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Elemental Composition of Muscle Tissue of Wild Animals from Central Region of Poland

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ABSTRACT: In the paper, the contents of calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), chromium (Cr), lead (Pb), cadmium (Cd), aluminium (Al), and nickel (Ni) were determined in the meat of roe deers, wild boars, and hares coming from central region of Poland. Measurements were carried out using atomic absorption spectrometry. The ranges of median values found for the concentration of elements were as follows: 89 – 121 (Ca), 235 – 241 (Mg), 19.2 – 28.6 (Zn), 0.93 – 2.07 (Cu), 26.3 – 39.1 (Fe), 0.10 – 0.38 (Mn), 0.04 – 0.31 (Cr), 0.07 – 0.48 (Pb), 0.004 – 0.010 (Cd), 0.31 – 1.26 (Al), and 0.05 – 0.13 (Ni) ìg/g, respectively. We stated that the concentrations of Ca, Mg, Cu, Fe, and Mn were comparable to their amounts in meat of farm animals. The smallest amounts of hazardous elements, except for Al, were determined in meat of hares, and the highest ones in roe deer meat. Moreover, a large variability in Cr, Pb, and Ni content was found. Generally, there were no significant correlations between the concentration of elements in the meat of wild animals and their age and weight, but some statistically significant and strong correlations between concentrations of elements were stated. Moreover, significant positive correlations were found to exist for the content of Fe and Mn in muscle tissue and liver of hares.

Key words: Wild animals, Muscle tissue, Bioelements, Heavy metals

INTRODUCTION

Free-living animals in natural ecosystems are specially exposed to various environmental factors. The environment is the main factor which determines health condition and population of wild game. The content of heavy metals in Polish soils remains at the level of natural concentration, except for areas where it is slightly increased (mainly in the southern regions). The major cause of the problem, is the acidification of soil which supports the release and transfer of heavy metals between subsequent links of the trophic chain. In plant samples taken from natural meadows located at various distances from motorways, the concentration of Pb, Cd, Ni, and Fe often exceeded the permissible upper limit. Legumes (*Fabaceae*) and plants classified as forage herbs accumulate much greater amounts of heavy metals than grasses (Dębska-Kalinowska *et al*., 1999). The levels of heavy metals depend on plant species and their environment, and they vary within wide limits. For example, in plants from forest areas in the central-eastern Poland, the concentration of particular elements was noted in the following ranges (mg/kg dry weight (dw)): Pb: 0.20 – 174.19, Cd: 0.05 – 2.62, Ni: 1.9 – 97.2, Cu: 5.1 – 32.6, Zn: 21 – 266, Mn: 313 - 11261, and Fe: 44 – 2628 (Mikos-Bielak and Tujaka, 1999). Roe deer and hare are herbivorous animals. Wild boar additionally feeds on animal food (including insects, small rodents, and molluscs). The main components of wildlife food are e.g. cultivated plants, herbs, grasses, leaves and stems of plants, bark of trees and shrubs, blueberries, and fungi. The mineral composition of wild animals foods is characterized by seasonal variability. Generally, such factors as habitat, food, condition, age, sex of animal, and season of sample collection (hunting) may affect the concentration of elements in animal tissues. (Pokorny *et al*., 2004; Pokorny and Ribarič-Lasnik, 2002; Ramírez *et al*., 1996; Sobańska, 2005). Essential elements are known to interact with toxic heavy metals *in vivo*. Toxic metals are relatively easy absorbed from diet. They rapidly accumulate in tissues (mostly in bones, muscles, liver, brain, and kidneys) and induce embryotoxic, mutagenic, carcinogenic, and teratogenic effects. A deficiency of bioelements in body fluids and tissues of animals and, on the other hand, an excess of heavy metals disrupts homeostasis, which leads to many disorders. So far, little data concerning the contents of elements in meat of wild animals exist in literature. The elemental composition of their tissues such as liver, kidney, brain, muscles is a good indicator

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of environmental pollution. Moreover, the appropriate concentration of both bioelements and heavy metals in game meat is important for consumers. Thus, the purpose of this study was to determine the levels of essential and toxic metals in muscle tissues of wild animals and to compare them with the concentrations of these elements in meat of farm animals.

