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A comparative analysis of the fatty acid profiles in the liver and muscles of male and female *Salmo trutta macrostigma*

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ABSTRACT

The fatty acid compositions of liver and muscle of male and female *Salmo trutta macrostigma*, in the Tohma River, Turkey, were determined by gas chromatography. There were quantitative differences between individual fatty acids in the tissues investigated, depending on the sex. The most abundant fatty acids in both tissues of both sexes were palmitic acid (C16:0; 19.0–21.6%), stearic acid (C18:0; 5.32–11.3%), C18:1 ω−7 (5.65–9.38%), oleic acid (C18:1 ω−9; 15.6–22.4%), eicosapentaenoic acid (EPA; C20:5 ω−3; 6.34–7.88%) and docosahexaenoic acid (DHA; C22:6 ω−3; 7.38–15.6%). The ω−3/ω−6 ratio in tissues were found to be 2.89 (♂) and 1.97 (♀) in liver, and 2.59 (♂) and 2.26 (♀) in muscle. *S. trutta macrostigma* may be a valuable food for human consumption in terms of fatty acids.

1. Introduction

Fish and fish oils contain omega−3 (ω−3) fatty acids; in particular, eicosapentaenoic acid (C20:5 ω−3; EPA) and docosahexaenoic acid (C22:6 ω−3; DHA) (Holub & Holub, 2004), which originate from phytoplankton and seaweed in the food chain (Visentainer et al., 2007). Recent studies have shown that EPA and DHA, present at abundant amounts in fish tissues, have beneficial effects on bone formation and metabolism, and in the prevention of cardiovascular disease (Lauritzen et al., 2000; Lombardo, Hein, & Chicco, 2007; Simopoulos, 1999; Su, Huang, Chiu, & Shen, 2003; Watkins, Li, Lippman, & Feng, 2003).

ω−3 polyunsaturated fatty acids (ω−3 PUFAs) cannot be synthesised effectively by humans (Jankowska, Zakes, Zmijewski, & Szczepkowski, 2003; Sushchik, Gladyshev, & Kalachova, 2007) and must be obtained by diet (Çelik, Diler, & Küçükgülmez, 2005). Like other vertebrates, fish also require EPA, DHA and arachidonic acid (C20:4 ω−6; ARA) for normal growth, development and reproduction (Rodriguez et al., 2004; Sargent, Bell, McEvoy, Tocher, & Estevez, 1999). Sargent et al. (1999) indicated that these PUFAs are necessary to maintain membrane structure integrity and are precursors of eicosanoids.

Variations in lipid and fatty acid compositions between and within fish species, depending on factors, such as food availability, the season, location, sex, diet and age, have been well documented by numerous authors (Exler, Kinsella, & Watt, 1975; Görgün & Akpınar, 2007; Rueda et al., 1997; Shearer, 1994). Lipid and fatty acid composition are known to vary even between tissues of fish. The liver is an important organ in terms of lipid metabolism and the main part of fish used for human nutrition is generally muscle. Thus, analysis of the fatty acid profiles of the tissues, such as muscle and liver, from fish living in their natural ecosystem, can yield valuable information (Kiessling et al., 2001; Rodriguez et al., 2004).

There are also differences in terms of fatty acid composition between freshwater and marine fish. Freshwater fish is generally recognised to contain lower levels of ω−3 PUFA than marine fish. However, chain elongation and desaturation processes are more efficient in freshwater fish than in marine fish. Thus, freshwater fish can be converted to a food having high nutritional value with feed (Jankowska et al., 2003; Steffens, 1997; Vujkovic, Karlovic, Vujkovic, Vörösbaranyi, & Jovanovic, 1999).

There is only one study (Aras, Haliloğlu, Bayır, Atamanalp, & Sirkecioğlu, 2003) about the fatty acids of mature *Salmo trutta macrostigma* in Turkey. Thus, the fatty acid dynamics of this wild species is not well known. The main objective of this study was to measure the liver and muscle fatty acid composition of *S. trutta macrostigma* females and males.

