A COMPARATIVE STUDY ON THE FATTY ACID PROFILES OF TOTAL LIPID, NEUTRAL AND POLAR LIPIDS IN THE LIVER AND MUSCLE OF *CAPOETA SIEBOLDII* (STEINDACHNER, 1864) AND *CAPOETA BALIKI* (TURAN, KOTTELAT, EKMEKÇİ, İMAMOĞLU, 2006) FROM TÖDÜRGE LAKE (SIVAS, TURKEY)

S. GÖRGÜN^{a,*}, N. AKPINAR^b and S. DIRICAN^c

^aDepartment of Biochemistry, Faculty of Science, Cumhuriyet University, 58140 Sivas. Turkey ^bDepartment of Biology, Faculty of Science, Cumhuriyet University, 58140 Sivas. Turkey ^eFisheries Department, Suşehri Vocational Training School, Cumhuriyet University, 58600 Suşehri, Sivas. Turkey

(Received: 12 October 2012; accepted: 26 November 2012)

The fatty acid compositions of the total lipid, neutral and polar lipid fractions in the liver and muscle of *Capoeta sieboldii* and *Capoeta baliki* from Tödürge Lake were determined. Major fatty acids found in total lipid (TL) and neutral lipid (NL) in liver and muscles were C16:0, C16:1 n-7, C18:1 n-9, C18:1 n-7, C20:4 n-6, C20:5 n-3, C22:5 n-3, and C22:6 n-3. Beside these acids, C18:0 was another notable fatty acid in polar lipid (PL) fraction of the tissues investigated. The n-3/n-6 ratio, which is an indicator of health benefits of fish oils, was between 2.89 (PLs of liver) and 5.84 (PLs of muscle) in *C. baliki*, while it was found between 1.43 (PLs of liver) and 2.52 (NLs of muscle) in *C. sieboldii*. *C. baliki* was the excellent species in terms of polyunsaturated fatty acid (PUFA) levels in TL (43.92% in muscle) and PLs (52.94% in muscle) and C22:6 n-3 amounts (docosahexaenoic acid; DHA) were responsible for these high percentages. These results suggest that *Capoeta* species investigated have high nutritive value in terms of polyunsaturated fatty acids for human nutrition.

Keywords: Capoeta sieboldii, Capoeta baliki, lipid fractions, fatty acids, liver, muscle, n-3/n-6 ratio, Tödürge Lake

One and very crucial result of the photosynthesis by phytoplankton and algae living in freshwater and marine areas is the production of n-3 form of polyunsaturated fatty acids (PUFA), which cannot be synthesized by humans. These long chain fatty acids are incorporated into the structure of the lipids of the fish from marine and freshwater environments (MOFFAT & MCGILL, 1993). However, freshwater fish is more able to synthesize PUFAs, such as docosahexaenoic acid (C22:6 n-3; DHA) and eicosapentaenoic acid (C20:5 n-3; EPA), from the unsaturated fatty acids by chain elongation and desaturation (JANKOWSKA et al., 2003). This means that freshwater fish is also able to use much more effectively the enzymatic processes required for the unsaturation and desaturation processes of fatty acids. All of these features make marine and freshwater fish important in terms of the nutrition with these essential acids.

A broad range of study focused on and showed the healing effects of the n-3 form of PUFAs, specially EPA and DHA, in some major health problems from metabolic syndrome (LOMBARDO et al., 2007) to cardiovascular (RUSSO, 2009) and neurodegenerative diseases (LAURITZEN et al., 2000). However, SARGENT and co-workers (1999) documented that EPA, DHA, and arachidonic acid (C20:4 n-6, ARA) are required for the growth and health of the

^{*} To whom correspondence should be addressed.

Phone: 90 3462191010, ext. 2945; fax: 90 3462191186; e-mail: sgorgun@cumhuriyet.edu.tr

fish. These kinds of studies have urged the scientists to determine the fatty acid compositions and PUFA levels of different fish species and organs. In this context, ARAS and co-workers (2003) compared the fatty acid composition of different tissues of *Salmo trutta macrostigma*. AKPINAR and co-workers (2009) investigated the effect of sex on the fatty acids of liver and muscle in the same species. HALILOĞLU and co-workers (2002) compared the fatty acid profiles of *Salvelinus alpinus, Oncorhynchus mykiss,* and *Salmo trutta fario*. Some studies reported the effect of season on the fatty acid pattern of *Cyprinus carpio* (GULER et al., 2008), *Vimba vimba tenella* (KALYONCU et al., 2009), and *Sander lucioperca* (UYSAL & AKSOYLAR, 2005) from Turkish freshwaters.

Phospholipids as a polar compounds are amphipathic molecules and play a central role in the membrane structure and very important for the fish to adapt to fluctuating temperatures (TOCHER et al., 2008). In fish body triacylglycerols, as a neutral lipid fraction, serve as storage sites and their compositions are affected by the diet taken (SATAR et al., 2012). All of these data suggest that the levels of these lipid fractions are influenced by the physiological and metabolic state (NIKOLAIDIS et al., 2006). It appears that only limited number of the studies on Turkish freshwaters dealt with the fatty acid composition of the lipid fractions of fish tissues. Recently two studies indicated that it is important to obtain these data together with that of total lipid to reveal the nutritive value of fish (CENGIZ et al., 2012; SATAR et al., 2012). Other fish species investigated in terms of polar and neutral lipid fractions were salmonids, including *Salmo trutta labrax, S. t. caspius,* and *S. t. macrostigma* (BAYIR et al., 2010).

The genus *Capoeta* from *Cyprinidae* family has been showing wide distribution in Asia and is represented with five species (*Capoeta capoeta, Capoeta tinca, Capoeta trutta, Capoeta pestai*, and *Capoeta barroisi*) in Turkey (ARAS et al., 2009). In this study, *C. sieboldii* and *C. baliki*, living in Tödürge Lake, have been used to determine the fatty acid compositions of total, neutral, and polar lipid fractions to reveal nutritive value in terms of n-3 PUFA levels together with n-3/n-6 ratios, which is an important indicator of the quality of fish lipids for human health.

