Original Research

Contents of Calcium, Magnesium, and Phosphorus in Antlers and Cranial Bones of the European Red Deer (*Cervus Elaphus*) from Different Regions in Western Poland

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Abstract

Annual shedding of antlers by males is a characteristic feature of most deer species. Regrowth is very fast, reaching 2 cm/day for some species and making them an interesting model for studying tissue regeneration processes. The aim of this study was to compare the contents of calcium, magnesium, and phosphorus in antlers and cranial bones of European red deer from Western Pomerania, Poland. We obtained 30 antlers from three forestry districts that differed in the extent of environmental pollution with fluorine compounds, SO_2, NO_x, CO, and CO_2 (Trzebież, Rokita and Gryfino). Deer were assigned to two age groups: from 2 to 4 years, and from 6 to 8 years. Powdered samples of cranial bones and antler base obtained with a dental drill were dissolved in nitric acid. Calcium and magnesium contents were measured by atomic absorption spectroscopy, while phosphorus was determined colorimetrically. The content of calcium was higher in antlers - (mean 133.96 mg/g) hardly surprising given the higher calcified cartilage and immature bone content in antler as opposed to cranial bone (mean 123.79 mg/g). Phosphorus content was slightly higher in bones than in antlers (84.62 mg/g and 83.58 mg/g, respectively), which suggests that Ca:P ratios are different in the mineral phase or that there is more P in bone compared to the antler matrix. No difference in magnesium content was noted (5.23 to 5.46 mg/g). Statistical analysis revealed significant differences depending on age of the animal and level of industrial pollution in the animal's habitat.

Keywords: calcium, magnesium, phosphorus, antler, deer, environmental pollution

Introduction

Deer antlers arouse interest not only because they are valued hunting trophies. They also have been subject of numerous scientific studies [1]. The process of antler formation might be a useful model for investigating mineral metabolism as well as bone diseases. In environmental studies, they may be a bioindicator of pollution with chemical substances, including radioactive ones [2]. Antlers are shed and regenerated each year. Polish deer include red deer (*Cervus elaphus*) and roedeer (*Capreolus capreolus* L.), and also fallow deer (*Dama dama* L.) and moose (*Alces alces* L.). A decrease in calcium content of postcranial bones (metatarsus, ribs), plus reversible osteoporosis in the period of antler mineralization in some of the cervid species have been reported (red deer, roe deer, fallow deer) [3, 4, 5]. Antler growth starts immediately after the shedding of old antlers. In elk, there is a relatively

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Table 1. Calcium, magnesium and phosphorus contents in bones and antlers of deer from three forestry districts (mean and standard deviation).

		"Trzebież" young	"Trzebież" old	"Gryfino" and "Rokita" young	"Gryfino" and "Rokita" old
Element	n	6	6	9	9
Ca bone (1b)	x	112.67	138.50	116.00	128.00
	SD	6.44	16.32	17.46	17.49
Ca antler (1a)	x	127.83	150.00	122.67	135.33
	SD	12.75	21.57	18.41	22.32
Mg bone (2b)	x	5.57	5.98	4.33	5.03
	SD	0.65	1.18	0.81	0.74
Mg antler (2a)	x	5.53	6.32	4.42	5.58
	SD	0.94	1.51	0.92	2.00
P bone (3b)	x	76.77	76.85	93.74	91.12
	SD	11.95	7.98	21.61	10.52
P antler (3a)	x	76.57	73.65	94.34	89.76
	SD	12.74	10.40	17.44	9.52

Age of young individuals group: 2-4 years and age of old individuals group: 6-8 years. Skull bone = b, antler = a.

long rest interval between shedding of the old antlers and the onset of new antler growth. In some species, longitudinal growth exceeds 2 cm per day, which makes antlers the fastest growing animal organ [6]. This extremely fast annual process of growth and ossification is hormonally controlled and is related to good blood perfusion and innervation [7, 8]. Hard antlers, already without their velvet covering, represent live bone tissue [9]. There are regions with active osteoblasts and osteoclasts in them, which lead to continuous bone remodelling [10]. Forty-four percent of the roedeer antler is composed of organic matter, mainly proteins (approximately 130 of them have been identified), and 56% of inorganic matter [11, 12]. The inorganic part contains 48% calcium phosphate, 5% calcium carbonate, 2% magnesium carbonate and the rest is made up by other mineral compounds [11]. The composition specified above means that calcium, phosphorus, and magnesium are the main inorganic components of antler tissue, the structure being similar to the inorganic part of other bone tissue in terms of composition. In the bone tissue, magnesium may be partly replaced with calcium ions, which is related to a change in the amorphous form of hydroxyapatite into a crystalline one. Due to the fact that antlers grow from skull bone, it was decided to compare proportions of the main inorganic constituents of the two tissues.