MATERIALS & METHODS

Samples of meat (femoral muscle) from roe deers (*Capreolus capreolus*), wild boars (*Sus scrofa*), and hares (*Lepus europaeus*) coming from the Lodz and Mazovia Voivodships (Provinces) (central regions of Poland) were collected from October 28 to December 30, 2009. The game animals lived in a rather uncontaminated areas of forests and meadows, far away from heavy industry. Location of sampling sites (obtained from GPS) is shown on Fig. 1. Before chemical analysis the samples of muscle tissues were stored at –18^{0}C in closed plastic containers. The following chemical reagents were used in this study: 65% HNO_3 (Merck, Suprapur®), 65% $HClO_4$ (Cheman, spectral grade), stock standard solutions of elements for AAS (Fluka, 1000 µg/mL), modifiers - NH_4NO_3 (Aldrich, 99.999% grade) and $NH_4H_2PO_4$ (Merck, Suprapur®), $LaCl_3$ as a buffer for Ca and Mg determination (Fisher Scientific, 99.999% grade), and deionized water (Cobrabid Aqua Company, 0.06 µS/cm). All steps during the processes of samples preparation and analysis were carried out under dust-free conditions corresponding to the requirements of AAS method.

The samples of muscle tissues (about 0.25 g) were mineralized in the mixture of HNO_3/$HClO_4$ acids (3:1, v/v). The residues after mineralization were transferred into volumetric flasks and were made up to 25 mL with deionized water. In such prepared solutions, the contents of selected elements were determined with the use of atomic absorption spectrometer AVANTA Σ (GBC Scientific, Dandenong, Australia). The concentrations of Ca, Mg, Zn, Cu, and Fe were analyzed in acetylene-air flame and amounts of Mn, Cr, Pb, Cd, Al, and Ni were measured in the graphite furnace GF3000 with the autosampler PAL3000, and the Ultra-Pulse background corrector. Measurements were performed in pyrolitic coated graphite tubes, in argon atmosphere. Samples were injected into the atomizer in amount of 20 µL. A peak area mode was used for calculating the concentration of elements. The parameters of instrumental analysis are given in Table 1. Two calibration methods (the calibration curve method and the method of standard additions) as well as analyses of certified reference material (bovine liver SRM 1577b) were applied to verify the accuracy of results. The details of quality control are presented in Table 1. The results obtained during this work are presented in form of arithmetic means, medians, and standard deviations (SD) (Table 2). In this table all details relating to age and body weight of animals are also listed. Correlations between age, weight and concentration of elements in the game meat as well as levels of elements in the muscle tissues were analyzed with application of the Spearman test (Tables 3 and 4). The values of $p<0.05$ were considered as significantly different. The statistical analyses were performed by means of STATISTICA 9.1 for Windows.

Fig. 1. Geografic location of sampling sites (areas near the cities: Gostynin, Bolimów and Siedlce)

Table 1. Data related to instrumental and analytical performance

Element	Wavelength (nm)	Pyrolisis/atomization temperature(°C)	Sensitivity[c] Characteristic mass[*](µg/mL)(pg)[*]	Accuracy(%)
Ca	422.7		0.07	103.4
Mg	285.2		0.004	104.3[d]
Zn	213.9		0.015	99.2
Cu	324.7		0.030	98.1
Fe	248.3		0.08	96.2
Mn	279.5	700/2400	0.32[*]	96.2[e]
Cr	357.9	1000/2500	1.44[*]	-
Pb[a]	283.3	900/2000	5.8[*]	96.9
Cd[b]	228.8	600/1800	0.36[*]	96.0
Al	309.3	1400/2400	8.6[*]	96.8
Ni	232.0	900/2400	4.82[*]	-

Modifiers used: [a] $NH_4H_2PO_4$, [b] NH_4NO_3

[c] Sensitivity (characteristic mass) defined as the concentration (mass) of an element giving an absorbance of 0.0044

