NUTRITIONAL COMPOSITION AND HEAVY METAL CONTAMINATION IN FISH FROM KARDZHALI DAM, BULGARIA

Violina R. Angelova*, Ljudmila N. Nikolova, Stanimir G. Balabanov, Georgi K. Georgiev

Agricultural Universty-Plovdiv, 12 Mendeleev str., Plovdiv, 4000, Bulgaria

Abstract

This study aimed to determine the nutritional value, heavy metals, and micro and macro elements in four species of fish from the Kardzhali dam (Carassius gibelio, Perca fluviatilis, Vimba melanops and Rutilus rutilus). Data on the content of proteins, lipids, heavy metals, and micro and macro elements were obtained, and the energy value in kcal/100g and kJ/100 g fresh weight was calculated. The analyzed species can be divided into two groups: with low lipid content (up to 2.0%) - perch (1 g/100g fresh weight); and with medium lipid content (2.0-8.0%) - Prussian carp, malamida, and roach (2.0 to 2.7 g/100g fresh weight). All freshwater fish examined from the Kardzhali dam belonged to the category of foods with medium and high energy content (more than 170 kJ/100g) and were a good source of protein (21.52-24.54%). All four fish studied are essential magnesium, potassium, and sodium sources. Zinc, iron, phosphorus, and calcium predominate in Prussian carp and roach, while copper and manganese in malamida. The content of heavy metals mercury, lead, cadmium, and zinc in all fish examined was below the maximum allowable concentration (MAC). All four tested fish can be recommended for consumption by the population in the area.

Keywords: *freshwater fish, proximate analysis, toxic metals, nutrients*

1. INTRODUCTION

Fish meat contains amino acids, protein, minerals, and vitamins. The proteins in fish act as protective agents against viral and bacterial infections and prevent protein-calorie malnutrition. Meat is also rich in polyunsaturated fatty acids, making fish helpful in treating many conditions associated with inflammation, such as cardiovascular disease, ulcerative colitis, and hyperlipidemia (Ali et al., 2022), as well as influencing food taste and texture (Ahmed et al., 2022). Water-soluble vitamins of the B-complex are precursors of coenzymes involved in metabolic pathways.

The meat of freshwater wild fish is high in branched-chain fatty acids (BCFA) (Yang et al., 2016), and marine and ocean fish are rich in sodium and potassium. Small freshwater fish are rich in calcium, iron, and manganese (Mohanty et al.,2016), with species, sex, and catch season influencing (Emre et al.,2018).

On the other hand, fish are a good indicator of heavy metal contamination in aquatic systems (Younis et al., 2021). Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), chromium (Cr), and arsenic (As) are of particular concern due to their toxicity and ability to bioaccumulate in aquatic ecosystems as well as their persistence (Chowdhury & Mamunur, 2021). Sources of heavy metal contamination of aquatic systems are mainly anthropogenic activities, domestic wastewater, agricultural activities, and discharge of petroleum products (Ayanda et al., 2019).

Fish from freshwater ecosystems, characterized by slow water exchange and high anthropic pollution, can be used to assess heavy metal pollution (Yousafzai et al., 2010). Heavy metals can accumulate in fish due to water and sediment contamination or along the food chain. Because they are located at a high trophic level in the food chain, they can accumulate large amounts of some metals from water at concentrations significantly higher than those in water (Yousafzai et al., 2010). The level and intensity of accumulation depend on many factors, such as fish species, size, sex, seasonal changes, and environmental factors (Yilmas, et al., 2010). Through fish consumption, heavy metals can enter humans.

This study aimed to determine the nutritional value, heavy metals, and micro and macro elements in four species of fish from the Kardzhali dam (Carassius gibelio, Perca fluviatilis, Vimba melanops and Rutilus rutilus).

2. MATERIALS AND METHODS

2.1. Materials

Kardzhali dam is located in the Eastern Rhodopes, near the town of Kardzhali. Four fish species were studied - Prussian carp (Carassius gibelio), perch (Perca fluviatilis), malamída (Vimba melanops), and roach (Rutilus rutilus). Caracus is a peaceful omnivorous fish, roach is a peaceful fish, feeding on crustaceans, insect larvae, worms, algae, and higher aquatic vegetation. Perch are predatory fish, with juveniles feeding on zooplankton, adults on benthic invertebrates, and only the largest fish feeding entirely on small fish. Malamida is a demersal fish. It feeds on benthic invertebrates and algae.

