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Mineral content in leg muscle and liver of coypu (*Myocastor coypus*)

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ABSTRACT

Leg muscle and liver samples of 25 coypu were used for analysis of mineral content. Total elements content was analyzed by flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS). The content of arsenic was analyzed by hydride generous atomic absorption spectrometry (HGAAS). Mean values and standard deviations of aluminium (Al), arsen (As), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), potassium (K), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr) and zinc (Zn) were calculated. For each mineral the contents in the liver were higher than in the muscle for all animals. The highest average contents had Na (muscle 2227.51 mg/kg, liver 4123.7 mg/kg) and K (muscle 1081.00 mg/kg, liver 3256.06 mg/kg). Zinc had the highest concentration of microelements in muscle, in decreased line follow the means of Fe, Cu, Mn, Se and Ni. The means of trace elements follow with Sr, Cd, Cr, Mo, Al, Co, Pb and As. The medians of the ratio content of liver to content of muscle were for Mn 5, for Mo and Se 4, for Cu and K 3, for Co and Fe 2.5, for the other minerals between 1.2 and 2. It couldn't be shown a correlation between liver and muscle mineral content. Some statistical significant differences between age and sex of coypu were found. These differences must be interpreted cautiously because of the situation of multiple tests.

Zawartość składników mineralnych w udźcu i w wątrobie nutrii (*Myocastor coypus*)

Słowa kluczowe: nutria, udziec, wątroba, zawartość składników mineralnych

STRESZCZENIE

Do analizy zawartości składników mineralnych wykorzystano próbki z udźca i wątroby 25 nutrii. Całkowitą zawartość składników analizowano metodami FAAS i ETAAS. Zawartość arsenu oznaczano metodą HGAAS. Obliczono wartości średnie i odchylenia standardowe glinu (Al), arsenu (As), kadmu (Cd), kobaltu (Co), chromu (Cr), miedzi (Cu), żelaza (Fe), potasu (K), manganu (Mn), molibdenu (Mo), sodu (Na), niklu (Ni), ołowiu (Pb), selenu (Se), strontu (Sr) i cynku (Zn). W wątrobie wszystkich zwierząt zawartość tych składników mineralnych była większa niż w ich udźcu. Największą średnią zawartość miał sód (Na): (udziec 2227,51 mg/kg, wątroba 4123,7 mg/kg) i potas (K): (udziec 1081,00 mg/kg, wątroba 3256,06 mg/kg). Największą koncentrację mikroelementów w udźcu miał cynk (Zn), innych średnie zmniejszały się w kolejności: Fe, Cu, Mn, Se i Ni. Średnie pierwiastków śladowych następowały w kolejności: Sr, Cd, Cr, Mo, Al, Co, Pb i As. Stosunek zawartości składników wątroby do składników udźca wynosił dla Mn 5, dla Mo i Se 4, dla Cu i K 3, dla Co i Fe 2,5, a dla innych składników między 1,2 i 2. Nie pojawiła się korelacja między zawartością składników mineralnych wątroby i udźca. Znaleziono niektóre, statystycznie istotne różnice, między wiekiem nutrii obu płci. Te różnice powinny być ostrożnie interpretowane z powodu sytuacji wielostronnego badania.

1. INTRODUCTION

The mineral content of animal muscle and liver is a standard for the physiological feeding. Sodium, potassium, iron and copper are parts of enzymes in the metabolism, and iron is the first element for the building of hemoglobin. On the other hand mineral content gives an example for incrimination of food with heavy metals caused by environment. In fact of contamination of the environment with cadmium, lead, mercury or arsenic the enrichment of heavy metals in meat and liver of animals were observed. Often the mineral content of bones, liver and kidney were higher than in muscle. The investigations about mineral content in meat were very rare for coypus.

2. LITERATURE

The biological cycles for toxic elements in the environment were studied by Wood [1, 2]. He had examined the toxicity of heavy metals. Iron cannot be critical for animals, but toxicity of soluble form were ascertained for cobalt, nickel, copper, zinc, tin, silver, cadmium, platin, gold, mercury, lead and arsenic. These toxic elements could accumulate in plants and in a secondary form in plant fed animals. Interactions between heavy metals and proteins, acidity of earth and contamination of air were factors for the incorporation and accumulation of critical contents in animals [3-6].