[d] at 202.6 nm

[e] FAAS (Flame Atomic Absorption Spectrometry)

Table 2. Concentration of essential and trace elements in the muscle tissue of wild animals (ig/g wet weight)

	Ca	Mg	Zn	Cu	Fe	Mn	Cr	Pb	Cd	Al	Ni
Roe deer (n = 13)											
Age: 2.4 years (1 – 4 years), Weight: 16 kg (7 – 21 kg)											
Mean	186	238	31.0	1.65	38.1	0.36	0.61	0.58	0.006	0.58	0.50
Median	121	241	27.5	1.32	39.1	0.38	0.31	0.48	0.006	0.31	0.13
SD	153	19	18.9	1.24	12.3	0.16	0.75	0.39	0.004	0.56	0.55
Range	66	194	11.2	0.43	19.9	0.12	0.04	0.05	0.002	0.12	0.05
	608	272	81.0	5.36	61.4	0.69	2.35	1.38	0.015	1.78	1.45
Wild boar (n = 11)											
Age: 2.2 years (1 – 5 years), Weight: 48 kg (21 – 110 kg)											
Mean	124	250	31.5	0.92	31.8	0.31	0.12	0.29	0.010	0.81	0.25
Median	99	240	28.6	0.93	26.3	0.15	0.10	0.25	0.010	0.54	0.10
SD	68	30	19.8	0.52	15.8	0.40	0.06	0.21	0.002	0.76	0.30
Range	48	207	10.4	0.15	15.0	0.08	0.04	0.05	0.006	0.21	0.02
	269	302	67.6	1.79	59.7	1.39	0.27	0.77	0.014	2.69	0.99
Hare (n = 11)											
Age: 2.4 years (1 – 4 years), Weight: 3.6 kg (2.0 – 5.0 kg)											
Mean	93	242	22.2	2.22	28.6	0.10	0.09	0.07	0.007	1.10	0.13
Median	89	235	19.2	2.07	27.7	0.10	0.04	0.07	0.004	1.26	0.05
SD	21	31	9.6	0.58	4.8	0.03	0.13	0.06	0.008	0.57	0.23
Range	67	217	11.4	1.42	21.8	0.07	0.02	0.01	<LOD[a]	0.10	0.01
	139	331	44.4	3.39	38.9	0.19	0.44	0.16	0.022	1.89	0.76

[a] LOD – limit of detection

RESULTS & DISCUSSION

In our study, the average contents of selected elements per 100 g of game meat were stated as follows: (mg) Ca – 13.4, Mg – 24.3, Zn – 2.8, Cu – 0.16, Fe – 3.3, and (in µg) Mn – 26.0, Cr – 23.0, Pb – 27.0, Cd – 0.77, Al – 83.0, and Ni – 29.0. Among the studied samples of muscle tissues the greatest concentrations of Ca were found in roe deer meat and the smallest amounts of this element in meat of hares. According to the Polish Food Composition Tables (Kunachowicz *et al*., 2005) the average content of Ca in mutton is 10 mg/100 g, pork - 5 mg/100 g, and rabbit – 19 mg/100 g. For the comparison, according to Danish (the Danish Food Composition Database) and Finnish (the Finnish Food Composition Database) standards, the analogous levels are (mg/100 g): 7.6, 7.0, 13.0, respectively and 7.0 (lamb), 6.0 and 13.0. In accordance with Slovak data (SFCDB, 2012) the concentration of Ca in game meat is (mg/100 g): 5 – roebuck, 11 – wild boar, and 16 – brown hare. The Danish and Finnish databases also include information about content of some other elements in the hare and reindeer meat. The Mg content in the meat of all tested wild animals is comparable. The dispersion of results and standard deviations are also small. The Mg contents in the meat of farm animals found in Polish tables (Kunachowicz *et al*., 2005) are

Table 3. Spearman's rank correlation coefficients between age and weight of free living animals and the elemental status of muscle tissue