1. Materials and methods

1.1. Sample collection

Mature and female individuals of *C. sieboldii* (Steindachner, 1864) and *C. baliki* (Turan, Kottelat, Ekmekçi, İmamoğlu, 2006) were hunted from Tödürge Lake in September 2011. Three fish were used to extract total lipids and fatty acids. The fork lengths and the weights of the fish used in the analyses were 26.00 ± 1.44 cm and $239\pm.17$ g in *C. baliki*, 23.60 ± 2.08 cm and 230 ± 3.56 g in *C. sieboldii*. Two grams of liver and muscle were used to extract the total lipid from the tissues. The muscle samples were taken from the area underneath the dorsal fin (AKPINAR et al., 2009).

1.2. Lipid extraction and lipid class purification

The method of FOLCH and co-workers (1957) was used to extract the total lipids from the tissues investigated. In order to reduce the autoxidation of unsaturated fatty acids, 50 μ l of 2% butylated hydroxytoluene in chloroform was used in each extraction procedure (CENGIZ et al., 2012). Separation of the polar and neutral lipid fractions from the total lipids by silicagel (Davisil[®] grade 633, pore size 60 Å, 200–425 mesh) column chromatography was carried out according to KOZLOVA and KHOTIMCHENKO (2000). Shortly, an equal aliquot of total lipids

in chloroform were applied to a column (8×1 cm). Neutral and polar lipids were eluted by repetitive washings with 40 ml of chloroform and methanol, respectively. Solvent was evaporated using rotary evaporator. The saponification procedure of both total lipid and the fractions was carried out by refluxing with methanol containing 5% NaOH for 1 h. From lipid samples, fatty acid methyl esters were obtained using the standard boron trifluoride-methanol (BF3) method of Moss and co-workers (1974). All experimental procedures included in the chromatographic steps were carried out in three replicates.

The fatty acid methyl esters (FAMEs) obtained in hexane/chloroform (4/1, v/v) were injected into a HP Agilent 6890N model gas chromatograph (GC) (Hewlett Packard, Palo Altu, CA, USA) fitted with a HP-88 capillary column (100 m, 0.25 mm ID, and 0.2 μ m) and equipped with a flame ionization detector (FID) (Agilent Technologies Inc., USA). The analyses and the identification of the fatty acid methyl esters (FAMEs) were carried out according to the method of GULER and co-workers (2010).

1.3. Statistical analyses

SPSS 15.0 for Windows (SPSS Inc., Chicago, IL) was used in statistical analyses. One-way analysis of variance (ANOVA) was carried out to analyse the data obtained. The results in the study are expressed as mean \pm standard error (S.E.) of the mean and the comparisons between means were carried out with post-hoc Tukey's test at P \leq 0.05 level.

2. Results and discussion

2.1. Fatty acid composition of total lipid

The average total lipid content (% wet weight basis) of *C. sieboldii* and *C. baliki* were determined to be 1.40% and 2.21% for livers and 1.29% and 1.17% for muscle samples, respectively. The fatty acid composition of total lipid in liver and muscle of *C. sieboldii* and *C. baliki* can be seen in Table 1. With quantitative differences, 38 fatty acids were determined in all tissues investigated. The highest and lowest levels of total SFA were determined in the muscle (32.89%) and liver (27.93%) of *C. baliki*, respectively. The values of total SFA of *C. sieboldii* were found to be 29.26% for the liver and 31.41% for the muscle. The major fatty acid of SFA was C16:0 (palmitic acid). The values obtained for this acid in muscles were 20.50% (*C. baliki*) and 18.38% (*C. sieboldii*), while in livers 16.24% for *C. sieboldii* and 15.83% for *C. baliki* (P≤0.05). It has been indicated that palmitic acid is an invaluable component of the fish tissue lipids (STEFFENS, 1997) and this finding is compatible with the data from other studies (GULER et al., 2008; AKPINAR et al., 2009). Other fatty acids exceeding 2% in the tissues investigated were C14:0 (myristic acid), C15:0 (pentadecanoic acid), C18:0 (stearic acid), and C21:0 (heneicosanoic acid).

Major fatty acids of MUFA class were C16:1 n-7 (palmitoleic acid), C18:1 n-7 (cisvaccenic acid), and C18:1 n-9 (oleic acid). C16:1 n-7 values determined for the liver (16.17%) and muscle (16.92%) of *C. sieboldii* were higher than for the liver (9.29%) and muscle (6.81%) of *C. baliki*. C18:1 n-9 percentages showed statistical differences in all tissues (P \leq 0.05). The highest and lowest percentages for this acid were determined in the liver (19.93%) and muscle (10.67%) of *C. baliki*. C18:1 n-7 values ranged between 3.83% (in the liver of *C. baliki*) and 7.96% (in the muscle of *C. sieboldii*). MUFA values in this study ranged from 23.10% to 40.34% in the muscles of *C. baliki* and *C. sieboldii*, respectively.