The fish were caught with a fishing line in the autumn (September- November). Specimens of average size for the given species were selected. The sampled specimens were transported to the Agricultural University-Plovdiv laboratory in an ice cooler bag. The fish were analyzed immediately or stored without compromising their integrity in a freezer, at -20ºC, until analysis. In the laboratory, fish were washed with distilled water to remove sand, silt, or algae and dried with filter paper. An ichthyometer and a precision balance were used to measure the length and weight of each fish, respectively. After morphometric measurement, fish were dissected with a stainless steel knife. The fish were filleted after removing the head, tail, and guts. The filleted tissue was minced and homogenized.

2.2. Methods

The content of heavy metals, macro, trace elements, proteins, and fat content of the examined fish from the lake was determined. Kardzhali. The microwave mineralization method (MARS 6 microwave system, CEM Corporation, USA) was used to prepare the fish for heavy metal, macro, and trace element analysis. The elements were determined using an ICP-OES spectrometer (Prodigy model 7, Teledyne Leeman Labs, USA). The mercury content was determined by mercury analyzer MA 3000. Protein content was determined according to BDS 9374 (1982), and moisture content according to BDS 5712(1974). In determining fat content, lipids were isolated by hydrolysis of the sample with hydrochloric acid, and the liberated fat was extracted with petroleum ether. The solvent was distilled off and calculated as a percentage by weight of the original sample (AOAC, 2012).

The energy value (FAO, 2003) was determined using the formula

$$\text{Energy value} = C \times 17 + F \times 38 + P \times 17 \quad (\text{kJ/100g})$$

$$\text{Energy value} = C \times 4 + F \times 9 + P \times 4 \quad (\text{kcal/100g})$$

C – the content of carbohydrate (%);

F – content of fat (%);

P – the content of protein (%);

The energy equivalent of carbohydrates is 17 kJ (4 kcal), of fat, is 38 kJ (9 kcal), and of protein is 17 kJ (4 kcal).

Analyses were performed in triplicate, and all data were expressed as mean values. All data were analyzed with Statistica software version 10.0.

3. RESULTS

The total body length in Prussian carp was 30.6±0.99 cm with a mean fish mass of 528.60±26.62 g; in malamída 24.74±0.89 cm with a mean fish mass of 186.32±18.04 g; in roach 22.62±0.37 cm and 180.12±12.94 g, respectively; in perch 24.40±0.76 cm and 259.16±30.45 g, respectively.

The results obtained for the chemical composition and energy value of the four fish species sampled during the autumn season (September-November) from the Kardzhali dam are presented in Fig. 1. The mineral content of the fish studied is presented in Fig. 2.