	Ca	Mg	Zn	Cu	Fe	Mn	Cr	Pb	Cd	Al	Ni
Roe deer											
Age	-0.13	-0.21	0.33	-0.53	-0.53	-0.10	-0.52	0.30	0.54[a]	-0.17	-0.38
Weight	-0.23	-0.21	0.16	-0.44	-0.24	-0.16	-0.17	0.32	0.22	-0.11	-0.30
Wild boar											
Age	-0.16	0.14	-0.20	0.44	0.29	0.10	0.08	0.49	-0.08	-0.19	0.10
Weight	-0.14	0.37	-0.25	0.53	0.30	0.21	0.25	0.29	0.17	-0.04	0.21
Hare											
Age	0.02	-0.06	0.14	-0.09	-0.07	0.07	0.21	0.01	-0.18	0.50	0.04
Weight	0.11	-0.06	0.10	-0.46	-0.22	-0.17	0.41	0.15	-0.08	0.10	0.43

[a] Value is statistically significant

Table 4. Spearman's rank correlation coefficients between elements' concentration in muscle tissues of wild animals (statistically significant correlations)

Roe deer		Wild boar		Hare	
Elements	**r**	**Elements**	**r**	**Elements**	**r**
Zn-Cu	-0.61	Ca-Pb	0.74	Ca-Cd	0.80
Zn-Al	0.58	Zn-Fe	0.74		
Cu-Fe	0.61	Mn-Cr	0.68		
Fe-Mn	0.66	Cd-Cr	0.85		
Fe-Cr	0.69				
Fe-Ni	0.61				
Cr-Ni	0.82				

as follows (mg/100 g): mutton - 23, pork - 21 rabbit – 26. The Danish and Finnish databases show the following values: 23 and 16.0 mg/100 g for mutton, 22 and 18 mg/100 g for pork, 23 and 28 mg/100 g for rabbit, respectively. The concentrations of Mg in game meat were stated at the following levels (mg/100 g): 29.0 (roebuck), 22.0 (wild boar), and 20.0 (hare) (SFCDB, 2012).In game meat, Zn was measured in smaller quantities as compared to those for Ca and Mg. Similar amounts of this element (3.2 – 3.7 mg/100 g) are provided by Polish, Danish and Finnish standards. The average content of Zn in the meat has been defined by authors at the level of 47.3 µg/g (Nardi *et al*., 2009) and 21 mg/kg (Santos *et al*., 2004). Comparable amounts (i.e. 17.5 - 39.6 µg/g, geometric mean - GM) of Zn in caribou and reindeer muscle tissues were described by other researchers (Asstrup *et al*., 2000). Also Medvedev (1999) found 36.8 µg Zn per 1 g of moose muscles and 21.2 µg Zn per 1g of reindeer muscle tissue. Authors (Zalewski *et al*., 2012) reported 26 – 54 µg Zn/g in the muscles of European beavers. Somewhat smaller amounts of Zn in game meat are present in the Slovak database i.e. 0.87 mg/100 g (roebuck) and 0.92 mg/100 g (brown hare).

As far as Cu content is regarded, a bit larger quantities of this element have been found in wild game meat compared to the meat of farm animals - 0.06 and 0.10 mg/100 g (Polish and Danish standards), 1.4 µg/g (Nardi *et al*., 2009), and 0.4 mg/kg (Santos *et al*., 2004). The results obtained in this study are comparable with those presented by other authors: 2.09 - 3.60 µg/g (GM, caribou and reindeer) (Asstrup *et al*., 2000), 1.63 µg/g (reindeer) and 3.80 µg/g (moose) (Medvedev, 1999), 0.41- 1.9 µg/g (beavers) (Zalewski *et al*., 2012). The Fe content in the meat of farm animals is greater than that for Cu and varies between 0.9 - 2.7 mg/100 g (Polish values) and 0.67 – 1.8 mg/100 g (Danish and Finish data). For moose and reindeer muscle tissues, Medvedev (1999) reported 51.16 µg Fe/g and 53.97 µg Fe/g, respectively. Similar values have been established in Slovak database (mg/100 g): 3.0 (roebuck), 3.8 (wild boar), and 4.04 (brown hare). The Mn concentration in the game meat was close to its content in the meat of farm animals provided by Polish and Danish standards (0.01 - 0.04 mg/100 g). The comparable amounts of this element were reported also by other authors: 0.26 µg/g (Nardi *et al*., 2009) and 0.2 mg/kg (Santos *et al*., 2004). Thus, the meat of wild and farm animals contains rather small amounts of Mn taking into account that the WHO/FAO recommends 2 – 9 mg of Mn for daily intake (Biego *et al*., 1998).

