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Impact of Season and Sex on Calcium and Phosphorus Content in the Meat of Roach (*Rutilus rutilus* L.) from the Brda River (Poland, Bydgoszcz)

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The aim of this work was to compare the concentrations of calcium and phosphorus and Ca/P ratio in the meat of females and males of the roach (*Rutilus rutilus* L.) caught from the Brda River. The study involved 40 roach individuals caught in fall and spring (10 females and 10 males from each season). The muscle samples for analyses were taken from the large side muscle of the fish body above the lateral line. Ca concentrations were determined by atomic absorption spectrophotometry; P content was analyzed by the colorimetric method. Calcium concentration in the meat of analysed roach was higher in samples collected from fish caught in spring and equaled 1.82 g kg^{-1} in females and 1.93 g kg^{-1} in males. Values for individuals from autumn amounted to 0.83 and 1.10 g kg^{-1}, respectively. Statistically significant differences in calcium content were detected between individuals caught in different seasons, but samples taken from females and males caught within one season did not differ substantially. The mean value of P in the meat of analysed roach caught in spring was higher than in fish from autumn, and it was respectively 2.24 g kg^{-1} in females and 2.30 g kg^{-1} in males from spring, and 1.89 g kg^{-1} in the tissue of females and 2.01 g kg^{-1} in males in fish from autumn. The ratio of calcium to phosphorus in the meat of analysed wild roach ranged from 0.43:1 to 0.82:1. A negative and statistically significant correlation between Ca and P concentrations was found in the meat and the body length of analysed roach from the Brda River.

Key words: Calcium, phosphorus, roach, meat.

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Trace elements are introduced into the environment through various routes such as smelting processes, fuel combustion and industrializations. They enter aquatic ecosystems (lakes, rivers) through atmospheric fallen dumping wastes and geological weathering (AL-YOUSUF *et al*. 2000). Fish meat is an important source of amino acids, protein, vitamins dissolvable in fat, long chain fatty acids and fat as a valuable energy source. Through its high nutritive value, fish meat should be a very significant part of the human diet. Fish are located at the end of aquatic food chains and may accumulate mineral compounds in their tissues.

There are many biological and environmental factors which influence the concentration of metals in fish (age, sex, size, feeding habits of fish, differences in aquatic organisms, the part of the fish body from which samples were collected, season of the catch and pH of the water, temperature and dissolved oxygen).

The aim of this work was to compare the concentrations of calcium and phosphorus and Ca/P ratio in the meat of females and males of the roach (*Rutilus rutilus*) caught in the Brda River during spring and autumn.

There are many publications on the influence of various factors on metal concentrations in different parts of fish bodies. The relationship between age and the value of metal bioaccumulation in tissues of four species of fish from Wojnowskie Lakes was investigated by DOBICKI and POLECHOŃSKI (2003). AL-YOUSUF *et al*. (2000) determined trace elements in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body and sex. Seasonal investigation of trace element contents in commercially valuable fish species from the Black Sea were carried out by MENDIL *et al*. (2010). ŁUCZYŃSKA and BORUCKA-JASTRZĘBSKA (2005) investigated the relationship between the content of heavy metals in muscle tissue and the size of fish from the Olsztyn Lake District. They analized pike (*Esox lucius* L.), Eurasian perch (*Perca fluviatilis* L.), roach and common bream (*Abramis brama* L.).

Calcium and phosphorus are essential elements for humans, meaning that these metals should be part of the human diet (CELIK *et al*. 2004). Ca and P play very important roles in several physiological processes and are directly involved in the development and maintenance of the skeletal system. In vertebrates Ca is complexed with P in hydroxyapatite to form the principal crystalline of bone (YE *et al*. 2006). Phosphorus is an element of DNA, RNA and phospholipides. One of its main roles is also in the process of photosynthesis, respiration and synthesis of organic compounds. In the water environment phosphorus is responsible for plant biomass extension during eutrophisation. In most fish species, phosphorus deficiency signs include poor growth, reduced feeding efficiency, poor bone mineralization, skeletal deformities and high lipid content in the entire body. In contrast to Ca, P should be supplemented with fish feeds because the concentration of this mineral is low in freshwater and seawater. Calcium deficiency causes osteroporosis and bone deformation.

Phosphorus in fish meal and cereals is not efficiently utilized by common carp (*Cyprinus carpio*) and the addition of soluble inorganic phosphates to a diet promotes body growth and increases the calcium and phosphorus content in the bones (NAKAMURA 1982).

As reported by YE *et al*. (2006), the Ca requirement of most fish is not by absorption from the water or from the composition of the natural diet. For example red sea bream require 0.34% Ca in the diet. But more excess dietary Ca inhibited P absorption in common carp. Dietary phosphorus requirements ranging from 0.50 to 0.8% have been reported for chum salmon, Atlantic salmon (*Salmo salar*), common carp, red sea bream (*Pagrus major*) and rainbow trout (*Oncorhynchus mykis* R.) (WILSON *et al*. 1982).

