Common Carp (*Cyprinus caprio*) and European Catfish (*Sillurus glanis*) from the Danube River as Sources of Fat Soluble Vitamins and Fatty Acids

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Abstract

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The total content of fat soluble vitamins and their percentages in the recommended daily intake for humans per 100 g portion, fatty acids composition, the atherogenic (IA) and thrombogenicity (IT) indices in two freshwater fish species – Common carp (*Cyprinus carpio*) and European catfish (*Sillurus glanis*) were investigated. Retinol contents in fresh edible tissues of the Common carp and European catfish were found to be 30.8 ± 3.4 mg/100 g wet weight (ww) for the Common carp 30.8 ± 3.4 μg/100 g ww and 1.9 ± 0.1 μg/100 g ww for the European catfish, cholecalciferol contents 14.8 ± 1.0 and 3.1 ± 0.1 μg/100 g ww, and α-tocopherol contents 2764.5 ± 44.0 and 2182.5 ± 31.5 μg/100 g ww, respectively. The sum of monounsaturated FA accounted for 50.02% (catfish) and 23.15% (carp). Polyunsaturated FA (PUFA) showed a higher level in the carp (36.75%) and a lower one in the catfish (21.64%). Both fishes are good sources of cholecalciferol in terms of the recommended daily intake of vitamins established in Bulgaria. Three fat soluble vitamins, n-3 PUFAs content, and IA value were higher in carp. IT values were similar for both species.

Keywords: RDI; atherogenicity index (IA); thrombogenicity index (IT); HPLC; GC-MS; human health

Fish are characterised by significant contents of various components beneficial for human health. They are one of the most important dietary sources of fat soluble vitamins and polyunsaturated fatty acids (PUFA), their contents, however, strongly depend on the fish species, gender, maturation, environmental factors, and feeding type (Tocher 2003; Steffens 2006).

Fat soluble vitamins are essential components of fish lipids and are exclusively provided by the diet. They control a variety of biologically important processes in the human body. All-trans retinol participates in photoreception, regulates the gene expression, bone growth, teeth development, reproduction, etc. Cholecalciferol promotes and enhances the absorption and metabolism of calcium and phosphorus. α-Tocopherol is an important antioxidant as it protects the membrane structures, essential fatty acids, and vitamins A from oxidation (Ribarova 2007; Anderson 2008).

Various epidemiological studies have demonstrated the key role of fish consumption in the prevention of coronary heart diseases (Kris-Etherton *et al.* 2003). The nutritional benefits of fish consumption are mainly attributed to the effects of ω-3 polyunsaturated fatty acids (n-3 PUFAs), which have several potential cardio protective effects along with their antithrombotic action. Numerous studies have explored and supported the antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFAs (Lee *et al.* 2006). PUFAs can affect platelet function by interacting with membrane proteins and serving as

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precursors for secondary messengers (Dutta-Roy 2002). Their effect depends on the fatty acid (FA) chain length and the degree of saturation. Individual saturated fatty acids (SFA) such as lauric (C12:0), myristic (C14:0), and palmitic (C16:0) increase LDL cholesterol and platelet aggregation (Kris-Etherton *et al.* 2003; Lee *et al.* 2006; FAO 2010).

It is well known that the seawater fish fatty acid (FA) composition is characterised by low levels of n-6 FA (linoleic acid – LA, C18:2n-6) and high levels of n-3 PUFA (eicosapentaenoic acid – EPA C20:5n-3; docosahexaenoic acid – DHA C22:6n-3). Several studies have shown that freshwater fishes have a high capacity for the transformation of C18 essential fatty acids (EFA); C18:3n-3 and C18:2n-6, into EPA, DHA, and C20:4n-6 (arachidonic acid – AA, respectively, and thus they could be a good source of such FA to a consumer (Tocher 2003; Steffens 2006).

Fish production in Bulgaria comes mainly from commercial fishing which includes two groups – marine fishing (Black Sea) and freshwater fishing (the Danube River and inland waters). The Danube River fishing is significant for some regional economies in Bulgaria where it is often the only source of income. Important species for fishing in the Danube River include: the European catfish (*Sillurus glanis*) and common carp (*Cyprinus carpio*) (NAFA 2007).

Common carp are warm water fish species. They live in slow waters overgrown with aquatic plants. Carp are omnivorous fish which feed on zooplankton, bottom invertebrates, algae, etc. European catfish are typical warm water fish. Catfish are carnivorous – they prefer to feed on fish, waterfowl birds, and small mammals (Karapetkova & Jivkov 2006).