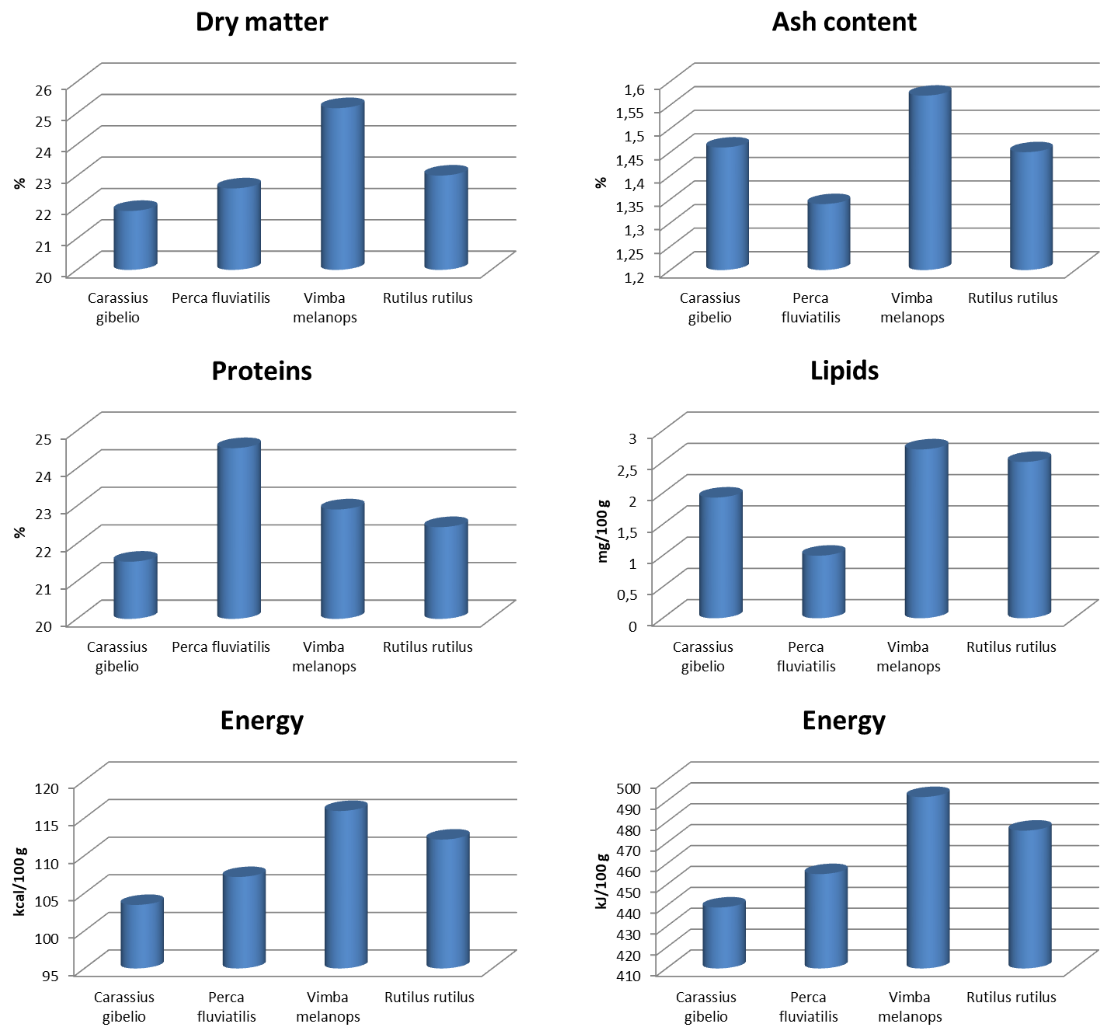
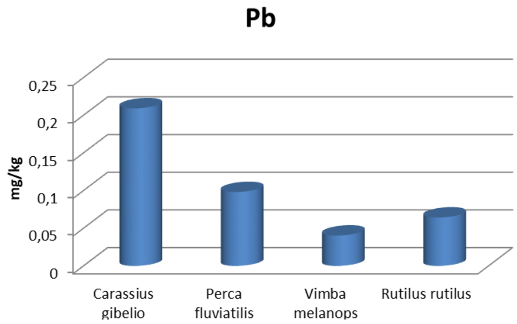


Fig. 1. Chemical composition and energy value of the fish species sampled during the autumn season (September-November) from the Kardzhali dam (Carassius gibelio, Perca fluviatilis, Vimba melanops, and Rutilus rutilus).