Fatty acids	C. sieboldii Liver	C. baliki Liver	C. sieboldii Muscle	C. baliki Muscle
	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
C8:0 ^c	0.06±0.00a	0.01±0.00b ^B	0.07±0.00a	0.27±0.04c
C10:0	0.02±0.00a	0.01±0.00a	0.02±0.00a	0.10±0.00b
C11:0	0.06±0.00a	0.01±0.00b	0.08±0.00c	0.29±0.05d
C12:0	0.12±0.02a	0.06±0.00b	0.06±0.00b	0.05±0.00b
C13:0	0.09±0.01a	0.05±0.00a	0.07±0.02a	0.05±0.00a
C14:0	2.95±0.23a	4.65±0.33b	2.97±0.18a	2.76±0.11c
C15:0	2.52±0.34a	0.54±0.10b	3.18±0.28c	0.76±0.09d
C16:0	16.24±0.44a	15.83±0.22b	18.38±0.36c	20.50±0.61d
C17:0	0.97±0.15a	0.48±0.06b	1.09±0.22a	0.93±0.14a
C18:0	2.96±0.12a	2.09±0.06b	2.67±0.14ab	4.82±0.32c
C19:0	0.37±0.06a	0.18±0.02b	0.35±0.02a	0.18±0.02b
C20:0	0.01±0.00a	0.01±0.00a	0.01±0.00a	0.02±0.00a
C21:0	2.62±0.17a	3.94±0.21b	2.34±0.13ac	1.90±0.20c
C22:0	0.02±0.00a	0.03±000a	0.02±0.00a	0.02±0.00a
C24:0	0.25±0.02a	0.04±0.01b	0.10±0.01b	0.24±0.03a
ΣSFA	29.26	27.93	31.41	32.89
C14:1 n-5	0.60±0.12a	0.15±0.03b	0.64±0.04a	0.32±0.07c
C15:1 n-5	0.44±0.07a	0.17±0.02b	0.43±0.09a	0.16±0.01b
C16:1 n-7	16.17±0.48a	9.29±0.15b	16.92±0.63c	6.81±0.10d
C17:1 n-8	0.39±0.08a	0.10±0.03b	0.37±0.05a	0.12±0.02b
C18:1 n-9	12.20±0.46a	19.93±0.53b	13.61±0.14c	10.67±0.19d
C18:1 n-7	7.93±0.32a	3.83±0.10b	7.96±0.63a	4.82±0.34c
C20:1 n-9	0.29±0.03a	0.11±0.01b	0.30±0.06a	0.08±0.02b
C22:1 n-9	0.03±0.00a	0.05±0.01a	0.03±0.00a	0.03±0.00a
C24:1 n-9	0.09±0.02a	0.04±0.00b	0.08±0.00a	0.09±0.01a
ΣΜUFA	38.14	33.67	40.34	23.10
C18:2 n-6	2.14±0.10a	2.88±0.08b	1.51±0.07c	1.61±0.03c
C18:3 n-6	0.97±0.08a	2.27±0.14b	0.97±0.20a	1.20±0.11a
C20:2 n-6	0.41±0.03a	0.04±0.01b	0.60±0.05c	0.06±0.01b
C20:3 n-6	0.30±0.0a	0.48±0.09b	0.41±0.06b	0.24±0.03a
C20:4 n-6	5.06±0.15a	1.40±0.07b	4.99±0.17a	2.73±0.19c
C22:2 n-6	0.06±0.01a	0.10±0.02a	0.10±0.02a	0.10±0.01a
C22:4 n-6	0.11±0.02a	0.03±0.00b	0.20±0.03c	0.66±0.10d
C22:5 n-6	0.33±0.02a	0.11±0.01b	0.32±0.03ac	0.23±0.03c
Σn-6 PUFA	9.38	7.35	9.10	6.83
C18:3 n-3	1.61±0.07a	4.73±0.23b	1.55±0.05a	0.79±0.01c

Table 1. Fatty acid composition (%) of total lipid in the livers and muscles of C. sieboldii and C. baliki^

Fatty acids	C. sieboldii Liver Mean±S.E.	<i>C. baliki</i> Liver Mean±S.E.	C. sieboldii Muscle Mean±S.E.	<i>C. baliki</i> [∧] Muscle Mean±S.E.
C20:3 n-3	0.08±0.01a	0.61±0.11b	0.09±0.02a	0.18±0.03c
C20:5 n-3	9.37±0.21a	12.00±0.19b	8.90±0.28a	15.33±0.32c
C22:3 n-3	0.39±0.04a	0.37±0.06a	0.57±0.05b	0.28±0.03c
C22:5 n-3	4.53±0.15a	3.93±0.25ab	3.44±0.10b	3.56±0.27b
C22:6 n-3	7.24±0.18a	9.45±0.31b	4.60±0.35c	16.89±0.56d
Σn-3 PUFA	23.22	31.09	19.15	37.09
ΣΡυγΑ	32.60	38.44	28.25	43.92
n-3/n-6	2.47	4.22	2.10	5.43

Table 1. Continued

^AAverage of three lots analysed; ^Bvalues reported are means± S.E.; ^c(a-b-c-d): values for each sample with different superscript letters in the same fraction are significantly different at P≤0.05. ΣSFA: total saturated fatty acid; ΣMUFA: total monounsaturated fatty acid; Σn-6 PUFA: total n-6 polyunsaturated fatty acid; Σn-3 PUFA: total n-3 polyunsaturated fatty acid; **SPUFA**: total polyunsaturated fatty acid

CAKMAK and co-workers (2012) studied the fatty acid profiles of the muscle lipids of six fish species, including in C. capoeta from Suğla Lake from Turkey, and found that C18:1 n-9 was the primary MUFA in all species investigated. In this study, it was reported that C16:1 n-7 was the other notable fatty acid in MUFA fractions of the species. Similar results were reported by KUCSKA and co-workers (2006) in a study carried out with Esox lucius.

The percentages of the total n-6 PUFA in the muscle (9.10%) and liver (9.38%) of C. sieboldii were higher than found in the muscle (6.83%) and liver (7.35) of C. baliki. Major fatty acids of n-6 form of PUFA in all groups were C18:2 n-6 (linoleic acid) and C20:4 n-6 (ARA). The amounts of C18:2 n-6 in the muscles of C. sieboldii (1.51%) and C. baliki (1.61%) did not show any statistical differences (P \geq 0.05). C18:3 n-6 (linolenic acid) amounts were determined to be same in the liver and muscle of the C. sieboldii with a percentage of $0.97 (P \ge 0.05)$ and this value was lower than found for the liver (2.27%) and muscle (1.20%) of C. baliki ($P \le 0.05$). There were statistical differences in ARA amounts in the liver (1.40%) and muscle (2.73%) of C. baliki ($P \le 0.05$) and the liver (5.06%) and muscle (4.99%) of C. sieboldii had higher level of this fatty acid ($P \ge 0.05$). Many studies reported similar results and low amounts of the total n-6 form of PUFA, as determined in our study, in the fish from Turkish freshwaters, including S. t. macrostigma (AKPINAR et al., 2009), C. carpio (GULER et al., 2008), V. v. tenella (KALYONCU et al., 2009), and S. lucioperca (UYSAL & AKSOYLAR, 2005). This phenomenon might be the result of the conversion of C18:2 n-6 and C18:3 n-6 to the longer chain fatty acids by the high enzymatic activity of elongases and desaturases in the freshwater fish.