2. Material and methods

2.1. Fish and sampling

Mature *S. trutta macrostigma* (Dumeril, 1858), used in this study were collected from Tohma River, which is a mountain creek at 1203

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meters in Sivas, Turkey, in September 2004. The geographic co-ordinates of the study area were 38°41′23.3″ North and 37°25′40.9″ East. The oxygen content of the water was 10.5 ± 0.23 mg l^{-1} with a pH value of 8.4 ± 0.38 and temperature of 12.3 ± 0.30 °C. Three male and 3 female fish (a total of 6 fish) were caught and used for total lipid extraction and fatty acid analyses. Lengths and weights of the fish used in the study were 32.1 ± 0.61 cm and 450 ± 8.65 g. One gram of each of liver and muscle sample were extracted for total lipid and fatty acid analysis. Muscle sample was directly taken from the area underneath the dorsal fin. Three 1 g replicates were taken of each sample and immediately placed in chloroform/methanol (2/1, v/v) and stored at −20 °C until analyses.

2.2. Lipid extraction and fatty acid analysis

Liver and muscle samples were homogenised in chloroform/methanol (2/1, v/v), using an Ultra-Turrax T25 homogenizer (Folch, Less, & Sloane-Stanley, 1957). The total lipids obtained were saponified by refluxing with methanol (50%) containing 5% sodium hydroxide for 1 h. The saponifiable lipids were converted to their methyl esters by using the standard boron trifluoride-methanol (BF_3) method (Moss, Lambert, & Mervin, 1974). The resultant mixture of fatty acid methyl esters (FAMEs) in hexane/chloroform (4/1, v/v) was injected into a Shimadzu GC 17 gas chromatograph (Shimadzu, Osaka, Japan) equipped with flame ionisation detector (FID), and a Macherey-Nagel Permabond capillary column (25 m × 0.25 mm; Macherey-Nagel GmbH & Co. KG, Düren, Germany), at 120 °C column temperature. The injection port and detector temperatures were 240 and 280 °C, respectively. Nitrogen was used as carrier gas at 2.5 ml min^{-1}. One microlitre of methyl ester solution was introduced onto the column. FAMEs were identified by comparison of retention times with standards from Sigma Chemical Co. (St. Louis, MO). Calculation of fatty acid ratios was carried out by GClass 2.01 software.

2.3. Statistical analysis

Statistical analyses were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). All analytical determinations were performed in triplicate and the mean values were reported. The percentages of fatty acid were compared by analysis of variance (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were evaluated as significant at $p \leqslant 0.05$.

3. Results and discussion

Table 1 shows liver and muscle fatty acid compositions of *S. trutta macrostigma* from the Tohma River in Turkey. Sixteen fatty acids were identified from the livers of male fish while 18 fatty acids were identified from female livers. Fifteen fatty acids were identified in the muscle tissue of both sexes with quantitative differences.

Palmitic acid (C16:0) was the major saturated fatty acid (SFA) in liver and muscle of both sexes. The amount of this acid was 19.0% and 19.1% in male and female livers, respectively ($p > 0.05$), and 21.2% (in males) and 21.6% (in females) in the muscle tissue ($p > 0.05$). Stearic acid (C18:0) was the fatty acid present at the second highest level. In the livers, females had a higher amount (11.3%) of C18:0 than males (7.87%) ($p < 0.05$). There was no difference between C18:0 percentages in male (5.32%) and female (5.64%) muscles. Pentadecanoic acid (C15:0) was only found in female livers in trace amounts (0.26%). As can be seen from Table 1, while very significant differences were determined between SFA fractions of male and female livers, smaller differences were found between SFA in male and female muscles.

Table 1
Comparison of liver and muscle fatty acid profiles of male and female *S. trutta macrostigma* (Dumeril, 1858)[A]