Material and Methods

Thirty antlers of the red deer were studied, which originated from scheduled hunting shoots in three forest divisions in Western Pomerania. The area of Trzebież forest division is industrialized as there is a large plant producing fertilizers located there. The two other areas are less contaminated as they are located approximately 40 km northeast (Rokita) or southeast (Gryfino) of Trzebież [13]. The antlers were divided into groups according to the age of the deer: younger group – individuals 2-4 years of age; older group – individuals aged 6-8 years. Samples in the form of bone powder were taken from the skull bone and antler base with the use of a dental drill. 10 mg of the collected material were dissolved in nitric acid to obtain the stock solution. The calcium and magnesium content was determined by atomic absorption spectrometry, using a Philips PU 9100X spectrometer [14]. The phosphorus content was measured with a spectrophotometric method using a reagent kit (Analco Co., Poland) [15]. Because the data deviated from a normal distribution, significance of differences between groups was checked with the non-parametric Mann-Whitney U-test, using $P<0.05$ as the significance level.

Results

Due to the relatively small number of antlers and the fact that Rokita and Gryfino forest divisions are located some 40 km from the more polluted Trzebież forest division, the antlers originating from Rokita and Gryfino were pooled [13]. Table 1 presents the average concentrations of calcium, magnesium, and phosphorus in the antlers and skull bone of deer from Trzebież forest division (T) – as a study group, and the material from Gryfino and

Rokita forest divisions (G + R) forming a single control group. Statistically significant differences are indicated in Figures 1 (calcium), 2 (magnesium), and 3 (phosphorus). Calcium contents in the antlers and skull bones of young individuals from the industrialized area were significantly different. Statistically significant differences were also recorded between young and old animals originating from both areas (Fig. 1). Statistically significant differences were noted for magnesium content (Fig. 2) in the antlers and skull bones of young individuals from the study group and the control group, and significant differences in the magnesium content in the skull bones of young and old animals from less contaminated areas. Phosphorus content in the antlers and skull bones of old animals from both areas was significantly different. Significant differences were also recorded for phosphorus content in the antlers of young animals from the industrialized area (T) and the control areas (G+R) (Fig. 3). In Table 2 values of Ca/P ratio and bone/antler indices for calcium, magnesium, and phosphorus are presented.

Discussion

Each year, antlers are shed and then regenerated. This makes it possible to investigate agents that have an impact on the intensive growth of bone tissue. For instance, button-like protuberances that sometimes are formed resemble neoplastic bone growth [16]. Purely biological issues such as the mechanism of antler shedding [17] or antler regeneration [18] seem to generate greater interest. In one of our earlier papers we analyzed the accumulation of fluorine in red deer antlers [13]. It was shown that the areas of Rokita and Gryfino forest divisions were characterized by the same level of contamination with fluorine compounds emitted from the distant Chemical Plant, located within the Trzebież forest division. That encouraged us to combine the material from the two forest divisions (Rokita and Gryfino) into one control group.

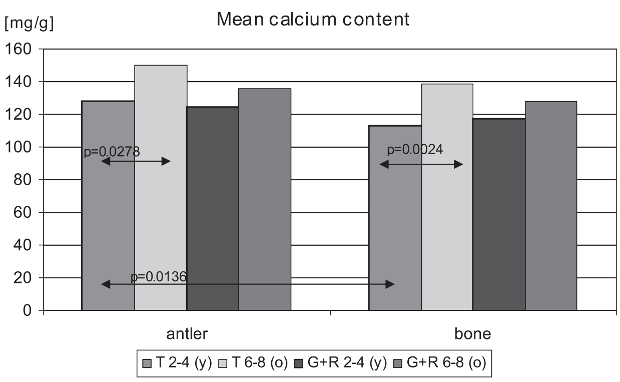

Fig. 1. Mean calcium content in antlers and skull bone of deer from different forestry districts. Arrows indicate statistically significant differences between groups. (y – younger age group; o – older age group; T – Trzebież (pollutant-exposed group); G+R – Gryfino and Rokita (control group).

Previously we compared changes in the calcium, magnesium, and phosphorus content in chicken bones [15]. The three macroelements (Ca, Mg, P) also constitute the main part of the inorganic antler component. Such an analysis is interesting also because the bone tissue and its constituents are subject to only minor changes during animal life – in contrast to antlers which are shed and regenerated each year. Mean contents of calcium and magnesium in deer skulls are lower than in deer mandibles studied by Dąbkowska [19]. The material which we received from hunters had been previously boiled and bleached in hydrogen peroxide. The bones were not defatted prior to analyses and the samples were dried at room-temperature (not oven-dried) during our resarch. Shultz et al. [20] reported that the deer bones contain 9% water, which is the reason for lower calcium and magnesium contamination in the studied material. In the course of our study we noticed that skull bone is more easily decalcified (over several days) than antler bone (over several months), which may suggest different types of mechanisms in chemical bonding of calcium in antlers.