The availability of phosphorus depends on the chemical form and solubility of the mineral, and further to dietary sodium chloride ratio and acid-base balance. As indicated by ALBREKTSEN *et al*. (2009) in Atlantic salmon, primary inorganic salts of phosphorus are more available than secondary salts, whole P bound to calcium in the bone tissue is the least available.

The daily dose of phosphorus in the diet of an adult should be 800 mg (SAPEK 2009), and approximately 1000 mg of calcium. Ideally, the same amount of phosphorus as calcium (calcium-phosphorus balance) should be consumed. A ratio between phosphorus and calcium greater than 3:2 may cause metabolic disorders. Fish meat is a rich source of phosphorus and can be on the order of 150-200 mg per 100 g^{-1} of product. This element occurs in almost all species of fish. Fish rich in calcium include sardines, herring, sprat and salmon (GAWĘCKI & HRYNIEWIECKI 2004). The approximate share of phosphorus and calcium in fish meat should be 1.8 % (dry weight) (KABATA-PENDIAS & PENDIAS 1999).

Material and Methods

The study involved 40 individuals of roach caught in fall and spring. The experimental fish were obtained in natural conditions from the Brda River, located within Bydgoszcz, near Iławska Street. Analyses were carried out on 10 females and 10 males caught from each season. Measurements of the mass of the fish body (BW) (± 0.01 g) and body length (Lc) (± 0.1 cm) and the total length (Lt) (± 0.1 cm) were taken from each individual. The muscle samples for analyses were taken from the large side muscle of the fish body above the lateral line. Individuals with similar biometric measurements were chosen for analysis. Body weight ranged from 60.49 to 138.48 g, and body length was from 14.0 to 17.5 cm.

The samples of fish meat were immediately frozen after preparation and kept in the deep freezer before analysis. All frozen samples were freeze dried in a Finn-Aqua Lyovac GT2 freeze drier (parameters: temperature -40°C, pressure 6 10^{-2} mbar, duration at least 48h).

The freeze dried samples were mineralized in a microwave mineralizator Ethos Plus, Milestone. For the mineralization, 0.1 g of tissue was weighed and then HNO_3 and H_2O_2 were added in a ratio of 4:1. During the first 10 minutes the temperature was increased to 190°C. During the next 7 minutes the temperature was kept at a level of 190±5°C. The mineralized samples were carried quantitatively to the measuring flask with a capacity of 50 ml.

Ca concentrations were determined by an atomic absorption spectrophotometer (Solar 969, Unicam). P content was analyzed by the colorimetric method (spectrophotometer Lambda 25, Parkin-Elmer) at wavelength 430 nm. Analyses were carried out according to PN-EN 13805/2003, PN-EN 15505/2009 and PN-ISO 13730. Tissue concentrations of minerals are reported as g kg^{-1} dry weight (g kg^{-1} d. w.).

The accuracy of the analyses was controlled by adding standard solutions: calcium standard solution $Ca(NO_3)_2$ in HNO_3 (Merck, Germany) and KH_2PO_4 (POCH S.A., Poland) dissolved in water. The results showed that the recovery percentage was in the range from 87 to 103% and these values were taken into account in the final results.

Data analyses were performed in Statistica 8.0 software (StatSoft, USA). Significance of differences in the average content of calcium and phosphorus in the meat of roach was calculated by two-way analysis of variance. Tukey's test was applied, and differences were evaluated as significant at $P \leq 0.05$.

Results and Discussion

Calcium concentration in the meat of analysed roach was higher in the samples collected from fish caught in spring and amounted to 1.82 g kg^{-1} in females and 1.93 g kg^{-1} in males. In individuals from autumn it was 0.83 and 1.10 g kg^{-1}, respectively (Table 1). The content of calcium differed significantly between individuals caught in different seasons, but differences between samples taken from females and males caught within one season were insignificant. The meat of the analysed roach is not a rich source of calcium. Large amounts of this mineral are accumulated in the bones, therefore fish eaten with bones (for example, sardines) are the best source of calcium.

A two-way analysis of variance indicated that the mean value of P in the meat of roach caught in spring was higher than in fish from autumn and amounted to 2.24 g kg^{-1} in females and 2.30 g kg^{-1} in males from spring. These values were not significantly different. In the meat of fish caught in autumn the mean value of P was 1.89 g kg^{-1} in the tissues of females and 2.01 g kg^{-1} in males, these differences were also not significantly different (Table 1).

A correlation analysis indicated a negative and statistically important correlation between Ca and P concentration in the meat and the body length of analysed roach from the Brda River (Fig. 1).