Wild animals were the best indicator organisms for the chronicle contamination of environment outside of industrial complexes [3, 6, 7]. Hare, rabbit, deer and sheep have a similar feeding as coypu and all animals which are endemic in a territory.

Kidney and liver of wild animals had often higher concentrations of lead, cadmium and mercury than those of domestic animals and as tolerated by FAO/WHO [8]. Kidneys concentration in relation to liver can be for cadmium at 10:1 to 11:1, whereas the meat content is low [6]. The origin of this effect is the accumulation of heavy metals with age [3, 6], the use of lead in petrol and the use of mercury-holding preparation of seeds before the 90th of the 20th century in Europe.

Wild meat which was shot with lead-holding ammunition showed higher concentration of lead. The bullet wound should be cut out in a wide range [8]. Reference values for wild meat are given in Austria: lead 0.25 mg/kg, cadmium 0.1 mg/kg, mercury 0.05 mg/kg.

Wild nutrias were investigated in USA [9]. The mean of ash content was lg/100 g wet weight. Iron content had an average value of 1.7 mg/100g, but the young animals had 1.2 mg, in contrast to the adults with 2.2 mg/100 g. Sodium content had an average value of 67 mg/100 g wet weight (young animals 64 mg/100 g, adults 70 mg/100 g). The mean of calcium was detected as 5.2 mg/100 g (young animals 5.6 mg/100g, adults 4.7 mg/100 g).

Mineral content of muscle homogenate of 15 female standard coypus at the age of 8 months was estimated. The highest concentration of macro-elements in dry matter was found in calcium [10]. (Ca: 27.9 ± 1.64 mg/kg) and potassium (K: 4.9 ± 0.33 g/kg). Sodium (Na) had a content of 1.69 ± 0.06 g/kg and magnesium (Mg) 1.13 ± 0.15 g/kg. The highest concentration of microelements was found in iron (Fe: 211.55 ± 16.53 mg/kg) and zinc (Zn: 69.59 ± 4.8 mg/kg). Manganese (Mn) had a content of 10.87 ± 1.21 mg/kg, copper (Cu) 7.24 ± 1.0 mg/kg, cadmium (Cd) 0.44 ± 0.33 mg/kg, cobalt (Co) 0.59 ± 0.03 mg/kg and lead (Pb) 1.57 ± 0.04 mg/kg. In relation to mineral content in female mink the contents of K, Na, Zn, Cu, Cd and Co in coypus were equal, but Mn, Ca and Pb were higher, Fe lower in body of coypus [11]. Statistical significant differences of mineral content in Musculus biceps femoris and liver and between liver and muscle of farm foxes were found. In silver foxes and arctic foxes significant differences in mineral content of some organs were found [12].

3. MATERIAL AND METHODS

Leg muscles and livers of 25 coypus (6 male, 19 female in two groups of age: 10 - 18 and 32 - 37 months) were used for own experiments. Collected samples of material in polypropylene containers (min. 60 g) were transported to the laboratory. To eliminate water content in samples the material were spread in an even layer on Petri dishes and dried in a laboratory electric drier for 48 h (or longer) at 105±5°C. After that, dry samples were transferred to an agate mortar and ground, screened with 0.5 - 1 mm sieve and subsamples (3 g) were mineralized in a closed Mars 5 Xpress microwave sample mineralization system by CEM in a closed system using HNO_3 and H_2O_2.

Concentration of total elements content was analyzed by flame atomic absorption spectrometry (FAAS) with AA 280FS spectrometer (fast sequential capability and deuterium background correction) and electrothermal atomic absorption spectrometry (ETAAS) with AA280 Zeeman instrument delivers (fast transverse Zeeman background correction and GTA120 graphite furnace). The content of arsenic was analyzed by hydride generous atomic absorption spectrometry (HGAAS).

