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CONTENT OF CALCIUM AND PHOSPHORUS IN THE MEAT, GILLS AND LIVER OF PERCH (*PERCA FLUVIATILIS* L.) FROM THE WIELKOPOLSKA LAKES DISTRICT (POLAND)

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Abstract

The aim of this work was to compare the concentration of calcium and phosphorus and Ca/P ratio in the meat, gills and liver of perch (*Perca fluviatilis* L.) caught from the Lake Góreckie, Strzeszyńskie and Wędromierz (the Wielkopolska Lake District). The study involved 30 individuals of fish caught in spring. The muscles samples for analyses were taken from the large side muscle of the fish body above the lateral line. Calcium concentration was determined by atomic absorption spectrophotometer Solar 969, Unicam. Phosphorus content was analyzed with colorimetric method, by spectrophotometer Lambda 25, Perkin-Elmer (at wavelength 430 nm). The mean content of Ca in the meat of fish caught from Lake Góreckie, Strzeszyńskie and Wędromierz was 3.175, 0,516 and 2.498 g kg^{-1}, respectively. The significantly higher amounts of Ca were determined in the gills than in the meat and liver and ranged from 63.09 g kg^{-1} (the Lake Góreckie) to 70.24 g kg^{-1} (the Lake Strzeszyńskie). The mean content of P was at the same level in the meat and in the liver and ranged from 2.703 g·kg^{-1} to 2.812 g kg^{-1}. Ratio of calcium to phosphorus in the meat of fish caught from the lake Góreckie, Wędromierz and Strzeszyńskie was 1.174:1, 0.254:1 and 0,888:1, respectively.

Keywords: calcium, phosphorus, organs, perch.

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ZAWARTOŚĆ WAPNIA I FOSFORU W MIĘSIE, SKRZELACH I WĄTROBIE OKONIA (*PERCA FLUVIATILIS* L.) Z POJEZIERZA WIELKOPOLSKIEGO

Abstrakt

Celem pracy było oznaczenie stężenia wapnia i fosforu oraz obliczenie stosunku Ca do P w mięśniach skrzelach i wątrobie okonia (*Perca fluviatilis* L.) odłowionego z Jezior: Góreckiego, Strzeszyńskiego i Wędromierz (Pojezierze Wielkopolskie). Badaniami objęto 30 osobników pozyskanych wiosną 2012 roku. Materiał do analiz stanowił mięsień najdłuższy grzbietu z nadosiowej części ciała, skrzela i wątroba. Stężenie wapnia oznaczono z użyciem spektrofotometru absorpcji atomowej Solar 969, Unicam. Stężenie fosforu oznaczono metodą kolorymetryczną stosując spektrofotometr Lambda 25, Perkin-Elmer (długość fali 430 nm). Średnia zawartość Ca w mięsniach ryb odłowionych z Jezior Góreckiego, Strzeszyńskiego i Wędromierz wynosiła odpowiednio: 3,175, 0,516 i 2,498 g kg^{-1}. Znacząco więcej Ca oznaczono w skrzelach, w porównaniu z próbami mięśni i wątroby; wartości te mieściły się w zakresie od 63,09 g kg^{-1} (Jezioro Góreckie) do 70,24 g kg^{-1} (Jezioro Strzeszyńskie). Średnia zawartość P była bardzo zbliżona w próbach mięśni oraz wątroby i wynosiła od 2,703 g kg^{-1} do 2,812 g kg^{-1}. Stosunek zawartości wapnia do fosforu w mięśniach ryb odłowionych z Jezior Góreckiego, Wędromierz i Strzeszyńskiego wynosił odpowiednio: 1,174:1, 0,254:1 i 0,888:1.

Słowa kluczowe: wapń, fosfor, narządy, okoń.

INTRODUCTION

Fish meat is an important source of amino acids, proteins, vitamins dissolved in lipids, long chain fatty acids and lipids as a valuable energy source. Through their high nutritive values fish meat should be a very significant part of the human diet, because it have low risk of coronary heart disease, hypertension and cancer (Mendil et al. 2010). Metals can be accumulated by fish, both through the food chain and water (Mendil, Uluözlü 2007). Fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human organism (Al-Yousuf et al. 2000). The level and intensity of the accumulation depends on many factors, such as: species of fish, size, sex, seasonal changes and environmental factors (Yilmaz et al. 2010). Trace elements are introduced into aquatic system (lakes, rivers) through atmospheric fallen dumping wastes and geological weathering (Al-Yousuf et al. 2000).