Fatty acids	Liver ♂	Liver ♀	Muscle ♂	Muscle ♀
C14:0	1.14 ± 0.27[ab,B]	1.10 ± 0.13[a]	2.02 ± 0.39[b]	2.20 ± 0.48[b]
C15:0	–	0.26 ± 0.14	–	–
C16:0[C]	19.0 ± 0.31[a]	19.1 ± 0.19[a]	21.2 ± 0.27[b]	21.6 ± 0.47[b]
C18:0	7.87 ± 0.49[a]	11.3 ± 0.16[b]	5.32 ± 0.15[c]	5.64 ± 0.38[c]
C16:1 ω−9	2.62 ± 0.23[a]	3.44 ± 0.38[a]	6.50 ± 0.15[b]	7.31 ± 0.19[b]
C18:1 ω−7	8.10 ± 0.26[a]	9.38 ± 0.51[b]	5.65 ± 0.14[c]	6.40 ± 0.21[d]
C18:1 ω−9	17.6 ± 0.36[a]	15.6 ± 0.57[b]	22.4 ± 0.17[c]	22.1 ± 0.49[c]
C20:1 ω−9	0.50 ± 0.13[a]	1.91 ± 0.22[b]	1.44 ± 0.31[b]	1.75 ± 0.44[b]
C22:1 ω−9	–	0.39 ± 0.31	–	–
C18:2 ω−6	2.77 ± 0.37[a]	2.02 ± 0.29[a]	4.82 ± 0.16[b]	5.19 ± 0.23[b]
C18:3 ω−6	0.77 ± 0.22[a]	1.25 ± 0.34[b]	0.99 ± 0.12[ab]	1.20 ± 0.60[ab]
C20:3 ω−6	0.59 ± 0.14[ab]	2.84 ± 0.25[c]	0.97 ± 0.24[ad]	1.35 ± 0.32[d]
C20:4 ω−6	6.21 ± 0.31[a]	5.65 ± 0.38[b]	3.00 ± 0.30[c]	2.34 ± 0.41[d]
C22:4 ω−6	–	0.60 ± 0.33	–	–
C22:5 ω−6	0.53 ± 0.21	–	–	–
C18:3 ω−3	2.80 ± 0.23[a]	1.15 ± 0.17[b]	5.71 ± 0.64[c]	5.52 ± 0.21[c]
C20:5 ω−3	7.16 ± 0.36[ac]	6.34 ± 0.22[b]	7.88 ± 0.59[ad]	6.45 ± 0.43[bc]
C22.5 ω−3	5.61 ± 0.41[a]	4.27 ± 0.30[b]	3.40 ± 0.27[c]	3.53 ± 0.36[c]
C22:6 ω−3	15.6 ± 0.49[a]	12.7 ± 0.38[b]	8.42 ± 0.27[c]	7.38 ± 0.16[c]
ΣSFA	28.0 ± 0.74[a]	31.8 ± 0.28[b]	28.5 ± 0.67[a]	29.4 ± 0.61[c]
ΣMUFA	28.8 ± 0.46[a]	30.7 ± 0.41[b]	35.9 ± 0.27[c]	37.5 ± 0.33[d]
Σ ω−6 PUFA	10.9 ± 0.25[ab]	12.4 ± 0.17[a]	9.78 ± 0.28[b]	10.1 ± 0.42[b]
Σ ω−3 PUFA	31.1 ± 0.44[a]	24.5 ± 0.29[bc]	25.4 ± 0.74[b]	22.9 ± 0.24[c]
ω−3/ω−6 ratio	2.89 ± 0.68[a]	1.97 ± 0.52[b]	2.59 ± 0.37[ab]	2.26 ± 0.22[ab]

[A] The data are expressed as percentages of total fatty acids and average of three lots analysed.
[B] Values reported are means ± SE.
[C] Values for each sample with different superscript letters in the same row are significantly different ($p < 0.05$).

Oleic acid (C18:1 ω−9) was identified as the major monounsaturated fatty acid (MUFA) in liver and muscle of both sexes. This fatty acid was at similar levels in muscle tissues of male (22.4%) and females (22.1%) but at higher levels than in the livers of male and females (17.6% and 15.6%, respectively; $p < 0.05$). C18:1 ω−7 was also at high levels in the MUFA fraction of male and females and amounts of this acid ranged from 5.65% to 9.38% in male and female livers and muscles ($p < 0.05$). No study has reported high levels of C18:1 ω−7. However, C18:1 ω−9 is a characteristic MUFA in fish tissues (Steffens, 1997). In *Oncorhynchus mykiss* C18:1 ω−9 is the predominant MUFA in both liver and muscle (Görgün & Akpınar, 2007; Haliloğlu, Bayır, Sirkecioğlu, Aras, & Atamanalp, 2004). C16:1 ω−9 was another notable fatty acid in the MUFA fraction of male (6.50%) and female (7.31%) muscles ($p > 0.05$). This acid was at lower amounts in male (2.62%) and female (3.44%) livers ($p > 0.05$). Aras, Haliloğlu, Bayır, et al. (2003) reported that the main MUFA of *S. trutta macrostigma* were C18:1 ω−9 and C16:1 ω−7. The discrepancy in ω−7 acids may be primarily caused by different ecological environments and the diets of the fish samples. It was found that the male and female tissues studied showed statistical differences in the total MUFA fraction ($p < 0.05$).