The most dominant fatty acids of n-3 form of PUFAs were C20:5 n-3 (EPA) and C22:6 n-3 (DHA) in all groups. The amounts of C20:5 n-3 ranged from 8.90% (in the muscle of C. sieboldii) to 15.33% (in the muscle of C. baliki) (P≤0.05). The amounts of C22:6 n-3 in all groups investigated showed statistical differences (P≤0.05), and the highest level of this important acid was determined in the muscle of C. baliki as 16.89%. However, C22:5 n-3 (docosapentaenoic acid, DPA) also was present in high amounts in the liver and muscles of the species, with the values ranging from 3.44% (in the muscle of C. sieboldii) to 4.53%

(in the liver of *C. sieboldii*). HOLUB and HOLUB (2004) indicated that long-term consumption of fish containing high amounts of EPA and DHA can be associated with lower primary and secondary heart attack ratios from cardiovascular diseases, and healthy individuals should take 650 mg/day of EPA/DHA. Fish are invaluable resources of these long-chain PUFAs, and it appears that *Capoeta* species in our study might have satisfying amounts of EPA and DHA. In this study, the highest total PUFA (n-6 plus n-3 PUFA) was determined in the muscle of C. baliki as 43.92%, while the lowest was found in the muscle of C. sieboldii with a value of 28.25%. It has been indicated that increased n-3/n-6 ratios in the human diet result in reduced plasma lipids and have protective effect on coronary structure (KINSELLA et al., 1990). For this reason, the n-3/n-6 ratio of fish tissues are accepted as a useful indicator in comparison of the nutritive value of fish oils (PIGGOT & TUCKER, 1990). When the n-3/n-6 ratios were considered in the present study, the highest ratios were recorded for the liver (4.22) and muscles (5.43)of C. baliki. These values were found to be 2.47 and 2.10 for the liver and muscle of C. sieboldii, respectively. In a study conducted by ARAS and co-workers (2009), C20:5 n-3 was a minor compound (changing from 0.25% to 0.57% in the seasons and groups investigated) of n-3 form of PUFA in Capoeta sieboldi umbla living in two different areas. However, the same study noted that C22:6 n-3 was the primary fatty acid of the n-3 PUFAs (as indicated in the present study), with values ranging from 5.14% to 15.86%, together with the high levels of total n-6 form of PUFA and the low levels of n-3/n-6 ratios (between 0.37 and 1.14). These discrepancies might be explained by the feeding behaviours of the species investigated, and this issue is well explained by the study of KUCSKA and co-workers (2006), using two different kinds of diet on E. lucius.

2.2. Fatty acid composition of neutral and polar lipid fractions

Fatty acid compositions of neutral lipids (NL) and polar lipids (PL) fractions can be seen in Tables 2 and 3, respectively. In both fractions, 38 fatty acids were determined with some quantitative differences.

Fatty acids	C. sieboldii Liver Mean±S.E.	<i>C. baliki</i> Liver Mean±S.E.	C. sieboldii Muscle Mean±S.E.	<i>C. baliki</i> Muscle Mean±S.E.
C8:0 ^c	0.03±0.00ac	$0.01{\pm}0.00a^{\rm B}$	0.08±0.03bcd	0.11±0.02d
C10:0	0.02±0.00a	0.01±0.00a	0.02±0.00a	0.11±0.03b
C11:0	0.03±0.00a	0.01±0.00a	0.11±0.02b	0.13±0.01b
C12:0	0.08±0.01a	0.06±0.01a	0.08±0.02a	0.09±0.03a
C13:0	0.10±0.00ab	0.05±0.02a	0.10±0.01ab	0.14±0.03b
C14:0	3.40±0.16a	5.00±0.31b	3.48±0.28a	5.12±0.32b
C15:0	2.84±0.17a	0.53±0.08b	3.34±0.20c	0.74±0.13b
C16:0	17.24±0.42a	16.15±0.14a	17.52±0.21a	17.03±0.59a
C17:0	0.89±0.10a	0.44±0.13b	0.94±0.07a	0.53±0.09b
C18:0	1.57±0.08a	1.59±0.13a	1.45±0.11a	2.85±0.23b
C19:0	0.60±0.07a	0.18±0.03b	0.46±0.13c	0.45±0.04c

Table 2. Fatty acid composition (%) of neutral lipids in the livers and muscles of C. sieboldii and C. baliki^A