Tissue metals concentrations are influenced by an environmental contamination. Fish from more contaminated or cooler lakes had lower indicators of physical condition than individuals from cleaner reservoirs (Eastwood, Couture 2002). There were carried out analyses about variations in heavy metals pollution of the Lake Balaton and accumulation capacity especially for Zn, Cu, Cd and Pb in common bream. There were observed significant positive correlations between the level of heavy metals accumulated in the organs of fish and the pollutant load of the water (Farkas et al. 2003). As Chen and Chen (2001) indicated, metal content in fish tissues are related to the pollution status 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 metals concentrations in the fish of Ann-Ping coastal were similar to those of slightly polluted regions. EASTWOOD and COUTURE (2002) investigated seasonal variations in the liver metal contamination of yellow perch (*Perca flavescens*) caught from seven northeastern Ontario Lakes. There were much higher concentrations of metals in the spring. It 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).

Phosphorus is one of the most essential mineral, necessary for normal growth, bone mineralization, reproduction and energy metabolism in fish (NAKAMURA 1982, ALBREKTSEN et al. 2009). The availability of phosphorus on the organism depends on the chemical form and solubility of the mineral. As ALBREKTSEN et al. (2009) indicated in Atlantic salmon, primary inorganic salts of phosphorus are more available than secondary salts, whole phosphorus bound to calcium in the bone tissue is the last available. Calcium and phosphorus are essential elements for human being which means that those metals should be part of human diet (CELIK et al. 2004). Calcium and phosphorus plays a very important roles in several physiological processes and are directly involved in the development and maintenance of the skeletal system. In vertebrates calcium is complexed with phosphorus in hydroxyapatite to form the principal crystalline of bone (YE et al. 2006). Ca/P ratio is the most important indicator for good bone health.

The daily dose of phosphorus in the diet of an adult should be 800 mg (SAPEK 2009), and approximately 1000 mg of calcium (1200 mg per day for women and men aged 25 years and younger, and 1100 mg per day for women over 60 years old due to progressing in loss in bone mineral pass) (LIDWIN-KAŹMIERKIEWICZ et al. 2009). The ideal would be to consume the same amount of phosphorus as calcium (calcium/phosphorus balance). The ratio between phosphorus and calcium greater than 3:2 may cause metabolic disorders. Fish meat is a rich source of phosphorus and those can be the order of 150-200 mg 100 g^{-1} of product. This element occurs in almost all species of fish. Species which are rich in calcium are sardines, herring, sprat and salmon. Approximate share of phosphorus and calcium in the fish meat should be at 1.8% (dry weight).

The aim of this work was to determine calcium and phosphorus content and Ca/P ratio in the meat, gills and liver of perch caught from Lake Góreckie, Strzeszyńskie and Wędromierz during spring and analyze content of the elements in the different organs. Muscles are not always a good indicator of the whole body fish metal contamination and, therefore, it is important to analyze other tissues, such as the liver and gills (JARIĆ et al. 2011).

MATERIAL AND METHODS

Study area

The Lake Góreckie is a dimictic lake with a surface area of ca. 104 ha, maximum and mean depths of 17.25 m and 8.97 m, respectively and a shore line of 8300 m. The degree of antropopressure from the annual nitrogen and phosphorus for the eutrophic Lake Góreckie is estimated at 9.5 g m^{-2} $year^{-1}$ for total nitrogen and 0.36 for total phosphorus g m^{-2} $year^{-1}$ (PEŁECHATY, OWSIANY 2003). The Lake Strzeszyńskie has got a surface area of 34.9 ha and is located in the administrative boundaries of the city of Poznań. The maximum depth is 18.8 m and the mean depth is 8.2 m. A shore line of 4500 m is mostly covered with deciduous and pine forests used mainly for recreational destination. The lake has purity class No. 2. and it is used by the Polish Fishing Association. The Lake Wędromierz is a flow reservoir, with an surface area of 73.8 ha, maximum and mean depths of 11.8 m and 4.9 m, respectively and a shore line covered with a mixed forests.