Hollow-cathode lamps (HCL), Varian and Perkin Elmer were exclusively used (lamps for one element only). For each analyzed element, the apparatus optimization procedure was performed. Content of elements in collected samples was determined with the procedures based on the guidelines for analyses of biological materials by atomic absorption spectrometry. Analyzed elements were selected mainly on the basis of their biological importance, after preliminary determination of 34 metals by ETAAS and FAAS as well as ICP-OES and ICP-MS.

Results were validated on the basis Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method with a Vista MPX apparatus by Varian in randomly selected samples (15). As additional verification of results, analysis of selected samples by accredited laboratories and comparison of the results was performed.

4. STATISTICS

PASW Statistics 17.0 was used for all statistical calculations. Because all samples were measured three times the repeatability of the method could be described with the help of coefficients of variation (CV). For the further analyses the mean values of the 3 measurements were used. Means and standard deviations (SD) were calculated for the 16 minerals measured in liver and muscle samples. Statistics were also given for subgroups split according to sex (male – female) and age (10-18 month – 32-37 months).

To analyze the relation between the results of liver and muscle the ratios liver/muscle were calculated. Because the distribution of a ratio cannot be assumed as symmetric, median, minimum and maximum is given. As a second descriptive statistic for the relationship between muscle and liver the coefficients of correlation were calculated.

The next steps involve the questions if there are obvious differences between sex and age. If the p-value of an appropriate t-test is lower than 0.05 differences were judged as remarkable in an explorative sense. In the tables the results are marked by *.

5. RESULTS

Table 1 shows the mean values and standard deviations for liver and leg muscle samples. For all 16 minerals the contents in liver were statistical significant higher than in muscle. The median of the ratio of liver to muscle content ranges from Sr (1.19) over Cu (2.85) and K (2.97) to Se (4.25) and Mn (5.09).

No statistical significant correlation between the measurements for liver and leg muscle could be shown.

In the following text the intervals mean ± standard deviation will be reported in addition to Table 1. The macroelement sodium had the highest content with 2128.75 mg/kg - 2326.27 mg/kg in muscle and 4052.25 mg/kg - 4195.15 mg/kg in liver. The second mineral was potassium with 1048.41 mg/kg - 1113.59 mg/kg in muscle and 3095.48 mg/kg - 3416.64 mg/kg in liver.

Among the microelements the contents of zinc had the highest values. The content was examined with 31.79 mg/kg - 39.13 mg/kg in muscle and 67.56 mg/kg - 75.84 mg/kg in liver. Iron content was detected at 10.26 mg/kg - 12.24 mg in muscle and 27.12 mg/kg - 29.64 mg/kg in liver. Copper content was registered by 1.95 mg/kg - 2.35 mg/kg muscle and 5.60 mg/kg - 6.78 mg/kg in liver. Manganese had a content of 0.52 mg/kg - 0.58 mg/kg in muscle and 2.65 mg/kg - 2.83 mg/kg in liver. Selenium was detected with 0.17 mg/kg - 0.23 mg/kg in muscle and 0.81 mg/kg - 0.91 mg/kg in liver. Nickel was detected with 0.81 mg/kg - 0.99 mg/kg in muscle and 1.53 mg/kg - 1.97 mg/kg in liver.

The highest values of trace elements were found for strontium with 0.70 mg/kg - 0.78 mg/kg in muscle and 0.84 mg/kg - 0.94 mg/kg in liver. Cadmium was detected with 0.20 mg/kg - 0.26 mg/kg in muscle and 0.35 mg/kg - 0.43 mg/kg in liver. Chrome content was 0.18 mg/kg - 0.24 mg/kg in muscle and 0.33 mg/kg - 0.39 mg/kg in liver. Molybdenum had 0.14 mg/kg - 0.18 mg/kg in muscle and 0.61 mg/kg - 0.69 mg/kg in liver. Aluminium was estimated with 0.09 mg/kg - 0.11 mg/kg in muscle and 0.16 mg/kg - 0.19 mg/kg in liver. Cobalt was found with 0.006 mg/kg – 0.009 mg/kg in muscle and 0.017 mg/kg - 0.020 mg/kg in liver. Lead was detected as 0.015 mg/kg - 0.020 mg/kg in muscle and 0.029 mg/kg - 0.036 mg/kg in liver. Arsenic was estimated with 3.52 µg/kg - 3.82 µg/kg in muscle and 5.41 µg/kg - 5.61 µg/kg in liver.