Fatty acids	<i>C. sieboldii</i> Liver Mean±S.E.	<i>C. baliki</i> Liver Mean±S.E.	<i>C. sieboldii</i> Muscle Mean±S.E.	<i>C. baliki</i> ^A Muscle Mean±S.E.
C20:0	0.02±0.01a	0.02±0.00a	1.00±0.28b	0.02±0.00a
C21:0	3.10±0.56a	4.13±0.18b	3.00±0.21a	3.84±0.15c
C22:0	0.01±0.00a	0.03±0.01a	0.03±0.01a	0.01±0.00a
C24:0	0.23±0.09a	0.06±0.01b	0.16±0.04a	0.22±0.03a
ΣSFA	30.16	28.27	31.77	31.39
C14:1 n-5	0.69±0.10a	0.16±0.04b	0.97±0.13c	0.64±0.07a
C15:1 n-5	0.46±0.08a	0.18±0.01b	0.47±0.17a	0.27±0.07ab
C16:1 n-7	18.87±0.50a	9.92±0.29b	19.41± .33a	12.00±0.35c
C17:1 n-8	0.46±0.12a	0.11±0.03b	0.72±0.05c	0.79±0.11c
C18:1 n-9	14.10±0.33a	21.49±0.58	15.00±0.40ac	16.01±0.28c
C18:1 n-7	8.16±0.10a	3.64±0.35b	7.93±0.16a	5.07±0.17c
C20:1 n-9	0.30±0.11a	0.10±0.02b	0.28±0.04a	0.17±0.03b
C22:1 n-9	0.02±0.00a	0.06±0.01b	0.06±0.00b	0.09±0.02c
C24:1 n-9	0.05±0.01a	0.07±0.02ab	0.04±0.02a	0.13±0.03b
ΣΜUFA	43.11	35.73	44.88	35.17
C18:2 n-6	2.53±0.08a	3.09±0.06b	1.62±0.12c	2.31±0.09a
C18:3 n-6	1.19±0.14a	2.48±0.06b	0.10±0.03c	2.27±0.13b
C20:2 n-6	0.46±0.07a	0.09±0.02b	0.54±0.01c	0.28±0.04d
C20:3 n-6	0.30±0.03a	0.50±0.09b	0.36±0.05a	0.31±0.08a
C20:4 n-6	3.18±0.22a	0.98±0.14b	3.42±0.24c	1.16±0.11d
C22:2 n-6	0.11±0.03a	0.11±0.01a	0.08±0.01a	0.09±0.02a
C22:4 n-6	0.12±0.05a	0.11±0.03a	0.26±0.09b	0.44±0.13c
C22:5 n-6	0.14±0.04a	0.04±0.00b	0.26±0.08c	0.11±0.03a
Σn-6 PUFA	8.03	7.40	6.64	6.97
C18:3 n-3	1.70±0.08a	5.22±0.33b	1.60±0.14a	1.28±0.09c
C20:3 n-3	0.10±0.02a	0.62±0.1b	0.15±0.01a	0.31±0.10c
C20:5 n-3	9.72±0.39a	11.59±0.37b	8.98±0.56a	13.33±0.64c
C22:3 n-3	0.29±0.11ab	0.34±0.07a	0.33±0.04a	0.27±0.06b
C22:5 n-3	3.16±0.17ac	3.63±0.34a	2.80±0.24bc	3.44±0.13ac
C22:6 n-3	3.59±0.11a	7.00±0.29b	2.88±0.51a	7.79±0.46b
Σn-3 PUFA	18.56	28.40	16.74	26.42
ΣΡυγΑ	26.59	35.80	23.38	33.39
n-3/n-6	2.31	3.83	2.52	3.79

Table 2. Continued

^AAverage of three lots analysed. ^BValues reported are means±S.E. ^C(a-b-c-d): Values for each sample with different superscript letters in the same fraction are significantly different at P \leq 0.05. Σ SFA: total saturated fatty acid; Σ MUFA: total monounsaturated fatty acid; Σ n-6 PUFA: total n-6 polyunsaturated fatty acid; Σ n-3 PUFA: total n-3 polyunsaturated fatty acid; Σ PUFA: total polyunsaturated fatty acid

Fatty acids	C. sieboldii Liver	C. baliki Liver	C. sieboldii Muscle	C. baliki ^A Muscle
<u> </u>	Mean±S.E.	Mean±S.E.	Mean±S.E.	Mean±S.E.
C8:0 ^c	0.28±0.06a	0.55±0.04b	0.48±0.09b ^B	0.11±0.02c
C10:0	0.15±0.03a	0.18±0.04a	0.57±0.13b	0.17±0.02a
C11:0	0.36±0.04ab	0.51±0.01a	0.23±0.07b	0.45±0.09ac
C12:0	0.14±0.01a	0.14±0.03a	0.18±0.02a	0.13±0.02a
C13:0	0.13±0.01a	0.25±0.07b	0.10±0.01a	0.30±0.05b
C14:0	0.98±0.13a	3.85±0.42b	1.43±0.20a	0.91±0.17a
C15:0	0.93±0.25a	0.77±0.09a	0.86±0.03a	0.54±0.11a
C16:0	12.34±0.19a	19.99±0.57b	16.90±0.52c	15.52±0.30c
C17:0	1.98±0.21a	1.45±0.05b	0.98±0.27c	1.45±0.16b
C18:0	17.69±0.73a	11.74±0.42b	12.81±0.60c	13.20±0.56c
C19:0	0.10±0.02a	0.10±0.04a	0.21±0.02b	0.14±0.04a
C20:0	0.02±0.00a	0.54±0.15b	0.13±0.06c	0.02±0.00a
C21:0	0.25±0.08a	0.78±0.14b	0.82±0.09b	0.45±0.11c
C22:0	0.05±0.01a	0.08±0.00a	0.12±0.02a	0.05±0.02a
C24:0	0.05±0.00a	0.24±0.06b	0.18±0.04bc	0.11±0.03ac
ΣSFA	35.45	41.17	36.00	33.55
C14:1 n-5	0.65±0.08a	0.80±0.02a	1.49±0.14b	0.84±0.07a
C15:1 n-5	0.26±0.04a	0.26±0.02a	0.25±0.07a	0.28±0.03a
C16:1 n-7	3.87±0.46a	4.89±0.58a	4.14±0.31a	2.08±0.22b
C17:1 n-8	0.58±0.09a	0.80±0.11a	1.36±0.26b	1.10±0.18ab
C18:1 n-9	3.77±0.22a	8.42±0.35b	6.28±0.59c	4.29±0.33a
C18:1 n-7	8.26±0.31a	6.11±0.36b	5.64±0.22bc	4.58±0.31c
C20:1 n-9	0.37±0.06a	1.53±0.16b	0.19±0.07c	0.16±0.01c
C22:1 n-9	0.09±0.02a	0.08±0.02a	0.21±0.05b	0.07±0.01a
C24:1 n-9	0.10±0.02a	0.14±0.04a	0.11±0.03a	0.10±0.01a
ΣΜUFA	17.95	23.03	19.67	13.50
C18:2 n-6	0.57±0.08a	1.05±0.22b	0.65±0.10a	0.76±0.12ab
C18:3 n-6	0.09±0.01a	0.28±0.03b	0.39±0.02c	0.34±0.07bc
C20:2 n-6	0.42±0.05ab	0.24±0.03a	0.56±0.13ab	0.77±0.18b
C20:3 n-6	0.37±0.09a	0.52±0.17a	0.47±0.10a	0.40±0.06a
C20:4 n-6	15.21±0.68a	5.27±0.28b	8.01±0.54c	3.91±0.21d
C22:2 n-6	0.09±0.01a	0.09±0.00a	0.28±0.04b	0.11±0.03a
C22:4 n-6	1.10±0.34a	1.40±0.27a	4.52±0.38b	1.05±0.19a
C22:5 n-6	1.24±0.18a	0.35±0.09b	0.70±0.21c	0.39±0.07b
Σn-6 PUFA	19.12	9.20	15.58	7.73
C18:3 n-3	1.84±0.24a	1.30±0.10b	1.25±0.12b	0.58±0.09c