The ratio of calcium to phosphorus in the meat of analysed wild roach is presented in Table 1 (values of this ratio are not high). Numerous studies show that the value of this ratio should be 1:1 in consumed products. If there is an excess of calcium over phosphorus P is not absorbed because this

Table 1

Calcium and phosphorus concentration (g kg^{-1} dry weight) in the meat of roach (*Rutilus rutilus*) caught in the spring and autumn from the Brda River

Season	Sex	n	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio
Spring	females	10	$1.82 \pm 0.68^{a,c}$	$2.24 \pm 0.26^{a,c}$	0.79:1
	males	10	1.93 ± 0.74^{a}	2.30 ± 0.26^{a}	0.82:1
Autumn	females	10	0.83 ± 0.41^{b}	1.89 ± 0.13^{b}	0.43:1
	males	10	$1.10 \pm 0.62^{b,c}$	$2.01 \pm 0.18^{b,c}$	0.53:1

Mean values denoted with various letters in the same column are significantly different ($P \leq 0.05$).

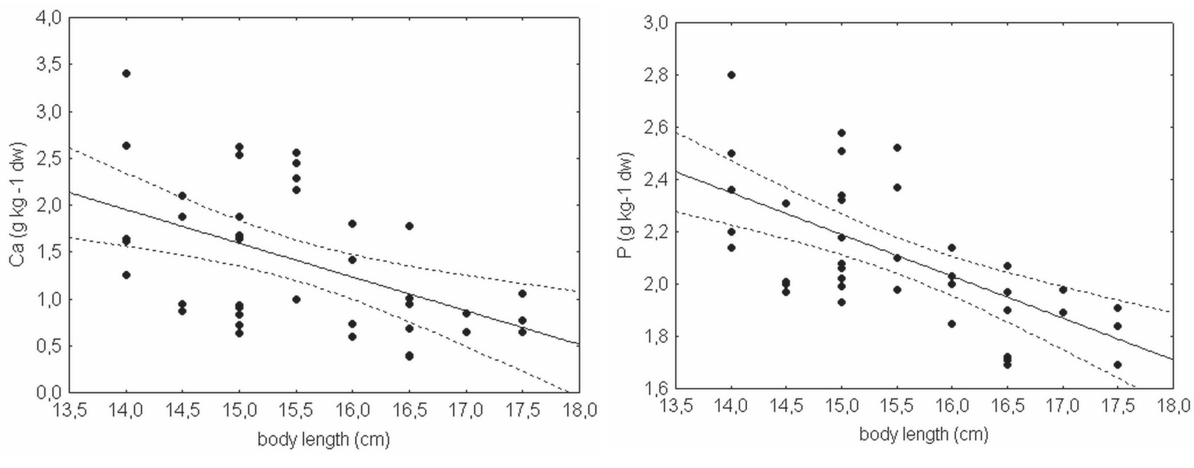

Fig. 1. Correlations between fish body length (cm) and Ca and P concentration (g kg^{-1} dry weight) in meat samples of roach (*Rutilus rutilus*) caught in the spring and autumn from the Brda River

form of calcium phosphate is not biologically available (Chavez-Sanchez *et al*. 2000). Porn-Ngan *et al*. (1993) investigated which portion of calcium phosphate and ratio of phosphorus to calcium inhibits zinc availability in rainbow trout by giving diets with different amounts of P and Ca. They observed that increased Ca levels slightly reduced average body weight. Nakamura (1982) investigated a negative relationship between the amount of P absorbed by carp and the dietary Ca content. Numerous studies show that excess phosphorus in the body causes calcium malabsorption which can lead to decalcification of the bones.

As indicated by the analyses, the muscle tissues are not considered to be the specific physiological sites for Ca and P (Al-Yousuf *et al*. 2000). Phosphorus and calcium accumulate in the largest amounts in bones. Borucka-Jastrzębska *et al*. (2009) determined micro- and macroelement concentrations in different tissues of freshwater fish (rainbow trout, common carp and Siberian sturgeon (*Acipenser baeri* B.). They reported that the calcium distribution followed the same pattern for all three analysed species in decreasing order: gills muscles skin liver kidney blood. As indicated by Perkowska and Protasowicki (2000) cadmium and lead accumulate in different ratios in the liver and kidney. In all analysed species the content of these metals were higher in gills than in muscles. This proves that the respiratory system is the main way of acquisition of these metals by fish. Higher cadmium concentrations were found in the liver of red gurnard (*Trigla cuculus*); whole lowest cadmium levels were always found in muscle tissue of the analysed fish. Lead concentrations were much higher than cadmium concentrations and the liver and gills accumulated large amounts of these metals (Canli & Atli 2003). In addition, a negative correlation between metal concentration and body length may be due to the dilution of these elements with an increase of body size, reducing the rate of nutrition, food ratios and mineral excretion by the gills, skin, and feces. Investigations have shown that the bioaccumulation of heavy metals (Cr, Mn, Ni) decreased as fish body length increased (Canli & Atli 2003). The main explanation of this negative correlation is a higher ability to metabolize compounds in younger fish than in older individuals. Another explanation may be that the mechanisms of neutralization of harmful metals are not developed sufficiently in young organisms. Therefore, larger amounts of minerals can be accumulated in their bodies (Kljaković *et al*. 2002). An analysis of the dependence of the content of mineral elements in particular organs and the body length of fish from Żnin Duże Lake indicated a positive correlation for Cu, Fe and Ni, and negative correlation for Zn and Mn (Stanek *et al*. 2005).