Trophic level of the lakes was based on Carlson's index (CARLSON 1977) calculated as an average for three seasons (spring, summer and autumn). The basis for the calculation were the results of analysis: Secchi disk visibility, chlorophyll concentration and the concentration of total phosphorus in the surface layer carried out in 2012.

The obtained values of Carlson index were for particular lakes: the Lake Góreckie – 71, the Lake Strzeszyńskie – 63 and the Lake Wędromierz – 83. On this basis, all the lakes were classified as eutrophic.

Fish samples

The study involved 30 individuals of perch (*Perca fluviatilis* L.) caught in spring (April, 13-25 2010). The experimental fish were obtained in natural condition from the Wielkopolska Lake District. Measurements of the mass of the fish body – BW (± 0.01 g) and body length – Lc (± 0.1 cm) and the total and body length – Lt (± 0.1 cm) were taken on the each individuals. The meat samples for analyses were taken from the large side muscle of fish body above the lateral line. Due to a relatively low amounts of liver and gills obtained from one individuals, the material was combined (about 2 pieces of each). There were chosen for analysis individuals with similar biometric measurements. Body weight ranged from 160 to 295 g, and body length and total length was from 20.0 to 23.5 cm and from 21.5 to 28.0, respectively (Table 1).

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

The freeze dried samples were mineralized in microwave mineraliza-

Table 1

The mean values of the biometric measurements of perch (*Perca fluviatilis* L.) from Lake Góreckie, Strzeszyńskie and Wędromierz

Place of catch	Total length (cm) (range and mean)	Body length (cm) (range and mean)	Body mass (g) (range and mean)
Lake Góreckie	21.5-27.5 25.5	18.0-22.5 20.0	170.5-270.5 182.2
Lake Strzeszyńskie	22.0-27.0 23.8	18.5-23.5 20.5	165.0-255.0 170.6
Lake Wędromierz	23.0-28.0 24.7	19.5-24.0 21.2	160.0-295.0 195.6

tor Ethos Plus, Milestone. For the mineralization 0.1 g of the tissue was weighted and then HNO_3 and H_2O_2 were added in ratio 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 have been carried quantitatively to the measuring flask with a capacity of 50 cm^3.

Calcium concentration was determined by atomic absorption spectrophotometer Solar 969, Unicam. Phosphorus content was analyzed with colorimetric method, by spectrophotometer Lambda 25, Perkin-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 have been reported as g kg^{-1} dry weight (g kg^{-1} d.w.).

The accuracy of the analyses was controlled by adding standard solutions. As a standards were used calcium standard solution $Ca(NO_3)_2$ in HNO_3 (Merck, Germany) and KH_2PO_4 (POCH S.A., Poland) dissolved in water.

Statistical analyses

Data analyses were performed by using the Statistica 8.0 software (StatSoft, USA). Significance of differences in the average content of calcium and phosphorus in the meat, gills and liver of perch were statistically analysed with the Tukey's test. In this case, a significance of differences in the average content of Ca and P (between the lakes within a single tissue) were calculated by one-way analysis of variance (Anova).

RESULTS AND DISCUSSION

It is very difficult to compare a metal concentrations between the same tissue of two different species or the same species lives in a different reservoir, because of different feeding habits, differences in aquatic environment, growing rates of the species, types of analyzed tissues and other factors (Yilmaz et al. 2010). As Brucka-Jastrzębska, Protasowicki (2006) indicated levels of bio-elements and harmful metals in the carp body depend on the culture method, water quality and type of the feed. The rate at which metals affect the organism is related to the metal transporting function of the blood.