In the ω−6 PUFA fraction, ARA (C20:4 ω−6) was the major ω−6 polyunsaturated fatty acid (PUFA) with levels of 5.65–6.21% in livers of female and male fish, respectively ($p < 0.05$). The C20:4 ω−6 percentage in the muscle tissue was higher in males (3.00%) than in females (2.34%) ($p < 0.05$). Linoleic acid (C18:2 ω−6) was at the highest amounts with values of 4.82% in male and 5.19% in female muscles ($p > 0.05$). There were not differences between male (2.77%) and female (2.02%) livers in terms of C18:2 ω−6 ($p > 0.05$). Similar results for liver and muscle of *S. trutta macrostigma* have been reported (Aras, Haliloğlu, Bayır, et al., 2003). C18:3 ω−6 and C20:3 ω−6 were found to be at low amounts in the ω−6 PUFA fractions of the tissues investigated, with the exception of female livers. It was found that C22:4 ω−6 (0.60%) and C22:5 ω−6 (0.53%) were present only in female and male liver, respectively. Total ω−6 PUFA level in female liver (12.4%) was not higher than

in male (10.9%) livers ($p > 0.05$), and male and female muscles did not show any differences in their total ω−6 PUFA levels ($p > 0.05$).

DHA (C22:6 ω−3) was found to be at the highest level in the ω−3 PUFA fraction in the female and male tissues studied. Male livers had substantial levels of C22:6 ω−3 with a value of 15.6%, higher than in female livers (12.7%; $p < 0.05$). EPA (C20:5 ω−3) was the fatty acid at the second high amount in ω−3 PUFA of female and male fish tissues. Male and female livers showed statistical difference in C20:5 ω−3 levels, as did male and female muscles ($p < 0.05$). High levels of EPA and DHA were reported by other studies in trout species, including *S. trutta macrostigma* (Aras, Haliloğlu, Ayık, & Yetim, 2003; Aras, Haliloğlu, Bayır, et al., 2003; Görgün & Akpınar, 2007; Haliloğlu, Aras, & Yetim, 2002) and marine fish species (Bayır, Haliloğlu, Sirkecioğlu, & Aras, 2006). The amounts of C22:5 ω−3 in male and female fish changed from 3.40% to 5.61%, and livers showed statistical differences ($p < 0.05$), while muscle tissues did not. Linolenic acid (C18:3 ω−3) was found to be at the lowest amounts in the ω−3 PUFA fraction. Total ω−3 PUFA level showed a clear differences in male (31.1%) and female (24.5%) livers, while a slight difference was present in male (25.4%) and female (22.9%) muscles ($p < 0.05$). The ω−3/ω−6 ratios in male and female livers were found to be 2.89 and 1.97 ($p < 0.05$), while in muscles of male and female fish the ratios were as 2.59 and 2.26 ($p > 0.05$), respectively. Güler, Aktümsek, Çitil, Arslan, and Torlak (2007) emphasised that ω−3/ω−6 ratio is a very useful index for comparing nutritional value of fish oils. ω−3/ω−6 ratios in dorsal muscle samples of *Salvelinus alpinus*, *Salmo trutta fario* and *O. mykiss* raised under the same conditions were 1.27, 0.95 and 1.58, respectively (Haliloğlu et al., 2002). In a seasonal study by Güler et al. (2007) conducted on *Sander lucioperca* from Beyşehir lake in Turkey, the highest ω−3/ω−6 ratio was determined as 1.49 in spring. Haliloğlu et al. (2004) found ω−3/ω−6 ratios of 1.94 and 1.83 for liver and muscle of *O. mykiss* living in fresh water, respectively. When compared with these species, the *S. trutta macrostigma* muscles used in our study have a higher ω−3/ω−6 ratio. However, Aras, Haliloğlu, Bayır, et al. (2003) reported higher ω−3/ω−6 ratios than our results for tissues of *S. trutta macrostigma*. Our study has revealed that *S. trutta macrostigma* is a wild fish species having a high nutritional value for human consumption due to its high ω−3/ω−6 ratio.

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