Pb
mg/kg
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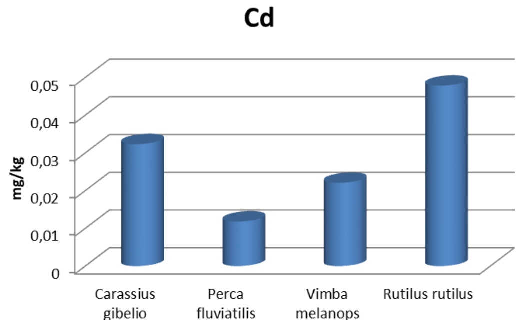
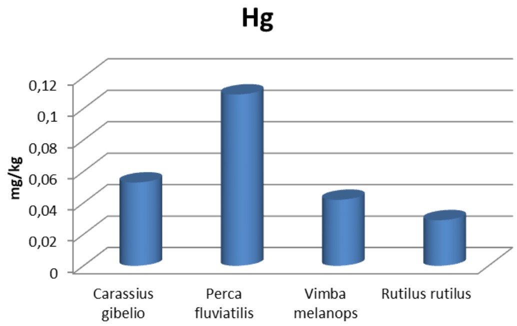
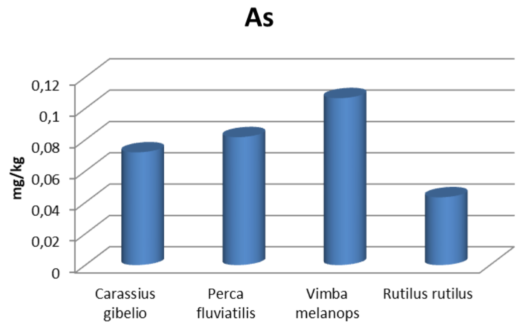
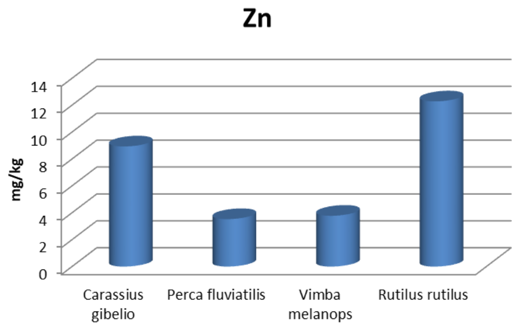
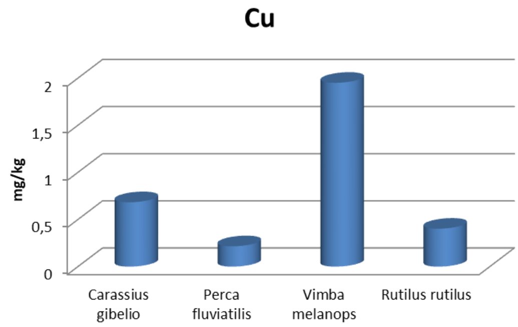
0,2
0,15
0,1
0,05
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus

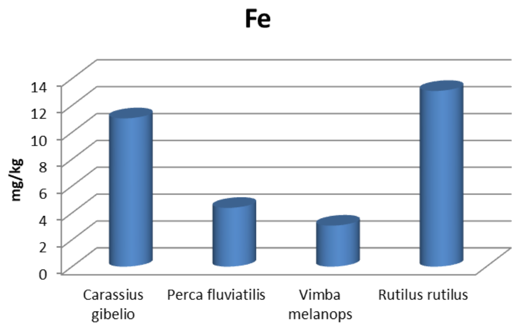

Cd
mg/kg
0,05
0,04
0,03
0,02
0,01
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus

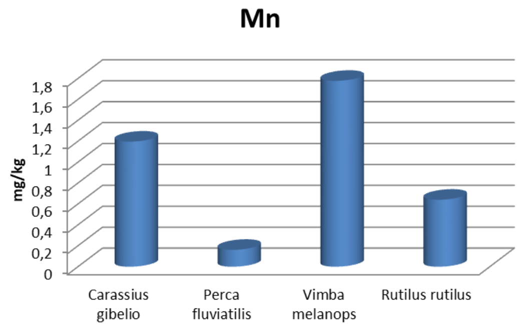

Hg
mg/kg
0,12
0,1
0,08
0,06
0,04
0,02
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus


As
mg/kg
0,12
0,1
0,08
0,06
0,04
0,02
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus


Zn
mg/kg
14
12
10
8
6
4
2
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus


Cu
mg/kg
2
1,5
1
0,5
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus


Fe
mg/kg
14
12
10
8
6
4
2
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus


Mn
mg/kg
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
Carassius gibelio
Perca fluviatilis
Vimba melanops
Rutilus rutilus