With regard to Danish standards, the Cr content in the meat remains within the range of 2.0 - 5.3 µg/100 g. However, authors reported 0.063 µg Cr/g (Nardi *et al*., 2009) and 52 µg Cr/kg (Santos *et al*., 2004). In our research, the highest Cr level was found in the meat of roe deer, and the lowest in the meat of hares. Attention should be paid to large dispersion of Cr contents results, especially for roe deer. This may result from varying environmental exposures. Meat (including poultry) and eggs are the principal source of Cr in food (23%). Game meat may contain high Cr amounts like canned foods i.e. 0.19 - 0.52 µg/g (Tunzen and Soylak, 2007), bean – 0.9 µg/g, Brazil nut – 1.3 µg/g, and chocolate – 0.44 µg/g (Nardi *et al.*, 2009).

In case of natural environmental exposure of healthy humans (first of all without disturbances of metabolic processes and with properly functioning kidneys) there is no danger of excessive accumulation of elements discussed above. But, as it is known, even small amounts of Pb, Cd and Al are dangerous to human health. In case of Ni, its role in allergic reactions has been proved. In the view of animal health, excessive accumulation of heavy metals may result in disorders of many vital functions, including disturbances in functioning of nervous, excretory, circulatory, respiratory, immune, reproductive and digestive systems. In our study, the contents of Pb and Cr in roe deer muscle were higher than those found in the meat of wild boar and hare. The concentration of Pb only in the meat of hares does not exceed the maximum levels i.e. 0.1 mg/kg established for meat (excluding offal) of bovine animals, sheep, pig and poultry (Commission Regulation (EC) No 1881/2006 of 19 December 2006). The Pb concentrations in animal meat have been reported by other authors at following levels: 15.6 ng/g (Nardi *et al.*, 2009), 34 µg/kg (Santos *et al.*, 2004), for caribou and reindeer <0.004 – 0.007 µg/g (Asstrup *et al.*, 2000), for wild boar 0.27 µg/g and reindeer 2.14 µg/g (Medvedev, 1999), for arctic hare 0.011 mg/kg dw, the values cited by authors: 0.195 mg/kg dw and 9.1 mg/kg dw (Pedersen and Lierhagen, 2006), and also 0.108 mg/kg (0.152 mg/kg, industrial region) - roe deers, 0.098 mg/kg (0.383 mg/kg, industrial region) – wild boars, 0.144 mg/kg (0.152 mg/kg, industrial region) – hares (Wrzesień *et al.*, 2000). Probably also in our research the environment has a significant impact on the Pb level in muscle tissue.The Cd concentration in the tested samples of game meat was low and did not exceed the permissible amount, i.e. 0.05 mg/kg (Commission Regulation (EC) No 1881/2006 of 19 December 2006). In literature one can find the following concentrations of Cd in meat: 0.21 ng/g (Nardi *et al.*, 2009), 1.6 µg/kg (Santos *et al.*, 2004), <0.003 – 0.003 µg/g (caribou and reindeer) (Asstrup *et al.*, 2000), 0.04 µg/g (wild boar) and 0.58 µg/g (reindeer) (Medvedev, 1999), 0.005 mg/kg dw (juvenile hares) and 0.082 mg/kg dw (adult hares) (Pedersen and Lierhagen, 2006), and 0.027 mg/kg (0.035 mg/kg, industrial region) - roe deers, 0.041 mg/kg (0.098 mg/kg, industrial region) – wild boars, and 0.021 mg/kg (0.038 mg/kg, industrial region) – hares (Wrzesień *et al.*, 2000). The low Pb and Cd concentrations were reported in the muscles of beavers (Zalewski *et al.*, 2012). In contrast to Cr, Pb, and Ni contents, the concentration of Al in the roe deer meat was lower than in the wild boar and hare meat. Generally, the amount of Al in the game meat was low and comparable with data presented by other authors: 0.12 µg/g (Nardi et al., 2009), 17 mg/kg (Santos *et al.*, 2004), and 0.93 – 2.86 µg/g (canned foods) (Tunzen and Soylak, 2007). The highest contents of Ni in the tested samples of meat were found in the muscle tissues of roe deer. The median values (0.13 and 0.10 µg Ni/g) for roe deers and wild boars meat tissues were similar. The Danish standards give the Ni content in the meat of 0.5 - 6.0 µg/100 g. The concentrations of Ni in the meat presented by other authors were as follows: 0.019 µg/g (Nardi *et al.*, 2009), 20 µg/kg (Santos *et al.*, 2004), 1.20 kg dw (beef) and 1.540 mg/kg dw (pork) (Onianwa *et al.*, 2000), 0.61 µg/g (pork) and 0.92 µg/g (calf) (Yerba *et al.*, 2008), 0.08 µg/g (moose) (Medvedev, 1999), and 0.18 – 0.75 µg/g (canned foods) (Tunzen and Soylak, 2007). We have also investigated the elemental composition of muscle tissue of red fox inhabiting the same central region of Poland as other wild animals tested (Długaszek and Kopczyński, 2012). Among the examined animals, taking into account the elemental composition, hare and fox muscle tissues are the most similar to each other.