The total commercial inland catch in 2005 comprised 361 t from the Danube River. This catch contained 40% carp and 10% catfish. The fish consumption in Bulgaria is traditionally low (4.5 kg per capita per year) compared to the levels of consumption in the neighbouring countries such as Turkey and Greece, and the average European levels (23 kg annually per capita per year). It would be beneficial to increase the fish consumption and the contents of the beneficial fatty acids and fat soluble vitamins in locally produced fish like wild common carp and catfish. Other sources of n-3 FA (including EPA and DHA) are scarce in the Bulgarian population diet but the fish demand has been increasing in recent years and consumers' interests turn off to the investigated freshwater species (Health of the Nation Report 2009). A few studies have examined the factors influencing FA composition in the cultured common carp (Circovic 2010; Mraz 2011, 2012). The biology of the common carp is quite well known mainly due to the importance of this species in some Central and Eastern European countries that have developed an intensive fish culture based on the genetically selected races of cyprinids. To our knowledge, there are no recent studies on similar aspects regarding the wild carp in the Danube River, especially for the area before the Danube Delta. Similar is the situation with some other wild Danube fish species like catfish (Ciolac 2004). Despite the importance of these species, the data concerning vitamin and fatty acid compositions and lipid quality indices on wild carp and catfish in the Bulgarian scientific literature are very scarce.

Having in mind all these facts, the aim of the present work was to determine and compare fat soluble vitamins contents as well as the relative daily intake of vitamins, fatty acid composition, atherogenic index, and thrombogenicity index, in two commonly consumed freshwater fish species in Bulgaria – Common carp and European catfish.

MATERIAL AND METHODS

Fish species. A total of twelve fresh Common carp and European catfish specimens were purchased from a fish market in Silistra, during the non-spawning season (spring 2011). Biological and biometrical characteristics of the species were determined and noted down (Table 1). The fishes were 2 days post – capture on arrival in ice at the laboratory. All the fish specimens were immediately frozen and stored in a fridge at –20°C prior to analysis (1 week).

Sample preparation. Six specimens of each fish species were used as a raw material for vitamin and fatty acid analysis. Prior to analysis, the fishes were gently defrosted. The head, tail, fins, and viscera were removed. The fishes were filleted and homogenised (at 800 rpm for 3 min, using Moulinex blender) with the skin and used for the preparation of random samples of fish tissue.

Table 1. Biometric and biologic characteristics of studied fishes (n = 6)

Parameter	Common carp	European catfish
Mean total weight (g ± SD)	1220.0 ± 60.0	3050.0 ± 70.0
Mean total length (cm ± SD)	55.0 ± 3.0	65.0 ± 5.0
Habitat	Demersal	Demersal
Food habits	Omnivorous	Carnivorous

Standards and reagents. All-trans-retinol was purchased from Fluka (Sigma-Aldrich GmbH, Buchs, Switzerland), cholecalciferol, α-tocopherol, and other HPLC-grade reagents came from Sigma-Aldrich™. Fatty Acid Methyl Esters (F.A.M.E.) mix standard (Supelco F.A.M.E. Mix C4-C24), nonadecanoic acid and methyl ester nonadecanoic acid standards were purchased from Sigma-Aldrich™ (Buchs, Switzerland). All chemicals used were of analytical, HPLC, or GC grades (Sharlau, Gato Perez, Spain).

Vitamin analysis. The sample preparation procedure for fat soluble vitamin analysis was performed using the modified method of Sanchez-Machado (Dobreva *et al.* 2011). An aliquot of the homogenised tissue (1.000 ± 0.005 g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 1M methanolic potassium hydroxide were added. Six parallel samples of edible fish tissue were prepared and saponified at 80°C for 20 minutes. After cooling, the analytes were extracted with *n*-hexane. The extracts, containing fat soluble vitamins, were evaporated under nitrogen. The dry residues were dissolved in methanol and injected (20 µl) into the HPLC system.

HPLC system used (Thermo Scientific Spectra SYSTEM) was equipped with RP analytical column (ODS2 Hypersil™ 250 × 4.6 mm, 5 µm) and UV and FL detectors (all Thermo Corporation, Waltham, USA). The mobile phase was composed of methanol:water (97:3) with a flow rate of 1.0 ml/minute. Qualitative analysis was performed by comparing the retention times of methanolic standard solutions of three fat soluble vitamins. Retinol and cholecalciferol were monitored by UV detection at λ_{max} = 325 nm and λ_{max} = 265 nm, respectively. α-Tocopherol was detected by fluorescence at λ_{ex} = 288 nm and λ_{em} = 332 nm. The quantification was done by the method of the external calibration.