Table 2

The mean content of calcium and phosphorus (g kg^{-1} dry weight) in the meat, liver and gills of perch (*Perca fluviatilis* L.) from Lake Góreckie, Strzeszyńskie and Wędromierz

Place of catch	Tissues	*n*	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio
Lake Góreckie	meat	10	3.175±0.508^a	2.633±0.520^a	1.174:1
	liver	5	2.043±0.067^a	2.703±0.013^a	0.756:1
	gills	5	63.09±1.969^a	7.079±0.407^a	8.912:1
Lake Strzeszyńskie	meat	10	0.516±0.058^b	2.028±0.270^b	0.254:1
	liver	5	1.870±0.399^a	2.703±0.216^a	0.692:1
	gills	5	70.24±3.061^b	7.054±0.511^a	9.958:1
Lake Wędromierz	meat	10	2.498±0.305^c	2.812±0.337^a	0.888:1
	liver	5	1.123±0.235^b	2.773±0.155^a	0.405:1
	gills	5	70.17±5.000^b	6.942±0.334^a	10.107:1

The values for the same tissues marked with different letters in the same column are significantly different ($p \leq 0,05$, Tukey's test).

As analyses indicated the mean content of Ca in the meat of perch caught from the Lake Góreckie, Strzeszyńskie and Wędromierz was 3.175, 0.516 and 2.498 g kg^{-1}, respectively. Those values differed statistically significantly (one-way Avova, Tukey test) – Table 2. The highest Ca concentrations in the liver were determined in fish collected from the Lake Wędromierz (1.123 g kg^{-1}) and this value differed statistically significantly from those indicated in the liver of fish from the Lake Góreckie and Strzeszyńskie. The mean content of Ca in the gills was significantly higher than in the meat and liver and ranged from 63.09 g kg^{-1} (the Lake Góreckie) to 70.24 g kg^{-1} (the Lake Strzeszyńskie). Analyses indicated that the mean content of Ca decreased in the following sequence: gills > meat > liver (the Lake Góreckie and Wędromierz) and gills > liver > meat (the Lake Strzeszyńskie) – Figure 1.

Łuczyńska et al. (2009) determined an essential mineral components in the muscles of six freshwater fish from Mazurian Great Lakes. They examined that the non-predatory fish (bream, roach and vendance) contained more calcium (74.6 mg) than the predatory fish (perch, pike and burbot) (59.5 mg), but there were no statistically significant differences between these values. The mean content of calcium in the meat of perch ranged from 43.7 to 94.8 mg 100 g^{-1}. A large amounts of calcium are accumulated in the bones, therefore the fish eaten with the bones (for example, sardines) are the best source of calcium. Analyses of the whole body of Prussian carp (*Carassius auratus gibelio*) indicated that the mean content of calcium was 5.63 g kg^{-1} wet weight (Stanek et al. 2013) – Table 3. Calcium concentra-