Table 3. Fatty acid composition (%) of polar lipids in the livers and muscles of C. sieboldii and C. baliki^A

Fatty acids	C. sieboldii Liver Mean±S.E.	<i>C. baliki</i> Liver Mean±S.E.	C. sieboldii Muscle Mean±S.E.	<i>C. baliki</i> [∧] Muscle Mean±S.E.
C20:3 n-3	0.11±0.03a	0.25±0.04b	0.40±0.09c	0.16±0.03ab
C20:5 n-3	3.85±0.20a	5.30±0.29b	7.85±0.59c	12.16±0.51d
C22:3 n-3	1.17±0.10a	0.82±0.16a	0.78±0.14a	0.45±0.04b
C22:5 n-3	6.70±0.41a	3.57±0.26b	5.92±0.33c	4.76±0.20d
C22:6 n-3	13.81±0.57ab	15.36±0.77b	12.53±0.47a	27.10±0.91d
Σn-3 PUFA	27.45	26.60	28.73	45.21
ΣΡυγΑ	46.57	35.80	44.31	52.94
n-3/n-6	1.43	2.89	1.84	5.84

Table 3. Continued

^AAverage of three lots analysed; ^Bvalues reported are means \pm S.E.; ^C(a-b-c-d): values for each sample with different superscript letters in the same fraction are significantly different at P \leq 0.05. Σ SFA: total saturated fatty acid; Σ MUFA: total monounsaturated fatty acid; Σ n-6 PUFA: total n-6 polyunsaturated fatty acid; Σ n-3 PUFA: total n-3 polyunsaturated fatty acid; Σ PUFA: total polyunsaturated fatty acid

In the fatty acid composition of NL and PL fractions, C16:0 was the main component of the SFA. In NL fraction, this acid did not show any statistical differences ($P \ge 0.05$) ranging from 16.15% (in the liver of C. baliki) to 17.52% (in the muscle of C. sieboldii). However, among the C16:0 amounts in the PL fraction, there were clear statistical differences ($P \le 0.05$) between C. sieboldii (12.34%) and C. baliki (19.99%) livers but not between muscles with the values of 16.90% and 15.52%, respectively in the species. C18:0 was another fatty acid having high levels in the SFA class of PL fraction and there were no statistical differences between muscles of C. sieboldii (12.81%) and C. baliki (13.20%). The highest level of this acid in PL was found to be in the liver (17.69%) of C. sieboldii with a significant statistical difference (P≤0.05) from the liver (11.74%) of C. baliki. The high percentages of C16:0 and C18:0 in PLs caused higher levels of SFA percentages, ranging from 33.55% to 41.17% than found in NL fractions changing between 28.27% and 31.39% (Tables 2 and 3). Our findings are in great agreement with the study carried out by BAYIR and co-workers (2010) on the three endangered S. trutta subspecies, investigating the fatty acid profile of polar and neutral lipids. They found that C16:0 was the main fatty acid in all seasons and the total SFA levels in phospholipids were higher than found in the neutral lipid fraction in all species. In another study, C16:0 was again the dominant fatty acid in the total SFA class of both PL and NL fractions in the muscle and liver of Comephorus baikalensis and C. dybowski. This study also showed that C18:0 is a little bit higher in the polar lipids than that found in the neutral lipids (Kozlova & Khotimchenko, 2000).

Total MUFA levels of the NL fraction (between 35.17% and 44.88%) were found to be higher than the values obtained for the PLs (between 13.50% and 23.03%) in all groups investigated (Tables 2 and 3). Major fatty acids of the MUFA were C16:1 n-7, C18:1 n-7, and C18:1 n-9 in polar and neutral lipid fractions of *C. sieboldii* and *C.baliki*. C16:1 n-7 and C18:1 n-9 were the fatty acids responsible for the higher levels of MUFA in the NL fraction, due to their higher levels. Lower percentages of these two acids were determined in the PL fraction in the liver and muscles of the species investigated. In studies carried out with different tissues of *Capoeta capoeta* (SATAR et al., 2012) and *E. lucius* (DESVILETTES et al.,

1997) it was revealed that C16:1 n-7 and C18:1 n-9 were to account for the more than 95% of total MUFA in triacylglycerol and polar lipids. At the same time, these studies emphasized that total MUFA levels were higher in the NLs than determined for the PLs. It seems that this finding on MUFA might be a typical feature of freshwater fish, and the data obtained in the present study are compatible with previous studies.

In the present study, the most important data standing out in terms of total n-6 PUFA was that C20:4 n-6 (ARA), which is the precursors of biologically active eicosanoids, had the highest levels in PL fractions with substantial statistical differences in all groups, ranging from 15.21% (in the liver of *C. sieboldii*) to 3.91% (in the muscle of *C. baliki*). The highest and lowest levels of this acid in the NL fractions were determined in the livers of *C. sieboldii* (3.18%) and *C. baliki* (0.98%), respectively. This result seems compatible with the data that phospholipids are depots of C20:4 n-6 in the biological membranes (Tocher et al., 2008) and the metabolism of this acid, participating in cell signalling events, results in the production of pharmacologically active eicosanoids (UYSAL et al., 2008; Le et al., 2009). However, C18:2 n-6 was other notable fatty acid exceeding 1% in the NL fractions of all groups.