Fatty acid analysis. Aliquots of freshly prepared homogenate (5.00 ± 0.05 g) were extracted by the method of Bligh and Dyer (1959) using chloroform/methanol/water in a ratio 2:2:1. BHT (2-terth-butyl-4-hydroxyanisole) was added to all samples as an antioxidant. After the phase separation, the chloroform extracts were removed, evaporated to dry residues in a Rotary Vacuum Evaporator Buchi R 205 (Buchi, Flawil, Switzerland) (40°C water bath), and quantified by weight. The total lipid content was determined gravimetrically for each fish species (n = 6). The results were expressed as g per 100 g wet weight. The dry residues (60 mg of extracted oil) were dissolved in 2 ml *n*-hexane and methylated by base-catalysed transmethylation using 1 ml 2M methanolic potassium hydroxide. After centrifugation (3500 rpm), the hexane layer was separated and analysed by GC-MS (BDS EN ISO 5509:2000). For the determination of the analytical recoveries, the samples of homogenised fish tissue were spiked with a methanolic solution containing a known amount of C19:0 (1 mg/ml). The gas chromatography analysis was performed by a model FOCUS Gas Chromatograph equipped with Polaris Q MS detector (Thermo Scientific, West Palm Beach, USA). The capillary column used was a TR-5 MS (Thermo Scientific, West Palm Beach, USA), 30 m length and 0.25 mm *i.d*. Helium was used as a carrier gas at a flow rate of 1 ml/minute. Peak identification was done by two parameters: retention time (RT) based on fatty acid methyl esters (FAME) mix standard, and mass spectra (ratio *m/z*) – compared to the internal Data Base (Thermo Sciences Mass Library; Thermo Corporation, Waltham, USA). FAMEs were quantified by the method of external standard. The FA content was expressed as a percentage of total FAs content (BDS EN ISO 5508:2000).

Indices of lipid quality (ILQ). Lipid quality parameters were calculated from the fatty acid composition data using the following equations:

Index of atherogenicity (IA) indicates the relationship between the sum of the main saturated and the main unsaturated FA, the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory systems), and the latteranti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macrocoronary diseases) (Ulbritcth & Southgate 1991; Senso *et al.* 2007):

$$IA = (12{:}0 + (4 \times 14{:}0) + 16{:}0)/(MUFA + \text{n-6 PUFA} + \text{n-3 PUFA})$$

Index of thrombogenicity (IT) shows the tendency to form clots in the blood vessels. This is defined as the relationship between the pro-thrombogenic (saturated) and the anti- thrombogenic FA (MUFA, n-6 PUFA, and n-3 PUFA) (Ulbritcth & Southgate 1991; Senso *et al.* 2007):

$$IT = (14{:}0 + 16{:}0 + 18{:}0)/((0.5 \times MUFA)+(0.5 \times \text{n-6 PUFA}) + (3 \times \text{n-3 PUFA}) + (\text{n-3PUFA/n-6 PUFA}))$$

Flesh-lipid quality (FLQ) indicates the percentage correlation between the main n-3 PUFA (EPA + DHA) and the total lipids. The higher value of this index is an indicator of the higher quality of the dietary lipid source (Abrami *et al.* 1992; Senso *et al.* 2007):

$$FLQ = 100 \times (\%\text{ of EPA} + \%\text{ of DHA})/(\%\text{ of total fatty acids})$$

Statistical analysis. Statistical analysis was done using Graph Pad Prizm 5 software (Graph Pad Inc., San Diego, USA). Column statistics were used for the calculation of the means, standard deviations, and the coefficients of variation. Unpaired *t*-test was used to evaluate the differences between the means. Statistical significance was indicated at $P < 0.05$.

RESULTS AND DISCUSSION

Vitamins content. In this study, significant differences ($P < 0.05$) in retinol, cholecalciferol, and α-tocopherol contents between the two fish species were estimated. The results were expressed as averages and standard deviations (mean ± SD). The amounts of vitamins are presented in Table 2 in μg/100 g ww.

The three analysed fat soluble vitamins were present in lower amounts in Catfish fillets. Considerable differences were observed with retinol contents – sixteen times higher in Common carp raw tissue. The cholecalciferol content in Carp fillets was specified as only four times higher than that in Catfish species. α-Tocopherol contents showed only a very slight distinction between the two analysed fishes. The results were close to each otherand statistically distinguishable.

The results presented are in a good agreement with those published by other authors. In comparison to the present study, Szlinder-Richert *et al*. (2011) found lower amounts for the same fat soluble vitamins – 7.69 μg/100 g ww for retinol, 7.46 μg/100 g ww for cholecalciferol, and only 280 μg/100 g ww for α-tocopherol in raw edible carp tissue. Ostermeyer and Schmidt (2006) studied cholecalciferol content in farmed Carp fish fillets. The result is considerably low (0.98 μg/100 g ww) compared to the presented data. Probably, these differences stem from feeding – natural or through diet. Wild Common carp naturally feeds on benthos and plankton that are good sources of vitamin D compounds, while aquaculture foods are mainly rich in carbohydrates (Sunita Rao 1996; Hadjinikolova 2004; Karapetkova & Jikov 2006).

Retinol and α-tocopherol contents in Common carp and European catfish were presented by Özyurt *et al.* (2009). Their research showed a similar correlation between the amounts of vitamins with both analysed fish species. The contents were 23.52 and 6.30 μg/100 g ww of retinol, and 460.0 and 800.0 μg/100 g ww of α-tocopherol for Carp and Catfish fillets, respectively. All the results shown, except those for the retinol amount in Catfish, demonstrate lower values than our data.