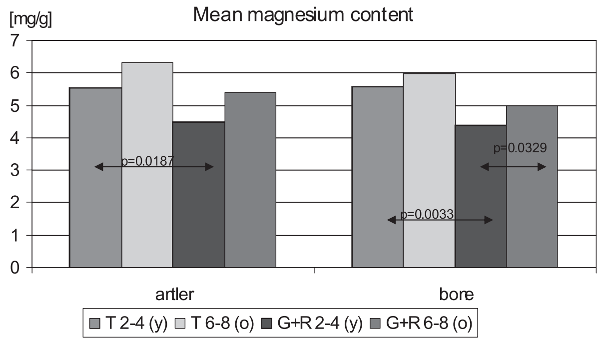

Fig. 2. Mean magnesium content in antlers and skull bone of deer from different forestry districts. Arrows indicate statistically significant differences between groups. (y – younger age group; o – older age group; T – Trzebież (pollutant-exposed); G+R – Gryfino and Rokita (control group).

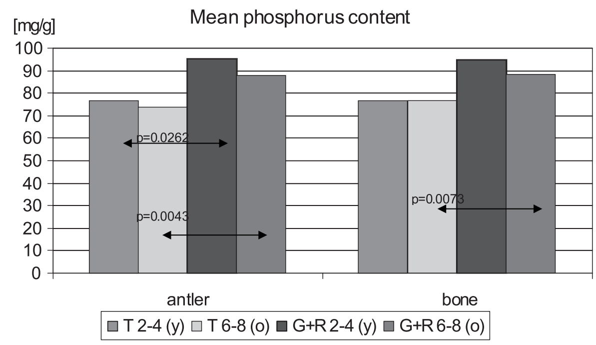

Fig. 3. Mean phosphorus content in antlers and skull bone of deer from different forestry districts. Arrows indicate statistically significant differences between groups. (y – younger age group; o – older age group; T – Trzebież (pollutant-exposed group); G+R – Gryfino and Rokita (control group).

Table 2. Comparing weight ratios of bone/antler for calcium, magnesium and phosphorus.

	Ratio bone/antler			Ratio Ca/P	
	Ca	Mg	P	bone	antler
"Trzebież" young	0.88	1.01	1.00	1.47	1.67
"Trzebież" old	0.92	0.95	1.04	1.80	2.04
"Gryfino" and "Rokita" young	0.95	0.98	0.99	1.24	1.30
"Gryfino" and "Rokita" old	0.95	0.90	1.02	1.41	1.51

Age of young individuals: 2-4 years; age of old individuals: 6-8 years. Bone is described by parameter b, antler – parameter a.

Small quantitative differences in the main inorganic constituents mentioned above between the two structures (skull bone and antlers) most probably result from the fact that antlers grow on the skull bones in a manner similar to that of the skull bone tissue itself, being provided with building elements by the blood system [21]. However, the two structures significantly differ in terms of the functions they fulfil in the deer's body [2]. Statistical analysis revealed significant differences in the content of elements in the two structures depending on the animal's age and the level of environmental contamination. Calcium content in antlers increases with age, particularly in older animals. Although it is known that skeletal bone depletion occurs during antler growth in deer [22]. Brockstedt Rasmussen [23] in his study shows a highly significant inverse correlation between porosity in the antlers and porosity in the metacarpal bones, indicating a necessary calcium mobilization from the bones for the mineralization of the antlers. Antler growth being accompanied by a form of physiologic, reversible osteoporosis in the skeleton by showing that there is a period when the antlers and skeleton are both undergoing net bone formation [22, 23]. ALP is produced in large quantity in the antler tissue during the growth period [24] and causes a rise in inorganic phosphate [25].

Magnesium behaved similar to calcium in antlers, although it plays a different role. It is hard to conclude whether magnesium takes part in other processes in addition to building functions.

Phosphorus differs from calcium and magnesium in behaviour. The phosphorus content is usually lower in older individuals than in young ones. That is yet more evidence of continuous quantitative changes and, in consequence, structural ones in the tissues under analysis throughout the animal's life.

The Ca/P weight ratios in bone differ among animal species and depend on age. In young chicken bones it oscillated from 1.31 (10 days old) to 2.04 (50 days old) [15]. In Piotrowska study [26] Ca/P ratio in deer antler is 2. On the basis of the results of those authors, it may be assumed that phosphorus is the most quantitatively stable ingredient of bones and antlers. The magnesium content in the bone/antler proportion declines with the animal's age, whereas calcium does not significantly fluctuate in the tissues of animals originating from less contaminated areas, and its content is always higher in antlers than in bones.

Conclusions

1. There is a considerable similarity between the quantitative behaviour of calcium, magnesium, and phosphorus in antlers and the deer skull bone tissue.
2. The dynamics of mineral rebuilding of antlers and skull bones is affected by the age of the animals and living regions.

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