimy w porównaniu z uzyskanymi pod koniec wiosny może być skutkiem utraty labilnych składników mięśni w okresie zimy i ich odbudowy w okresie wiosny.