Our results are close to those published by the Danish Food Composition Databank (2009), providing the data on the vitamins contents in raw edible catfish tissue. According to these data, the amounts of retinol, cholecalciferol, and α-tocopherol are 18.0, 1.3, and 2400.0 μg/100 g ww, respectively. Retinol and cholecalciferol contents are in the same magnitude with the contents obtained in this study, especially those for cholecalciferol, while tocopherol amount is higher.

The quantities of fat soluble vitamins provided by 100 g raw fish tissue and calculated as percentages of the average daily allowance (ADA) are presented in Table 2.

Dietary standards for fat soluble vitamins intake in Bulgaria are the same as those approved by the European Union (EFSA 2006; FNB 2011). An exception is the recommended daily intake (RDI) of cholecalciferol in our country (Ordinance No. 23/19.07.2005). The rate for the RDI of vitamin D in European Union was updated in 2011. This update has not been done in Bulgaria yet.

According to the Bulgarian dietary standards for ADA of fat soluble vitamins, both analysed fishes show very low percentages of the RDI of retinol, while those of α-tocopherol in the two species provide close amounts, accounting to about 10%. In contrast, the cholecalciferol content in both fishes studied over half of the recommended daily needs: for a portion (100 g) of Catfish, this is only 62%, whereas a portion

Table 2. Total content (mean ± SD) and percentage of the daily recommended intake of fat soluble vitamins

Analyte	Total content (μg/100 g ww)		RDI (%)	
	carp	catfish	carp	catfish
Retinol	30.8 ± 2.1	1.9 ± 0.1***	4.1^{+}	0.3^{+}
Cholecalciferol	14.8 ± 0.7	3.1 ± 0.1***	**296.0**	**62.0**
α-Tocopherol	1713.0 ± 107.1	1470.6 ± 97.6***	11.4	9.8

***$P < 0.001$; $^{+}$percent of average value (750 μg) of the RDI for adults (male and female)

of Carp provides almost three times higher quantity than the RDI (296%).

Total lipids. In this study, the total lipids (TL) content ranged from 4.17 ± 0.15 g/100 g ww for catfish to 12.15 ± 0.45 g/100 g ww for common carp. Depending on the species, size, and nutrition, the lipid level in the flesh of freshwater fishes can vary considerably (Stefens 2006; Mraz 2012). In previous investigations, Hadjinikolova (2004) and Kalyoncu *et al.* (2010) found low TL values in Carp from fish farm, while Jabeen *et al.* (2011) found a result similar to that received in the present investigation (12 g/100 g ww) for wild Carp from the Indus river. Vranić *et al.* (2011) reported a significantly higher TL content (11.4 g/100 g ww) for Catfish from Serbian fish farm in comparison to our results. Steffens (2006) and Mraz (2011) investigated Common carp which were reared in ponds on the basis of natural food and fed on supplementary grain, and found significant differences in the TL content of the flesh. The lipid content of wild fish cannot be manipulated and is mainly influenced by the prey type and availability, whereas in the farmed species higher lipid contents are usually found, mostly because of the accessible and well formulated diets. Fish fillet is heterogeneous and is composed of different muscles and tissues (Mraz 2012). However, the lipids are not equally distributed in the fillet, and probably due to this fact they were observed to differ in TL content in the same species (Steffens 2006).

Fatty acid composition. The fatty acid composition of TL of the two fish species obtained from the Danube River showed significant differences. The present study revealed that total SFA, total MUFA, and total PUFA levels varied between the species (Table 3).

Hadjinicolova (2004) and Stancheva and Merdzhanova (2011) presented the relative decreasing sequence MUFA > SFA > PUFA for Common carp from local fish farm in Bulgaria. Guller *et al.* (2008) showed the same results for Common carp from local fish farm in Turkey. In the present investigation, a deflection of this pattern was observed for the Danube River Common carp (SFA > PUFA > MUFA), in which SFA content was significantly higher than those of MUFA ($P < 0.001$) and PUFA ($P < 0.001$). Significantly higher quantities of MUFA compared to SFA ($P < 0.001$) and PUFA ($P < 0.001$) were found for catfish (MUFA > SFA > PUFA).