In the paper, no significant relations have been found between the concentration of elements in the game meat and the age as well as weight of animals except for the correlation between the content of Cd in the muscle tissue and age of roe deers. It was found that the Fe correlates with the highest number of elements (Zn, Cu, Mn, Cr, and Ni). Strong correlations (re”0.5), however not statistically significant, were established between the following pairs of elements: Ca-Zn, Ca-Mn, Ca-Cr, Mg-Cu, Zn-Mn, Cu-Fe, Cu-Ni, Mn-Pb, Pb-Cr (wild boar muscle tissue) and Ca-Zn, Fe-Cr (-), Mn-Pb, Pb-Al (-), Pb-Ni (hare muscle tissue). Elemental analysis of liver is one of the methods of evaluation the body burden of elements. Table 5 presents correlation coefficients between the elements contents in the liver and muscle tissues of examined wild animals (Długaszek and Kopczyński, 2011). Statistically significant relations were found only for Fe and Mn in the tissues of hares. Also for Mn, significant correlations were stated between its concentration in muscle tissue and hair of red fox (Długaszek and Kopczyński, 2012).

Table 5. Spearman's rank correlation coefficients between elements' concentration in muscle tissue and liver of wild animals

	Ca	Mg	Zn	Cu	Fe	Mn	Cr	Pb	Cd	Al	Ni
Roe deer	0.38	0.01	0.21	-0.02	0.35	0.21	-0.36	0.31	0.39	0.21	0.32
Wild boar	0.28	0.22	-0.27	-0.28	0.35	0.21	-0.48	0.07	0.15	-0.26	0.32
Hare	0.27	-0.08	-0.19	0.09	0.61[a]	0.70[a]	0.11	0.60	0.49	-0.05	0.10

[a]Values are statistically significant

CONCLUSION

Our findings indicate that the contents of particular elements in the game meat are comparable to values described by other authors and contained in the European food composition databases. Concentrations of Ca, Mg, Cu, Fe, and Mn were comparable with their amounts in the meat of farm animals. The smallest amounts of heavy metals, except for Al, were determined in the meat of hares, and the highest in roe deer meat. The great variability found in concentrations of metals such as Cr, Pb, Al, and Ni may be caused by environmental exposures. With one exception, we found no correlations between the concentration of elements and both age and body weight of animals.

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