C20:5 n-3, C22:5 n-3, and C22:6 n-3 were the most abundant fatty acids of the n-3 form of PUFA in both NL and PL class in all groups investigated. C20:5 n-3 values changed between 8.98% (in the muscle of C. sieboldii) and 13.33% (in the muscle of C. baliki) (P≤0.05) and it was the dominant n-3 form of PUFA in the NL fraction. The percentages of this acid in the PL fraction were between 3.85% (in the liver of C. sieboldii) and 12.16% (in the muscle of C. baliki) with statistical differences ($P \le 0.05$). The highest C22:6 n-3 amount in the NLs was determined for the muscle of C. baliki as 7.79%. However, the levels of this acid in PLs were found to be between 12.53% in the muscle of C. sieboldii and 27.10% in the muscle of C. baliki. This data also might be associated with the explanations made for C20:4 n-6 amounts found in PLs. Total PUFA amounts of polar and neutral lipids in the livers of C. *baliki* were found the same as 35.80%. The n-3/n-6 ratios of the livers of C. sieboldii (2.31) and C. baliki (3.83) in the NL fractions were higher than determined for the livers of C. sieboldii (1.43) and C. baliki (2.89) in PLs. However, the n-3/n-6 ratios of 5.84 (the highest ratio) and 1.84 were found in the muscles of C. baliki and C. sieboldii in the PLs, respectively. In NLs, these values were 2.52 for C. sieboldii and 3.79 for C. baliki in the muscle samples. Previous studies revealed that C22:6 n-3 was the most dominant fatty acid in both the neutral and polar lipids of the freshwater fish, together with some quantitative differences according to season in n-3 PUFA (MENDEZ, 1997; BAYIR et al., 2010; CENGIZ et al., 2012; SATAR et al., 2012). The n-3/n-6 ratios determined in this study seem to be higher than determined for C. sieboldi umbla (ARAS et al., 2009) and Silurus triostegus (CENGIZ et al., 2012). However, our results on the n-3/n-6 ratios seem in agreement with the ratios determined for Capoeta trutta (SATAR et al., 2012). These phenomena can be related to the feeding regimes and the metabolism of the species under investigation.

3. Conclusion

The complete fatty acid analyses covering total lipid, neutral and polar lipids of *C. sieboldii* and *C. baliki* from Tödürge Lake have been carried out. When n-3 PUFA amounts and the n-3/n-6 ratios in the muscles were considered, these two species appear to be nutritious fish, especially *C. baliki*. At the same time, neutral lipid analyses have been suggesting that the livers of these species also are rich in n-3 PUFA. Muscle fatty acid composition results

suggest that *Capoeta* species investigated have high nutritive value in terms of polyunsaturated fatty acids and might be good food items for human nutrition.

*

The authors thank to G. ZENGIN (Selcuk University) for his helps during the analyses of the fatty acids.