Many authors have found great variations in FA content with predomination of palmitic (C16:0) and stearic (C18:0) SFA among the fish species (Tocher 2003; Steffens 2006; Guler *et al.* 2008). The present results also revealed that the dominating SFAs in both studied fishes follow the distribution: C16:0 > C18:0 > myristic acid (C14:0) (Table 3). A higher level of C16:0

Table 3. Fatty acid compositions (% of total FA) and lipid quality indices of carp and catfish (mean ± SD)

FA (% of total FA)	Common carp	European catfish
C12:0	3.55 ± 0.50	0.43 ± 0.05
C13:0	nd	0.26 ± 0.03
C14:0	4.67 ± 0.42	1.43 ± 0.38
C16:0	13.94 ± 0.92	19.16 ± 1.10
C17:0	1.26 ± 0.32	0.53 ± 0.05
C18:0	6.27 ± 0.45	4.03 ± 0.61
C20:0	4.09 ± 0.30	0.46 ± 0.04
C21:0	nd	0.31 ± 0.02
C22:0	4.61 ± 0.60	0.52 ± 0.05
C23:0	1.83 ± 0.25	0.30 ± 0.02
C24:0	4.83 ± 0.71	0.51 ± 0.06
Σ SFA	**46.37** ± 2.10	**28.34** ± 1.45***
C14:1n-5	0.61 ± 0.07	0.32 ± 0.10
C16:1n-7	4.00 ± 0.50	12.09 ± 0.94
C17:1n-8	0.41 ± 0.05	0.59 ± 0.09
C18:1 n-9	9.15 ± 0.85	33.99 ± 1.85
C20:1n-9	2.44 ± 0.37	2.25 ± 0.25
C22:1 n-9	2.90 ± 0.51	0.39 ± 0.12
C24:1n-9	1.55 ± 0.09	0.38 ± 0.05
Σ MUFA	**21.05** ± 1.30	**50.02** ± 2.51***
C18:3n-6	3.56 ± 0.30	0.38 ± 0.03
C18:2n-6	3.38 ± 0.27	3.35 ± 0.25
C18:3n-3	4.77 ± 0.50	1.31 ± 0.20
C20:5n-3	4.16 ± 0.51	1.85 ± 0.32
C20:4n-6	3.04 ± 0.30	6.42 ± 0.55
C20:3n-6	nd	0.45 ± 0.05
C20:2	3.07 ± 0.36	0.78 ± 0.10
C20:3n-3	2.06 ± 0.41	0.50 ± 0.03
C22:6n-3	4.68 ± 0.55	6.17 ± 0.35
C22:2	3.03 ± 0.45	0.43 ± 0.10
Σ PUFA	**32.58** ± 1.58	**21.64** ± 1.15***
Σ n-3	15.67 ± 1.10	9.83 ± 0.51***
Σ n-6	9.98 ± 0.81	10.6 ± 0.35
n-3/n-6	1.57 ± 0.12	0.93 ± 0.07***
PUFA/SFA	0.71 ± 0.08	0.76 ± 0.10
IA	0.65 ± 0.04	0.36 ± 0.05***
IT	0.36 ± 0.03	0.41 ± 0.04
FLQ	6.84 ± 0.40	8.02 ± 0.55***

***$P < 0.001$; nd – not detected; IA – index of atherogenicity; IT – index of thrombogenicity; FLQ – flesh-lipid quality

was measured for catfish (19.16%), while common carp presented a lower value (12.94%). Similar results for internal SFA distribution for Catfish and Carp were obtained by other authors (Steffens 2006; Guler *et al.* 2008; Stancheva & Merdzhanova 2011; Vranich *et al.* 2011; Yeganeh *et al.* 2012).

The major classes of unsaturated fatty acids (UFA) in nature are n-9, n-6, and n-3, represented by oleic (C18:1n-9), linoleic (C18:2n-6), and α-linolenic acids (C18:3n-3); freshwater fish species generally contain higher levels of these C18 UFAs (Henderson & Tocher 1987; Tocher 2003; Steffens 2006). As a consequence, the percentages of these FA do not appear to be similar in the observed fishes (Table 3). A higher total MUFAs value was found for catfish (50.02%), whereas common carp presented a significantly lower value (21.05%, $P < 0.001$). This was due to the high concentration of C18:1 (9.15–33.99%), which was the main MUFA (especially in catfish). Our results for FA distribution in MUFAs group for Common carp are in agreement with the data presented by Hadjinikolova (2004), Kalyoncu *et al.* (2010), Stancheva and Merdzhanova (2011), and Yeganeh *et al.* (2012). C18:1n9 was the main MUFA in artificially fed freshwater fish due to fact that the fish are able to biosynthesise this FA, but oleic acid also has an exogenous origin and its content reflects the type of the fish diet (Fajmonova 2005; Circović 2011; Mraz 2011; Yeganeh *et al.* 2012).

Fish is known to be a rich source of the unique PUFAs of n-3 series, including both EPA and DHA. In the analysed species, significant differences were found for FA distribution within PUFAs group. The major FAs identified as PUFA in catfish was AA followed by DHA and LA. PUFA profile in Danube River Common carp presented a different FA pattern: α-linoleic FA (ALA) > DHA > EPA. The maximum value of DHA obtained for catfish was 6.17% (which is 28.5% of total PUFAs), whereas carp contained 4.68% (which is 14.3% of total PUFAs). Deflections of these results for carp PUFAs were reported by Hadjinikolova (2004) and Stancheva and Merdzhanova (2011) who found significantly lower values for DHA and EPA. Vranić *et al.* (2011) showed different FA distribution in the farmed Catfish from Serbian fish farm: DHA > LA > EPA, and a significantly lower ARA content in comparison to the present results were obtained for same species. Probably, the difference in the profile is due to the dietary sources, since freshwater fish feed on fresh water algae, crustaceans, and aquatic larvae of insects that are rich in C18:2n-6, C18:3n-3 and C18:1n-9 (Steffens 2006).