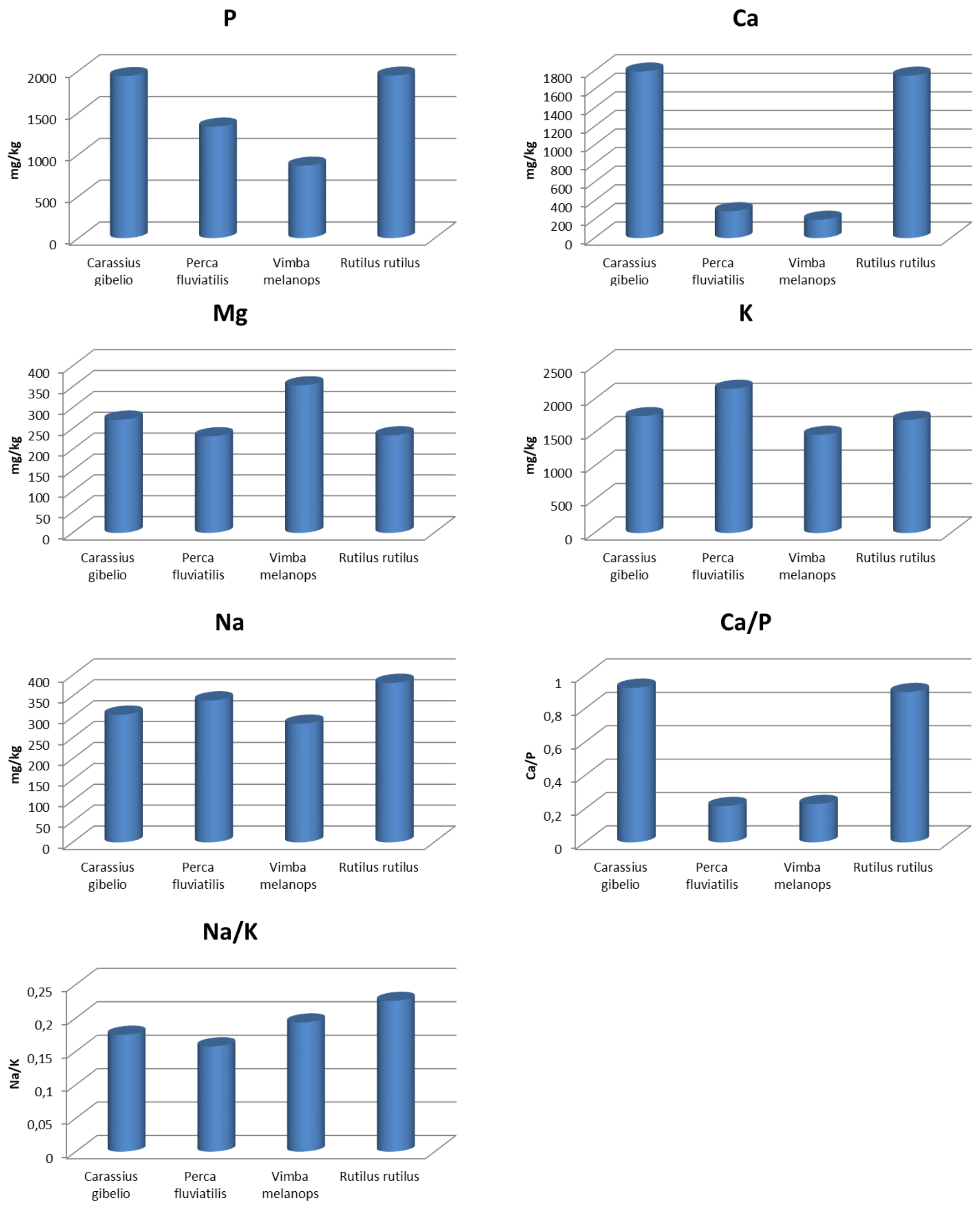


Fig. 2. Mineral content of the fish species sampled during the autumn season (September-November) from the Kardzhali dam (Carassius gibelio, Perca fluviatilis, Vimba melanops, and Rutilus rutilus).