References

- AKPINAR, M.A., GÖRGÜN, S. & AKPINAR, A.E. (2009): A comparative analysis of the fatty acid profiles in the liver and muscles of male and female Salmo trutta macrostigma. Fd Chem., 112, 6–8.
- ARAS, N.M., HALILOĞLU, H.İ., BAYIR, A., ATAMANALP, M. & SIRKECIOĞLU, A.N. (2003): Karasu havzası Yeşildere Çayı olgun dere alabalıkları (*Salmo trutta macrostigma*, Dumeril, 1858) 'nda farklı dokuların yağ asidi kompozisyonlarının karşılaştırılması. (Comparison of the fatty acid composition of different tissues in mature trout (*Salmo trutta macrostigma*, Dumeril, 1858) in Yeşildere Creek in the Karasu basin.) *Turk. J. Vet. Anim. Sci.*, 27, 887–892.
- ARAS, N.M., GUNEŞ, M., BAYIR, A., SIRKECIOĞLU, A.N. & HALILOĞLU, H.İ. (2009): Tuzla Çayı ve Tercan Baraj Gölü'ndeki *Capoeta caopeta umbla* HECKEL, 1843'nın bazı biyo-ekolojik özellikleri ile total yağ ve yağ asitleri kompozisyonlarının karşılaştırılması. (The comparison of total fat and fatty acid profiles with some bio-ecological features of *Capoeta capoeta umbla* HECKEL, 1843 living in Tuzla Stream and Tercan Dam Lake.) *Ekoloji*, 19, 55–64.
- BAYIR, A., SIRKECIOĞLU, A.N., ARAS, N.M., AKSAKAL, E., HALILOĞLU, H.İ. & BAYIR, M. (2010): Fatty acids of neutral and phospholipids of three endangered trout: Salmo trutta caspius Kessler, Salmo trutta labrax Pallas and Salmo trutta macrostigma Dumeril. Fd Chem., 119, 1050–1056.
- CAKMAK, Y.S., ZENGIN, G., GULER, G.O., AKTUMSEK, A. & OZPARLAK, H. (2012): Fatty acid composition and Ω3/ Ω6 ratios of the muscle lipids of six fish species in Sugla Lake, Turkey. Arch. Biol. Sci., Belgrade, 64, 471–477.
- CENGIZ, E.İ., ÜNLU, E., BASHAN, M., SATAR, A. & UYSAL, E. (2012): Effects of seasonal variations on the fatty acid composition of total lipid, phospholipids and triacylglicerol in the dorsal muscle of Mesopotamian catfish (*Silurus triostegus* Heckel, 1843) in Tigris River (Turkey). *Turk. J. Fish Aquat. Sci.*, 12, 33–39.
- DESVILETTES, C., BOURDIER, G. & BRETON, J.C. (1997): Changes in lipid class and fatty acid composition during development in pike (*Esox lucius* L.) eggs and larvae. *Fish Physiol. Biochem.*, 16, 381–393.
- FOLCH, J., LESS, M. & SLOANE-STANLEY, G.H.A. (1957): Simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, *226*, 497–509.
- GULER, G.O., KIZTANIR, B., AKTUMSEK, A., CITIL, O.B. & OZPARLAK, H. (2008): Determination of the seasonal changes on total fatty acid composition and $\omega 3/\omega 6$ ratios of carp (*Cyprinus carpio* L.) muscle lipids in Beyşehir Lake (Turkey). *Fd Chem., 108,* 689–694.
- GULER, G.O., CAKMAK, Y.S., ZENGIN, G., AKTUMSEK, A. & AKYILDIZ, K. (2010): Fatty acid composition and conjugated linoleic acid (CLA) content of some commercial milk in Turkey. *Kafkas Univ. Vet. Fak. Derg.*, 16, 37–40.
- HALILOĞLU, H.İ., ARAS, N.M. & YETIM, H. (2002): Comparison of muscle fatty acids of three trout species (Salvelinus alpinus, Salmo trutta fario, Oncorhynchus mykiss) raised under the same conditions. Turk. J. Vet. Anim. Sci., 26, 1097–1102.
- HOLUB, D.J. & HOLUB, B.J. (2004): Omega-3 fatty acids from fish oils and cardiovascular disease. Mol. Cell Biochem., 263, 217–225.
- JANKOWSKA, B., ZAKES, Z., ZMIJEWSKI, T. & SZCZEPKOWSKI, M. (2003): A comparison of selected quality features of the tissue and slaughter yield of wild and cultivated pikeperch Sander lucioperca (L.). Eur. Fd Res. Technol., 217, 401–405.
- KALVONCU, L., KISSAL, S. & AKTUMSEK, A. (2009): Seasonal changes in the total fatty acid composition of *Vimba vimba tenella* (Nordmann, 1840) in Eğirdir Lake, Turkey. *Fd Chem., 116,* 728–730.
- KINSELLA, J.E., LOKESH, B.&STONE, R.A. (1990): Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. Am. J. Clin. Nutr., 52, 1–28.
- KOZLOVA, T.A. & KHOTIMCHENKO, S.V. (2000): Lipids and fatty acids of two pelagic cottoid fishes (*Comephorus* spp.) endemic to Lake Baikal. *Comp. Biochem. Physiol. B., 126,* 477–485.
- KUCSKA, B., PAL, L., MÜLLER, T., BODIS, M., BARTOS, A., WAGNER, L., HUSVETH, F. & BERCSENYI, M. (2006): Changing of fat content and fatty acid profile of reared pike (*Esox lucius*) fed two different diets. *Aquac. Res.*, 37, 96– 101.

- LAURITZEN, I., BLONDEAU, N., HEURTEAUX, C., WIDMANN, C., ROMEY, G. & LAZDUNSKI, M. (2000): Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J.*, 19, 1784–1793.
- LE, H.D., MEISEL, J.A., DE MEIJER, V.E., GURA, K.M. & PUDER, M. (2009): The essentiality of arachidonic acid and docosahexaenoic acid. *Prostag. Leukotr. Ess.*, 81, 165–170.
- LOMBARDO, Y.B., HEIN, G. & CHICCO, A. (2007): Metabolic syndrome: Effects of n-3 PUFAs on a model of dyslipidemia, insulin resistance and adiposity. *Lipids*, 42, 427–437.
- MENDEZ, E. (1997): Seasonal changes in the lipid classes and fatty acid compositions of hake (Merluccius hubbsi) liver oil. J.A.O.C.S., 74, 1173–1175.
- MOFFAT, C.F. & MCGILL, A.S. (1993): Variability of the composition of fish oils: Significance for the diet. Proc. Nutr. Soc., 52, 441–456.
- Moss, C.W., LAMBERT, M.A. & MERVIN, W.H. (1974): Comparison of rapid methods for analysis of bacterial fatty acids. *Appl. Microbiol.*, 28, 80–85.
- NIKOLAIDIS, M.G., PETRIDOU, A. & MOUGIOS, V. (2006): Comparison of the phospholipid and triacylglycerol fatty acid profile of rat serum, skeletal muscle and heart. *Physiol. Res.*, 55, 259–265.
- PIGGOT, G.M. & TUCKER, B.W. (1990): Effects of technology on nutrition. Marcel Dekker, New York, 362 pages.
- Russo, G.L. (2009): Dietary n-6 and n-3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochem. Pharmacol.*, 77, 937–946.
- SARGENT, J., BELL, G., MCEVOY, L., TOCHER, D. & ESTEVEZ, A. (1999): Recent developments in the essential fatty acid nutrition of fish. Aquaculture, 177, 191–199.
- SATAR, E.İ., UYSAL, E., ÜNLÜ, E., BAŞHAN, M. & SATAR, A. (2012): The effects of seasonal variation on the fatty acid composition of total lipid, phospholipids, and triacylglycerol in the dorsal muscle of *Capoeta trutta* found in the Tigris River (Turkey). *Turk. J. Biol., 36*, 113–123.
- STEFFENS, W. (1997): Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. *Aquaculture*, 151, 97–119.
- TOCHER, D.R., BENDIKSEN, E.A., CAMPBELL, P.J.& BELL, J.G. (2008): The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, 280, 21–34.
- UYSAL, K. & AKSOYLAR, M.Y. (2005): Seasonal variations in fatty acid composition and the n-6/n-3 fatty acid ratio of pikeperch (*Sander lucioperca*) muscle lipids. *Ecol. Fd Nutr.*, 44, 23–35.
- UYSAL, K., BÜLBÜL, M., DÖNMEZ, M. & SEÇKIN, A.K. (2008): Changes in some components of the muscle lipids of three freshwater fish species under natural extreme cold and temperate conditions. *Fish Physiol. Biochem.*, 34, 455–463.