The results observed in this study showed that Common carp contains higher n-3 levels in comparison with n-6 PUFAs (Table 3). Mraz (2011) supposed that the origin of the n-3 PUFA in the carp muscle is most likely the natural food, plankton, and benthos, present in the ponds. Seasonal variations in plankton FA composition were observed, and there was a decreasing trend in n-3 PUFA level in the spring. This trend could be explained by the changes in the planktonic community. At the beginning of the vegetation season, the zooplankton biomass was dominated by *cladocera* (which is rich in EPA) and *copepoda* (DHA is more frequent) (Mraz *et al.* 2011). It was reported that the carp, in contrast to seawater fish species, are able to bio-convert ALA to EPA and DHA (Tocher 2003).

Previously, our study on farmed carp (Stancheva & Merdzhanova 2011) showed significantly lower levels of n-3 and higher of n-6 PUFAs, whereas a content comparable to our study of n-3 PUFA (13.30%) was detected in wild common carp from the Caspian Sea (Yeganeh *et al.* 2012). Being a carnivorous species, catfish showed the opposite trend, and this resulted in significant differences in omega PUFA levels between the species ($P < 0.001$). In addition, carnivorous fish have low delta 5 desaturase activities which lead to lower n-3 PUFAs content (Tocher 2003). The results for catfish n-3 and n-6 PUFAs contents are in agreement with the data presented by Vranić *et al.* (2011) for naturally fed catfish. Fish generally need PUFAs to tolerate low water temperatures; therefore, higher PUFA (especially DHA) concentrations are expected in fish that live in cold environments (Tocher 2003). Carp and catfish are warm water living species, and despite the fact that carp is more tolerable to large variations in the quality of ambient conditions, both species showed lower content of n-3 FAs compared to seawater fish species.

The n-3/n-6 ratio has been suggested to be a useful indicator when comparing relative nutritional values of fish. An increase in the human dietary n-3/n-6 PUFA ratio is essential to help prevent coronary heart disease by reducing plasma lipids and reduce the risk of cancer (Kris-Eterton *et al.* 2003; Tocher 2003). According to Tocher (2003) and Steffens (2006), the FA composition of freshwater species reflects, to a large extent, that of the diet, so the n-3/n-6 ratio varies between 0.5 and 3.8. Ćirković *et al.* (2010) established the relationship of these FAs and it was 0.54 for common carp fed on natural food only. A similar ratio (0.5) was found by Fajmonova *et al.* (2003). In the present study, common carp from the

Danube river showed n-3/n-6 PUFAs ratio considerably higher than 1, whereas this value for catfish was near to 1. Similarly, Yeganeh *et al.* (2012) found that n-3/n-6 PUFAs ratio of wild common carp from the Caspian Sea is higher than 1 (approximately 1.5).

Another useful key factor for the evaluation of the fish nutritional quality is PUFA/SFA ratio. The values of PUFA/SFA ratio higher than 0.45 are recommended by the Department of Health (1994). The present results are in agreement with this requirement and show higher PUFA/SFA ratios for both species studied (Table 3). In our previous study (Stancheva & Merdzhanova 2011) on farmed Common carp, a lower PUFA/SFA ratio (0.52) was found due to a lower PUFA level.

The dietetic value of fish meat is also determined by the lipid quality indices, which depend on the relative proportions of some individual saturated and unsaturated fatty acids. These indices indicate the global dietetic quality of lipids and their potential effects on the development of coronary disease (Senso *et al.* 2006; Jankowska *et al*. 2010; Stanec *et al.* 2011). With regard to the quality indices considered (Table 3), IA and FLQ showed statistical differences ($P < 0.001$) between the analysed species, as FLQ presented lower values in the Common carp, coinciding with the lower DHA level. Common carp presented significantly higher values for IA (0.65) than catfish ($P < 0.001$), due to higher levels of C14:0. IT showed the opposite trend – a higher value (0.41) in catfish than in carp (0.36). These values were higher than those obtained for crayfish species from the Lake Goplo (Stanec *et al.* 2011) and lower than those found in lamb, beef, and rabbit meats (Morbidini *et al.* 2000). FLQ amounts obtained for both species in this study are lower than those reported for seawater gilthead sea bass (Senso *et al.* 2007) due to lower DHA levels in the analysed Danube River species. Higher values of IA and IT (> 1.0) are detrimental to human health (Ouraji *et al*. 2009) while the values presented are beneficial in view of human nutrition and clearly show the differences in FA patterns in edible fish tissues in both wild species.