4. DISCUSSION

4.1. Nutrients

Moisture, protein, fat, and ash are the significant components of fish flesh, and the analysis is called "proximate composition" (Love, 1970). Like any other meat, moisture, protein, fat, and ash (mainly minerals) constitute the four major constituents of fish meat. Carbohydrates and non-protein compounds are important constituents but are present in small amounts and are usually ignored during analysis (Love, 1980). Fresh fish are characterized by 70-80% moisture, 20-30% protein, and 2-12%

fat (Love, 1980). However, these values can vary considerably within and between species and also with size, sex, diet, season, and environmental conditions. The estimated composition is usually used as an indicator of the nutritional value of the food.

The dry matter content of the fish ranged from 21.88 % in Prussian carp to 25.16% in malamida, and the ash content ranged from 1.34% in perch to 1.57% in malamida (Fig.1). The mineral and trace element contents that comprise the total ash content depend on factors such as feeding behavior, environment, etc.

According to Ali et al. (2022) freshwater fish contain, on average 220.2 g/kg dry matter, 12.4 g/kg crude ash, 176.2 g/kg crude protein, 32.6 g/kg ether extract, and 104.6 kcal.

The results obtained for lipid content of fish species ranged from 1 g/100 g fresh weight (perch) to 2.7 g/100 g fresh weight (malamida) (Fig.1).

According to Orban et al. (2007), the perch is characterized by total lipid content ranging from 0.64 g to 1.20 g/100 g, depending on the season and location of capture, and good protein levels (17.06 - 18.99 g/100 g). Many authors have suggested that lipid content should also be used as an indicator of the nutritional and ecological status of the catching region due to the direct relationship between lipid content and available food. Three species - Prussian carp, malamida, and roach can be classified as having medium lipid content (2.0-8.0 g/100g fresh weight), and perch as a low lipid species (up to 2.0 g/100g fresh weight).

The flesh of all four fish species contains relatively high protein levels (21.52-24.54%). The average protein content of the species allows them to be ranked in the following order: perch>malamida> roach> Prussian carp (Fig.1).

4.2. Energy value of fish species

Fish contain negligible amounts of carbohydrates (less than 0.5%) and minerals, which are not considered when determining their nutritional value. Based on the lipid and protein content data of these species, the energy value was also calculated. The energy value of the fish studied ranged from 439 to 492 kJ./100 g fresh weight (Fig.1).

The relative distribution of energy input via lipids and proteins in the consumption of the analyzed fish species was determined. A greater relative energy percentage input of the total energy value was attributed to protein in all four species. The proportion of energy percentage imported from the entire energy value of total lipids was relatively low (4-11%). The highest values were recorded for malamidaa (11%) and the lowest for perch (4%).

4.3. Mineral content

4.3.1. Macroelement content

Fish flesh is an excellent source of essential minerals (Njinkoue et al., 2016). Phosphorus, potassium, and calcium were the dominant macronutrients in the flesh of the fish studied.

Calcium is involved in bone and teeth strengthening, blood clotting, and muscle contraction. It is also involved as a cofactor in metabolic and enzymatic processes. The calcium content of the fish studied varied over an extensive range, from 198.3 mg/kg in malamida to 1747.97 mg/kg w.w. in roach and 1792.7 mg/kg w.w. in Prussian carp (Fig.2).

Phosphorus contributes to the absorption of calcium by bones and teeth. It is a constituent of nucleic acids, nucleoproteins and is involved in converting food into energy and its release into cells. Fish is known to be a rich source of phosphorus. The phosphorus content varies from 865.7 mg/kg w.w. in malamida to 1937.4 mg/kg in Prussian carp and 1942.9 mg/kg w.w.in roach(Fig.2).

The Ca/P ratio is the most critical indicator of bone health. Calcium and phosphorus play a significant role in many physiological processes and are directly involved in the development and maintenance of the skeletal system. Calcium binds to phosphorus in the form of hydroxyapatite. Hydroxyapatite is the major inorganic component of bone and tooth enamel, primarily crystalline (Ye, et al., 2006). The

Ca/P ratios in the flesh of the fish analyzed ranged from 0.22:1 to 0.99:1 (Fig.2). Higher levels of phosphorus compared to calcium in perch and malamida species studied resulted in low Ca/P ratios for these fish (0.22:1-0.23:1). In Prussian carp and roach the ratio was about 1:1. As numerous studies have shown, the value of this ratio should be 1:1 in consumed products because, over calcium over phosphorus, phosphorus is not absorbed. After all, this form of calcium phosphate is not bioavailable (Chavez-Sanchez, et al., 2000). A phosphorus-to-calcium ratio greater than 3:2 can lead to metabolic disorders.