The comparisons of the subgroups show p-values of the t-tests which are less than 0.05 in sporadic cases. Old male coypus had higher values for cadmium than younger coypus both muscle and liver (Tab. 2). Young male coypus show higher values for copper and selene in muscle than old animals for copper and selene, nickel contents in muscle are higher for old coypus (Tab. 2). Young female coypus had larger content of cobalt than the old ones, and lower sodium both in liver (Tab. 3). Referring to weight three small p-values were found.

Though we found small p-values with the help of statistical tests these results should be interpreted in an exploratory sense, because it must be considered the problem of multiple tests on the same data. It also must be discussed for which population the sample is representative.

6. DISCUSSION AND CONCLUSIONS

The iron content in the own examinations were lower in leg muscles (11.25 mg/kg) than by Tulley et al. [9] 17 mg/kg and Mertin et al. [10] 211.55 mg/kg. For young coypus lower iron content was found than for adults [9]. In the own examinations it couldn't be shown a difference between age. The sodium contents in the own results were higher (2227.5 mg/kg) than by Tulley et al. [9]: 670 mg/kg and Mertin et al. [10]: 1690 mg/kg. A lower content in young coypus than in adults was found [9]. In own investigations female coypus show a higher sodium content for adults. Zinc content by Mertin et al. was higher than in own examinations [10]. The content of manganese, copper, cadmium, cobalt and lead in own examinations were lower than by Mertin et al. [10]. For minerals Al, As, Cr, Mo, Ni, Se and Sr the own experiments were the first investigations in muscle of coypus. The liver contents of mineral elements and their relation to content in muscle were examined for coypus as well the first time. Standard for the healthy examination of contaminants in human food with lead, cadmium and mercury is the "provisional tolerable weekly intake" of FAO/WHO from 1972th year [13, 14]. For chemical contaminants in meat exists a list

Table 1 Statistics of mineral contents in coypus (n=25)

Tabela 1 Statystyka zawartości składników mineralnych u nutrii (n=25)

Mineral	liver mean (SD)	muscle mean (SD)	ratio liver/muscle median (min – max)	correlation liver - muscle (p-value)
Aluminium (Al) [mg/kg]	0.17 (0.015)	0.10 (0.008)	1.73 (1.43 - 2.17)	-0.084 (0.690)
Arsen (As) [µg/kg]	5.51 (0.101)	3.67 (0.152)	1.50 (1.38 - 1.64)	0.054 (0.796)
Cadmium (Cd) [mg/kg]	0.39 (0.039)	0.23 (0.028)	1.71 (1.12 - 2.33)	0.078 (0.711)
Cobalt (Co) [mg/kg]	0.02 (0.002)	0.01 (0.002)	2.25 (1.80 - 4.00)	0.252 (0.224)
Chrome (Cr) [mg/kg]	0.36 (0.027)	0.21 (0.027)	1.71 (1.42 - 2.17)	0.352 (0.084)
Copper (Cu) [mg/kg]	6.19 (0.592)	2.15 (0.198)	2.85 (2.25 - 3.59)	0.153 (0.465)
Iron (Fe) [mg/kg]	28.38 (1.261)	11.25 (0.986)	2.55 (2.08 - 2.87)	0.277 (0.180)
Potassium (K) [mg/kg]	3256.06 (160.580)	1081.00 (32.586)	2.97 (2.79 - 3.57)	-0.171 (0.415)
Manganese (Mn) [mg/kg]	2.74 (0.089)	0.55 (0.032)	5.09 (4.42 - 5.90)	-0.028 (0.893)
Molybdenum (Mo) [mg/kg]	0.65 (0.038)	0.16 (0.018)	3.93 (3.21 - 4.85)	0.347 (0.089)
Sodium (Na) [mg/kg]	4123.70 (71.448)	2227.51 (98.763)	1.85 (1.69 - 1.99)	0.328 (0.109)
Nickel (Ni) [mg/kg]	1.75 (0.219)	0.90 (0.092)	1.93 (1.47 - 2.65)	0.047 (0.823)
Lead (Pb) [mg/kg]	0.03 (0.003)	0.02 (0.003)	1.87 (1.42 - 2.43)	0.207 (0.322)
Selenium (Se) [mg/kg]	0.86 (0.047)	0.20 (0.025)	4.25 (3.38 - 5.87)	0.022 (0.917)
Strontium (Sr) [mg/kg]	0.89 (0.052)	0.74 (0.040)	1.19 (1.03 - 1.48)	-0.169 (0.418)
Zinc (Zn) [mg/kg]	71.70 (4.138)	35.46 (3.666)	1.98 (1.68 - 2.43)	-0.102 (0.627)