CONCLUSION

The three fat soluble vitamins were found in higher amounts in the Danube River Common carp. Both fish species analysed are characterised by a high cholecalciferol content. A portion (100 g) of the Common carp fillets provides three times higher amounts of cholecalciferol compared to the RDI established in Bulgaria. Carp species contain higher n-3 PUFA levels and AI values, whereas catfish present greater amounts of MUFAs. Regarding the lipid contents, the n-3/n-6 and PUFA/SFA ratios and lipid quality indices, we can conclude that both fish species from the Bulgarian part of the Danube River are good sources of the identified biologically active substances with beneficial effects on human health.

References

Abrami G., Natiello F., Bronzi P., McKenzie D., Bolis L., Aggrade E. (1992): A comparison of highly unsaturated fatty acid levels in wild and farmed eels (*Anguilla anguilla*). Comparative Biochemistry and Physiology, **1/2**: 71–82.

American Heart Association. (2004): Heart Attack and Related Diseases. American Heart Association, Washington, Available at http://www.americanheart.org/

Anderson J., Young L. (2008): Fat-Soluble Vitamins. Food and Nutrition Series. Colorado State University. 9.315.

BDS EN ISO 5508 (2000): Animal and vegetable fats and oils – preparation of methyl esters of fatty acids.

BDS EN ISO 5509 (2000): Gas chromatographic analysis of methyl esters of fatty acids.

Bligh E., Dyer W.J. (1959): A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, **37**: 913–917.

Ciolac A. (2009): Migrations of fishes in Romanian Danube River (№1). Applied Ecology and Environmental Research, **2**: 143–163.

Cirkovic M., Ljuboevic D., Dobrevic V., Novakov N., Petronievic R. (2012): Chemical composition of body including fatty acids of four cyprinidis fish species cultured at the same conditions. Archiva Zootechnica, **15** (2): 37–50.

Danish Food Composition Databank (2009): Department of Nutrition, National Food Institute. Available at http://www.foodcomp.dk/v7/fcdb_details.asp?FoodId=0878

Department of Health (1994): Nutritional aspects of cardiovascular disease: Health and social subsects. HMSO, London

Dobreva D.A., Galunska B., Stancheva M. (2011): Liquid chromatography method for the simultaneous quantification of fat soluble vitamins in fish tissue. Scripta Scientifica Medica, **43**: 276–279.

Dutta-Roy A.K. (2002): Dietary components and human platelet activity. Platelets, **13**: 67–75.

EFSA (2006): Tolerable upper intake levels for vitamins and minerals, Scientific Committee on Food Scientific Panel on Dietetic Products, Nutrition and Allergies. Available at http://www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf

Fajmonova E., Zelenka J., Komprda D., Kladroba D., Sarmanova I. (2003): Effect of sex, growth intensity and heat treatment on fatty acid composition of common carp (*Cyprinus caprio*) fillets. Czech Journal of Animal Science, **48**: 85–92.

FAO (2005–2011): Fisheries and Aquaculture topics. Composition of fish. FAO Fisheries and Aquaculture Department. Available at http://www.fao.org/fishery/topic/12318/en

FAO (2010): Fats and fatty acids in human nutrition Report of an Expert Consultation, Paper 91. FAO, Food and Nutrition, Rome.

FNB (2011): Dietary reference intakes for calcium and vitamin D. Available at http://books.nap.edu/openbook.php?record_id=13050

Guler G., Kiztanir B., Aktumsek A. (2008): Determination of the seasonal changes on total fatty acid composition and ω3/ω6 ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beysehir Lake (Turkey). Food Chemistry, **108**: 689-694.

Hadjinikolova L. (2004): The influence of nutritive lipid sources on the growth and chemical and fatty acid composition of carp (*Cyprinus carpio* L.). Archives of Polish Fisheries, **12**: 111–119.

Health of the Nation Report (2009): Available at http://ncpha.government.bg/files/Doklad%20Zdrave%20na%20naciata%202009.pdf

Hearn T.L., Sgoutas S.A., Hearn J.A., Sgoutas D.S. (1987): Polyunsaturated fatty acids and fat in fish flesh for selecting species for health benefits. Journal of Food Sciences, **52**: 1209–1215.

Henderson R.J., Tocher D. (1987): The lipid composition and biochemistry of freshwater fish. Progress in Lipid Research, **26**: 257–276.

Jabeen F., Chaudhry A. (2011): Chemical compositions and fatty acid profiles of three freshwater fish species. Food Chemistry, **125**: 991–996.

Jankowska B., Zakes Z., Zmijewski T., Szczepkowski M. (2010): Fatty acid profile of muscles, liver and mesenteric fat in wild and reared perch (*Perca fluviatilis*). Food Chemistry, **118**: 764–768.

Kalyoncu L., Yaman Y., Aktumsek A. (2010): Seasonal changes on total fatty acid composition of carp (*Cyprinus carpio* L.) in Ivriz Dam Lake Turkey. African Journal of Biotechnology, **9**: 3896–3900.