Magnesium is involved in the composition of intracellular enzymes and is essential for normal metabolism and energy production processes. Magnesium content ranges from 231.5 mg/kg w.w.in perch to 353.5 mg/kg w.w. in malamida (Fig.2).

Sodium helps maintain osmotic pressure, acid-base balance, and enzyme activation. Sodium content ranges from 284.1 mg/kg w.w. in malamida to 281.8 mg/kg w.w.in roach (Fig.2).

Potassium is involved in enzyme activation, muscle contraction, osmotic regulation, membrane transport, maintenance of osmotic pressure, and acid-base balance. Potassium content varied among the fish studied. The potassium content changed from 168. 9 mg/kg in malamida to 2156.3 mg/kg in perch (Fig.2).

The Na/K ratio in the studied fish varied from 0.16 to 0.23 (Fig.2). In food, this ratio is usually less than 1. The results indicate that these fish can be recommended for consumption by people with hypertension (high blood pressure) and cardiovascular disease.

4.3.2. Microelement content

Zinc is involved in immune responses, taste perception, wound healing, and fetal development during pregnancy and interacts with sex and thyroid hormones. Zinc deficiency leads to impaired immune function, growth, acne, and skin problems. The permissible maximum Zn content in fish and fish products is 50 mg/kg fresh weight according to the Bulgarian legislation (Bulgarian Ministry of Health, 2004) and 30 mg/kg (FAO, 1983). The zinc content was much lower than the established legislative standards in all samples analyzed. The lowest values were found in perch (3.53 mg/kg w.w) and the highest in roach (12.3 mg/kg w.w). Significantly higher results were obtained for perch from Kardzhali dam by Velcheva (2006) (11.0-18.1 mg/kg) and Arnaudova (2008) (26.7 mg/kg).

Copper is involved in energy production, iron metabolism, neuropeptide activation, connective tissue synthesis, and neurotransmitter synthesis. It also helps fight oxidative stress and is part of many oxidation-reduction enzymes. The high copper content in foods can cause adverse health problems, such as liver and kidney damage (Velcheva et al., 2006).The Cu content of the analyzed samples ranged from 0.22 mg/kg w.w. in perch to 1.95 mg/kg w.w. in malamida. The copper content of the fish examined was lower than the values for copper (10 mg/kg w.w) regulated in Regulation 31 of 2004. Similar results were obtained from Orban et al. (2007) for malamida (1.65-2.09 mg/kg) and for roach (0.69 mg/kg) by Zhelyazkov et al. (2014)

Manganese is essential for carbohydrate, amino acid and cholesterol metabolism, connective tissue formation, bone formation, blood clotting and energy production in the body. Manganese is also important for the function of the nervous and immune systems.

Manganese content varies significantly ($p < 0.05$) among species, from 0.16 mg/kg w.w. in perch to 1.78 mg/kg w.w. in malamida. Similar results were obtained for perch from Velcheva (2006) (0.48 mg/kg).

Iron is a constituent of hemoglobin, ensuring oxygen transport and retention in cells. Iron is involved in the synthesis of hormones and neurotransmitters. According to Bulgarian and European legislation, there are no set maximum levels for Fe in fish and fish products. Still, the American Academy of Sciences sets the total amount of Fe in canned fish not to exceed 30 μg/g w.w (USEPA, 2011).

The minimum value of Fe is found in malamida (3.06 mg/kg w.w.) and the maximum in roach (13.08 mg/kg w.w.) (Fig.2). Lower values for Fe were found in roach samples 6.59 mg/kg from (Zhelyazkov et al. (2014) in Zhrebchevo dam.

4.3.3. Heavy metal content

To ensure food safety and protect the consumer's health, maximum contaminants in food, referred to as "standards," have been introduced under European and national legislation. This is the maximum amount of a given pollutant in raw materials and foodstuffs considered not to pose a toxicological risk to human health at a given acceptable daily product intake or when ingested at a given single dose. Maximum levels for various contaminants in food, including toxic metals, are regulated in Regulation (EC) No 1881/2006. In Bulgarian legislation, these limits are laid down in Regulation No 31 of 29.07.2004. According to both regulations, foodstuffs intended for human consumption must not contain contaminants in quantities higher than these limits.