of highest concentration by weakly intake [15]. For adult persons with 70 kg body mass 3.5 mg lead, 0.35 mg mercury and 0.525 mg cadmium weakly are tolerable. The WHO/FAO guide value in meat 1986 was 0.25 mg/kg lead, 0.1 mg/kg cadmium and 0.03 mg/kg mercury. These values were fixed in the German Meat Inspection Law as highest values. When the heavy metal content in meat is double as these values, it is to confiscate [4].

The lead and cadmium accumulation in kidney of animals over 2 years is a reason for kidney confiscation by slaughter horses, breeding sows and boars, and cattle in meat inspection in Germany [16]. In the EU a regional rule is valid: when the environment is contaminated with heavy metals, the liver and kidney of more as 2 years old slaughter animals are to confiscate (VO (EG) 854/2004 Anh.I, Absch.II, Kap. V) [17]. The contents of heavy metals in investigated samples of coypus were not dangerous for human consumption.

Table 2 Content of mineral elements in liver and leg muscles in relation to age of male coypus (n=6)
Tabela 2 Zawartość składników mineralnych w wątrobie i w udźcu w relacji do wieku samców (n=6)

Mineral	Liver			Muscle		
	10-18 months (n = 3) mean (SD)	32-37 months (n = 3) mean (SD)	p-value mean (SD)	10-18 months (n = 3) mean (SD)	32-37 months (n = 3) mean (SD)	p-value mean (SD)
Aluminium (Al) [mg/kg]	0.17 (0.006)	0.18 (0.015)	0.349	0.09 (0.01)	0.10 (0.002)	0.150
Arsen (As) [µg/kg]	5.43 (0.136)	5.57 (0.04)	0.154	3.51 (0.135)	3.60 (0.128)	0.465
Cadmium (Cd) [mg/kg]	0.37 (0.021)	0.43 (0.021)	**0.035 ***	0.21 (0.006)	0.24 (0.006)	**0.008 ***
Cobalt (Co) [mg/kg]	0.02 (0.003)	0.02 (0.002)	0.872	0.01 (0.003)	0.01 (0.001)	0.834
Chrome (Cr) [mg/kg]	0.36 (0.032)	0.36 (0.018)	0.907	0.21 (0.029)	0.21 (0.024)	0.807
Copper (Cu) [mg/kg]	5.66 (0.415)	6.53 (0.391)	0.059	2.25 (0.098)	1.94 (0.064)	**0.011 ***
Iron (Fe) [mg/kg]	28.23 (1.672)	28.95 (0.544)	0.515	11.43 (0.617)	10.55 (0.694)	0.175
Potassium (K) [mg/kg]	3230.64 (72.356)	3219.46 (46.241)	0.833	1047.35 (33.94)	1057.82 (31.155)	0.714
Manganese (Mn) [mg/kg]	2.75 (0.05)	2.79 (0.02)	0.268	0.54 (0.032)	0.56 (0.021)	0.417
Molybdenum (Mo) [mg/kg]	0.64 (0.032)	0.62 (0.036)	0.450	0.15 (0.015)	0.17 (0.02)	0.184
Sodium (Na) [mg/kg]	4100.29 (94.497)	4096.18 (98.807)	0.961	2324.81 (75.597)	2150.61 (137.531)	0.127
Nickel (Ni) [mg/kg]	1.76 (0.046)	1.70 (0.219)	0.667	0.89 (0.025)	1.03 (0.045)	**0.008 ***
Lead (Pb) [mg/kg]	0.03 (0.006)	0.03 (0.001)	0.315	0.02 (0.003)	0.02 (0.001)	0.420
Selenium (Se) [mg/kg]	0.87 (0.03)	0.85 (0.04)	0.527	0.22 (0.012)	0.19 (0.006)	**0.035 ***
Strontium (Sr) [mg/kg]	0.88 (0.057)	0.89 (0.055)	0.734	0.73 (0.029)	0.73 (0.055)	1.000
Zinc (Zn) [mg/kg]	68.74 (3.235)	70.11 (1.7)	0.552	36.15 (2.01)	32.89 (2.38)	0.144