The obtained results indicate that environmental conditions such as the season of fish catch, temperature and the chemical composition of water affect the physiological status of fish and the ability to accumulate metals in their organs.

Cooling water discharged from power plants contains various substances essential for proper functioning of ecosystems. Concentrations of Ca in the meat of common carp attained a value of 2.73 g kg^{-1}. These levels were not high because the fish were reared in cooling water within the West Pomeranian Province (Borucka-Jastrzębska *et al*. 2009).

Metal concentrations in tissues are influenced by environmental contamination. Fish from more contaminated or cooler lakes had lower indicators of physical condition than individuals from cleaner reservoirs (Eastwood & Couture 2002). Variation in heavy metal pollution of Lake Balaton and accumulation capacities for Zn, Cu, Cd and Pb have been studied in common bream. Significant positive correlations were observed between the levels of heavy metal accumulated in the organs of fish and the pollutant load of the water (Farkas *et al*. 2003). As indicated by Chen and Chen (2001), metal content in fish tissues are related to the pollution of the environment. Except for Zn and Mn concentrations in the muscles of *Sardinella lemuru* being higher than those of the slightly polluted Chi-Ku Lagoon, the heavy metal concentration in the fish of Ann-Ping coastal were similar to those of slightly polluted regions. Eastwood and Couture (2002) investigated seasonal variations in liver metal contamination of yellow perch (*Perca flavescens*) caught from seven northeastern Ontario Lakes. Much higher concentrations of metals were detected in the spring. This may be due to increased metal input or bioavailability caused by snowmelt events or lake turnover that affect water quality parameters. The same results were observed by Laitinen (1994). The mean values of Ca concentration were significantly higher in the muscle of Eurasian perch caught in spring relative to samples taken from fish caught in winter and autumn. Mendil *et al*. (2010) reported that the concentrations of most analysed metals were higher in fish samples from summer than other seasons. The seasonal variations in the heavy metal loads of bream could be attributed rather to the seasonal change in the factor condition of fish than to variations in the pollutant load of the site (Farkas *et al*. 2003).

Analysed roach caught from the Brda River were in two age groups (5+ and 6+). There were significant differences in calcium and phosphorus content in the meat between five and six-year-old individuals ($P>0.05$). The concentration of minerals was determined at the level of 1.49 g Ca kg^{-1}

and 2.15 g P kg^{-1} in younger fish and 1.31 g Ca kg^{-1} and 2.04 g P kg^{-1} in the meat of older fish. DOBICKI and POLECHOŃSKI (2003) analysed the relationship between age of fish and level of metal bioaccumulation in tissues of four fish species. Many studies have shown that this interdependence varied and depended on the characteristics of individual species. In most cases a negative correlation between age and metal concentration was observed in their tissues. Similar results were observed by ŁUCZYŃSKA and BORUCKA-JASTRZĘBSKA (2005). They investigated the relationship between the content of heavy metals (lead and cadmium) in the muscle tissue and the size of the four fish species.

The analyses indicated that there were insignificant differences between samples taken from females and males of roach caught within each season. As reported by VASNJIC-JEFTIC *et al.* (2010) there was a significant difference in heavy metals between the males and females of Pontic shad in both muscle and liver. Mg content in the muscle was higher in males, concentrations of As and Mg in liver were higher in females, while content of Cd, B, Ba, Fe, Zn and Li in liver were higher in males. Higher concentration of Mg in liver of females could be explained by its ability to bind to vitellogenin.

Conclusions

1. The mean values of Ca and P concentrations were significantly higher in the muscle of fish caught in spring, relative to samples taken from fish caught in autumn.

2. There were insignificant differences between samples taken from females and males caught within each season.

3. The ratio of calcium to phosphorus in the meat of analysed wild roach ranged from 0.43:1 to 0.82:1.

4. There was a negative and statistically important correlation between Ca and P concentration in meat and body length of analysed roach from the Brda River.

5. The meat of examined roach was not a rich source of calcium.

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