Karapetkova M., Jivkov M. (2006): Fishes in Bulgaria. Gea-Libris, Sofia.

Kris-Etherton P., Harris W., Appel L. (2003): Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology, **23**: 20–30.

Lee K.W., Blann A.D., Gregory Y., Lip H. (2006): Effects of omega-3 polyunsaturated fatty acids on plasma indices of thrombogenesis and inflammation in patients post-myocardial infarction. Thrombosis Research, **118**: 305–312.

Mitchell M., Vanberg J., Sipponen M. (2010): Commercial inland fishing in member countries of the European Inland Fisheries Advisory Commission (EIFAC). Available at www.fao.org/docrep/015/an222e/an222e.pdf

Morbidini L., Sarti D.M., Pollidori P., Valigi A. (2001): Carcass, meat and fat qualiti in Italian Merino derived lambs obtained with organic farming systems. Opinions Mediterraneenes. Serie A: Seminares Mediterraneens. **46**: 29–33.

Mraz J. (2011): Lipid Quality of Common Carp (*Cyprinus carpio*) in Pond Culture. [Licentiate Thesis.] Swedish University of Agricultural Sciences Uppsala: 52.

Mraz J. (2012): Lipids in common carp (*Cyprinus Carpio*) and effects on human health. [Doctoral Thesis.] Swedish University of Agricultural Sciences Uppsala.

NAFA (2007): National Strategic Plan for Fisheries and Aquaculture 2007–2013. Ministry of Agriculture and Forestry, National Agency for Fisheries and Aquaculture. Sofia. Available at http://www.mzgar.government.bg/mz_eng/default.asp

Ordinance №23/19.07.2005 on the physiological feeding of population. Available at http://bg.wikipedia.org/wiki/Физиологични_норми_за_хранене#cite_note-echo-9

Ostermeyer U., Schmidt T. (2006): Vitamin D and provitamin D in fish. European Food Research and Technology, **222**: 403–413.

Ouraji H., Shabanpur B., Abediankenari A., Shabani A., Nezami A., Sudagar M., Faghani S. (2009): Total lipid, fatty acid composition and lipid oxidation of Indian white shrimp (*Fenneropenaeus indicus*) fed diets containing different lipid sources. Journal of the Science of Food and Agriculture, **89**: 993–997.

Özogul Y., Özogul F. (2007): Fatty acid profiles and fat content of commercially important seawater and freshwater fish species of Turkey: A comparative study. Food Chemistry, **100**: 1634–1638.

Özyurt G., Polat A., Loker G.B. (2009): Vitamin and mineral content of pike perch (*Sander lucioperca*), common carp (*Cyprinus carpio*), and European catish (*Silurus glanis*). Turkish Journal of Veterinary and Animal Sciences, **33**: 351–356.

Ribarova F. (2007): Food and Vitamins. 1st Ed. Infopress, Sofia.

Senso L., Suarrez M.D., Ruiz-Cara T., Garciŕa-Gallego M. (2007): On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). Food Chemistry, **101**: 298–307.

Szlinder-Richert J., Usydus Z., Malesa-Ciecwierz M., Polak-Juszczak L., Ruczynska W. (2011): Marine and farmed fish on the Polish market: Comparison of the nu-

tritive value and human exposure to PCDD/Fs and other contaminants. Chemosphere, **85**: 1725–1733.

Stancheva M., Merdzhanova A. (2011): Fatty acid composition of common carp, rainbow trout and grey mullet fish species. Agriculture Science and Technology, **3**: 285–289.

Stanec M., Borejszo Z., Dabrowski J., Janicki B. (2011): Fat and cholesterol content and fatty acid profiles in edible tissue of spiny-cheek crayfish, *Orconectes limosus* (Raf.) from Lake Goplo (Poland). Archives of Polish Fisheries, **19**: 241–248.

Steffens W. (2006): Freshwater fish – wholesome foodstuffs. Bulgarian Journal of Agricultural Sciences, **12**: 320–328.

Sunita Rao D., Raghuramulu N. (1996): Food chain as origin of vitamin D in fish. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology, **114**: 15–19.

Tocher D. (2003): Metabolism and functions of lipid and fatty acid in teleost fish. Reviews in Fisheries Sciences, **11**: 107–184.

Ulbricht T., Southgate D. (1991): Coronary heartdisease: seven dietary factors. Lancet, **338**: 985–992.

Vranić D., Dinović-Stojanović J., Spirić A. (2011): Rainbow trout (*Oncorhynchus Mykiss*) from aquaculture – meat quality and importance in the diet. Tehnologija Mesa, **52**: 122–133.

Yeganeh S., Shabanpour B., Hosseini H., Imanpour M., Shabani A. (2012): Comparison offarmed and wild Common carp (*Cyprinus caprio*): Seasonal variations in chemical composition and fatty acid profile. Czech Journal of Food Sciences, **30**: 503–511.

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