Table 3 Content of mineral elements in liver and leg muscles in relation to age of female coypus (n=19)

Tabela 3 Zawartość składników mineralnych w wątrobie i w udźcu w relacji do wieku samic (n=19)

Mineral	**Liver**			**Muscle**		
	10-18 months (n = 3) mean (SD)	32-37 months (n = 16) mean (SD)	p-value mean (SD)	10-18 months (n = 3) mean (SD)	32-37 months (n = 16) mean (SD)	p-value mean (SD)
Aluminium (Al) [mg/kg]	0.17 (0.026)	0.17 (0.014)	1.000	0.10 (0.006)	0.10 (0.008)	0.482
Arsen (As) [µg/kg]	5.49 (0.112)	5.51 (0.099)	0.673	3.75 (0.206)	3.69 (0.138)	0.503
Cadmium (Cd) [mg/kg]	0.38 (0.061)	0.38 (0.038)	0.905	0.22 (0.032)	0.23 (0.032)	0.479
Cobalt (Co) [mg/kg]	0.021 (0.001)	0.018 (0.001)	**0.002 ***	0.01 (0.002)	0.01 (0.002)	0.513
Chrome (Cr) [mg/kg]	0.35 (0.014)	0.36 (0.031)	0.711	0.19 (0.034)	0.21 (0.027)	0.258
Copper (Cu) [mg/kg]	6.07 (0.352)	6.25 (0.645)	0.659	2.15 (0.136)	2.16 (0.219)	0.946
Iron (Fe) [mg/kg]	27.68 (1.29)	28.43 (1.318)	0.375	10.37 (0.555)	11.51 (1.033)	0.084
Potassium (K) [mg/kg]	3214.24 (29.869)	3275.53 (197.495)	0.607	1112.84 (25.174)	1085.69 (27.772)	0.135
Manganese (Mn) [mg/kg]	2.78 (0.051)	2.72 (0.103)	0.317	0.56 (0.049)	0.54 (0.031)	0.370
Molybdenum (Mo) [mg/kg]	0.62 (0.05)	0.66 (0.036)	0.133	0.15 (0.017)	0.17 (0.017)	0.142
Sodium (Na) [mg/kg]	4045.43 (74.48)	4147.92 (53.139)	**0.010 ***	2216.62 (79.459)	2225.72 (91.528)	0.875
Nickel (Ni) [mg/kg]	1.68 (0.183)	1.77 (0.252)	0.570	0.91 (0.079)	0.87 (0.088)	0.462
Lead (Pb) [mg/kg]	0.03 (0.003)	0.03 (0.003)	0.328	0.02 (0.003)	0.02 (0.003)	0.374
Selenium (Se) [mg/kg]	0.84 (0.051)	0.86 (0.052)	0.482	0.18 (0.044)	0.21 (0.024)	0.131
Strontium (Sr) [mg/kg]	0.90 (0.042)	0.89 (0.057)	0.719	0.72 (0.031)	0.74 (0.043)	0.472
Zinc (Zn) [mg/kg]	72.86 (1.308)	72.34 (4.737)	0.856	38.75 (7.849)	35.20 (2.887)	0.516

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