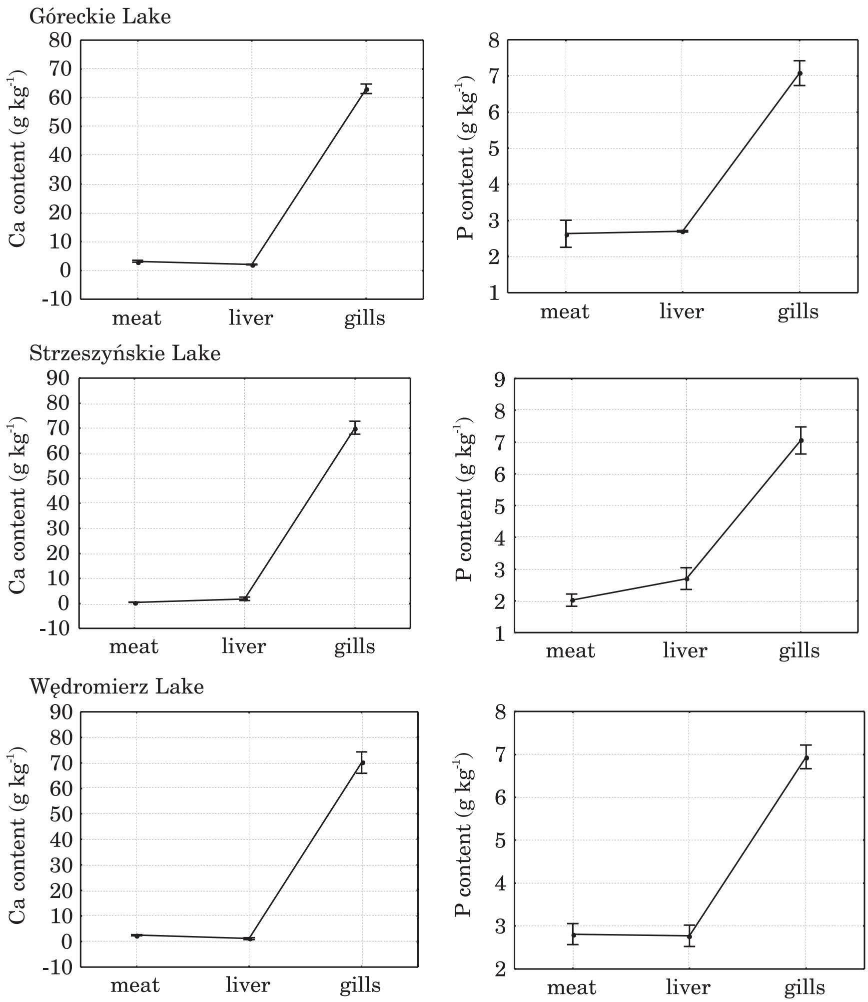

Fig. 1. The mean content of calcium and phosphorus (g kg^{-1} dry weight) in the meat, liver and gills of perch (*Perca fluviatilis* L.) from Lake Góreckie, Strzeszyńskie and Wędromierz

tion in the large side muscle of the fish body above the lateral line of roach from the Brda River was 1.82 g kg^{-1} in females and 1.93 g kg^{-1} in males (in spring). In the individuals from autumn it was 0.83 and 1.10 g kg^{-1}, respectively (STANEK, JANICKI 2011). These analyses indicated that calcium concentration was much higher in the whole body of fish than in a different parts of body. Content of calcium differed statistically significant between individuals caught in a different seasons, but there were not statistically significant differences between samples taken from females and males caught within one season. Meat of the analysed roach were not a rich source of calcium.

Analyses of variance indicated that the mean content of P (g kg^{-1} wet weight) was in the same level in the meat and liver of perch and ranged from 2.028 to 2.812 g kg^{-1} and from 2.703 to 2.773 g kg^{-1}, respectively. The average content of phosphorus in the gills was significantly higher than in the other tissues and ranged from 6.942 to 7.079 g kg^{-1} (Table 2). Analyses indicated that the mean content of P decreased in the following sequence: gills > meat > liver (the Lake Wędromierz) and gills > liver > meat (the Lake Góreckie and Strzeszyńskie) – Figure 1.

Łuczyńska et al. (2009) examined that the mean content of phosphorus in the meat of perch ranged from 1068.3 to 1265.4 mg 100 g^{-1}. Analyses carried out by Stanek et al. (2013) indicated that the mean concentration of phosphorus in the whole body of Prussian carp was 2.38 g kg^{-1}. Two-way analyses of variance indicated, that the mean value of phosphorus in the meat of analyzed roach from Brda River caught in spring was higher than in the fish from autumn, and it was 2.24 g kg^{-1} in females and 2.30 g kg^{-1} in males from spring, respectively. There were no statistical significant differences between those values. In the meat of fish caught in autumn the mean value of phosphorus was 1.89 g kg^{-1} in the tissue of females and 2.01 g kg^{-1} in males. There were no statistical significant differences between these values (Stanek, Janicki 2011).