Lead is a highly toxic metal that lowers the immune response, leads to brain disorders, and strongly affects the nervous system. Lead content ranges from 0.04 in malamida to 0.209 in Prussian carp. According to Regulation No 31 of 29 July 2004 on maximum levels of contaminants in foodstuffs, the standard for Pb in fresh, chilled, or frozen fish, fish fillets, and other fish meat fit for human consumption is 0.2 mg/kg fresh product. For the fish species Prussian carp, the value for lead is close to the standard of 0.2 mg/kg fresh product. Velcheva (2006) reported lead content in perch range from 0.14 mg/kg to 0.168 mg/kg. According to Arnaudova (2008) the lead content in perch reaches 3.579 mg/kg, and these values are significantly higher compared to this study (0.098 mg/kg)

The accumulation of cadmium in the human body leads to increased blood pressure, anemia, kidney and liver diseases, and many other problems, including chronic fatigue. The cadmium content varies considerably between fish species, from 0.01 mg/kg w.w. perch to 0.047 mg/kg w.w. in roach. According to European Union (WHO, 2011) and Bulgarian (Bulgarian Ministry of Health, 2004) standards, the maximum permissible concentration for Cd in fish is 0.05 mg/kg fresh weight. The Cd content was lower than the allowable limits in all fish samples analyzed. Higher values for Cd for perch from Kardzhali dam lake were found by Velcheva (2006) (0.094 mg/kg) and Anrnaudova (2008) (0.199 mg/kg)

Mercury leads to depression, irritability, and tremors. As it accumulates in the body, degenerative diseases of the brain, liver, and kidneys progressively develop, and it can lead to impaired fetal development, reproductive problems, and coma.

The total mercury content in the fish species studied was highest in perch (0.109 mg/kg w.w.) and lowest in roach (0.029 mg/kg w.w.). The maximum permissible mercury concentration in fish for Bulgaria is 0.5 mg/kg fresh weight (Bulgarian Ministry of Health, 2004). The mercury content was lower than the allowable limits in all the fish samples analyzed. Velcheva (2006) (less than 0.3 µg/kg) obtained significantly lower values for mercury in perch.

Chronic arsenic exposure can cause disorders in the cardiovascular, nervous, respiratory, and renal systems, liver and prostate cancer, and hyperpigmentation. Arsenic content ranges from 0.04 mg/kg w.w. in roach to 0.11 mg/kg w.w. in malamida. The results obtained by Velcheva (2006) for perch (0.082-0.084 mg/kg) from Kardzhali dam is similar to those found in this study (0.082 mg/kg). The maximum permissible arsenic concentration in Bulgaria's freshwater fish is 1.0 mg/kg fresh weight (Bulgarian Ministry of Health, 2004). The arsenic content was lower than the allowable limits in all fish samples analyzed.

5. CONCLUSIONS

For the four fish species from Kardzhali dam data on the content of dry matter, proteins, lipids, heavy metals, micro and macro elements were obtained and the energy value in kcal/100g and kJ/100 g fresh weight was calculated. According to the results of lipid content, the analyzed species can be divided into two groups: with low lipid content (up to 2.0%) - perch (1 g/100g fresh weight); and with medium lipid content (2.0-8.0%) - Prussian carp, malamida and roach (2.0 to 2.7 g/100g fresh weight).

All freshwater fish examined from Kardzhali dam belonged to the category of foods with medium and high energy content (more than 170 kJ/100g) and were a good source of protein (21.52-24.54%).

All four fish studied are important sources of magnesium, potassium and sodium. Zinc, iron, phosphorus and calcium predominate in prussian sagrta and goash, while copper and manganese in malamida. The content of heavy metals mercury, lead, and cadmium in all fish examined was below the maximum allowable concentration (MAC) according to Regulation 1881/2006.

All the four tested fish can be recommended for consumption by the general population in the area.

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