As indicated analyses about perch and those previous about roach and Prussian carp, the muscle tissue are not considered to the specific physiological sites for calcium and phosphorus (Al-Yousuf et al. 2000). Phosphorus and calcium accumulate in the largest amounts in bones. Borucka-Jastrzębska et al. (2009) determinated micro- and macroelements concentration in the different tissues of freshwater fish (rainbow trout, common carp and Siberian sturgeon (*Acipenser baeri* B.). And they reported that calcium distribution followed the same pattern for all the three analyzed species in decreasing order: gills > muscles > skin > liver > kidney > blood. As Perkowska, Protasowicki (2000) indicated metals accumulate in a different ratio in the liver and kidney. Thus, fish livers represent a good biomonitors of metals present in the surrounding environment. Analyses showed that high levels of the heavy metals were in the liver while the lowest ones were in the muscles. The difference in accumulation potential between these two tissues can be explained by the activity of metallothioeins, proteins that are present in liver but not in the muscle, which have the ability to bind certain metals and thus allow the tissue to accumulate them at a high degree (Visnjic-Jeftic et al. 2010). Liver as a particularly metabolically active tissue, has a tendency to accumulate metals to higher degree than muscle tissue (Yilmaz et al. 2010). Elements have a tendency to react with the oxygen carboxylate, amino group, nitrogen and/or sulphur of the mercapto group in the metallothionein protein which is at highest concentration in the liver (Al-Yousuf et al. 2000). In all fish species analyzed by Roméro et al. (1999) the content of metals were higher in the gills than in the muscles. It proves that the respiratory system is the main way of asquisition of this metals by fish. Gills and liver are chosen as target organs for assessing metal accumulation. The highest concentrations of elements (for Fe, Cu, Mn, Sr, Cr, Cd, Co and Pb) were found in the liver and skin, while the lowest ones were in the muscle. Main concentrations of other studied metals (e.g. Na, As) were found more in the muscle tissue than in liver and skin. Analyses of the heavy metals carried out by CANLI, ATLI (2003) indicated that the highest cadmium concentrations were found in the liver of red gurnard (*Trigla cuculus*), whole the lowest cadmium levels were always found in muscles tissues of the analyzed fish. Lead concentration were much higher than cadmium and the liver and gills accumulated the great amounts of this metal. As JARIĆ et al. (2011) indicated, liver was the main heavy metal storage tissue, while the muscle had the lowest amounts of the analyzed metals.

EASTWOOD, COUTURE (2002) investigated seasonal variations in liver metal contamination of yellow perch (*Perca flavescens*) caught from seven northeastern Ontario Lakes. There were much higher concentration of metals in the spring. It may be due to increased metal input or bioavailability caused by snowmelt events or lake turnover that affect water quality parameters. DRĄG-KOZAK et al. (2011) determined heavy metals concentration in some tissues and organs of rainbow trout (*Oncorhynchus mykiss*) and they reported that higher concentration of the metals were found in the spring compared with those during the autumn season. 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. As investigated MENDIL et al. (2010) concentration of most determined metals have been reported higher in the fish samples of summer season than other. The seasonal variations in the heavy metals load 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, DURAL et al. 2006).

As analyses indicated Ca/P ratio in the meat of fish caught from the Lake Góreckie, Wędromierz and Strzeszyńskie was 1.174:1, 0.254:1 and 0.888:1, respectively (Table 2). As numerous studies show the value of this ratio should be 1:1 in the consumed products, because when there is an excess of calcium over phosphorus, phosphorus is not absorbed, because this form calcium phosphates is not biologically available (CHAVEZ-SANCHEZ et al. 2000). Ca/P ratio in the meat of Prussian carp was 2.37:1 (STANEK et al. 2013) – Table 3. Ratio of calcium to phosphorus in the meat of wild roach from Brda River was ranged from 0.43:1 to 0.82:1 (STANEK, JANICKI 2011) – Table 3. PORN-NGAN et al. (1993) investigated which portion of calcium phosphate and ratio phosphorus to calcium inhibits zinc availability in rainbow trout by giving diets with different amounts of P and Ca. They observed that increase Ca levels slightly reduced the average body weight. NAKAMURA (1982) investigated a negative relationship between the amount of P absorbed by carp and the dietary Ca content. And numerous studies show

Table 3

The mean content of calcium and phosphorus (g kg^{-1} dry weight) in the meat of Prussian carp (*Carassius auratus gibelio*) from Lake Gopło and roach (*Rutilus rutilus*) from Brda River

Species	Place of catch	*n*	Ca (g kg^{-1})	P (g kg^{-1})	Ca:P ratio
Prussian carp* *Carassius auratus gibelio*	Lake Gopło	14	5.63 ± 1,24	2.38 ± 0,31	2.37:1
Roach** *Rutilus rutilus*	Brda River	40	1.41 ± 0,76	2.11 ± 0,27	0.67:1

* Stanek et al. (2013)
** Stanek and Janicki (2011)

that excess of phosphorus in the body causes calcium malabsorption, which can lead to decalcification of the bones.

CONCLUSION

1. Perch caught from lakes of similar trophic level were characterized by differences in the content of calcium in the muscle, gills and liver.
2. There were no statistically significant differences in the mean content of phosphorus determined in the liver and gills of fish collected from the lake Góreckie, Strzeszyńskie and Wędromierz.

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