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Full Length Research Paper

Determination of the seasonal changes on total fatty acid composition of rainbow trout, *Oncorhynchus mykiss* in Ivriz Dam Lake, Turkey

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Total fatty acid compositions and seasonal variations of *Oncorhynchus mykiss* in Ivriz Dam Lake, Turkey were investigated using gas chromatographic method. A total of 38 different fatty acids were determined in the fatty acid composition of rainbow trout. Polyunsaturated fatty acids (PUFAs) were found to be higher than saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in all seasons. Oleic acid (C18:1 ω9) is the major MUFA in all seasons. Palmitic acid (C16:0) was identified as the major SFA in four seasons. Docosahexaenoic acid (C22:6 ω3), linoleic acid (C18:2 ω6) and eicosapentaenoic acid (C20:5 ω3) had the highest levels among the PUFAs. In the present study, ω3 /ω6 ratios were found to be 1.24, 1.68, 0.61 and 0.98 in spring, summer, autumn and winter, respectively.

Key words: Fatty acid composition, seasonal changes, freshwater fish, Turkey.

INTRODUCTION

Fish naturally contain high levels of polyunsaturated fatty acids (PUFAs) of the ω6 series and especially of ω3 series such as eicosapentaenoic acid (EPA, C20:5 ω3) and docosahexaenoic acid (DHA, C22:6 ω3) that are recognised as essential biochemical components of human diet because of their beneficial effects on human health (Sushchik et al., 2007). Today, it is known that omega 3 (ω3) fatty acids, or a balanced ω3/ω6 ratio in the diet are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, diabetes, hyper-tension and cancer. They also affect neurodevelopment in infants, fat glycemic control, learning ability and visual function (Kinsella et al., 1990). The ω3 and ω6 PUFAs are considered essential to the growth and development of children and they are precursors of composite hormones known as eicosanoids, involved in several metabolic processes of great importance for the human body, mainly related to cardiovascular activity (Eder, 1995; Inhamuns and Franco, 2008). Long chain n-3 PUFAs cannot be synthesised by humans and must be obtained through the diet (Alasalvar et al., 2002).

The ω3 fatty acids are always present in fish flesh even in lean fish (Ackman, 2002). The fat content and the fatty acid composition of the fish are not constant (Zlatanos and Laskaridis, 2007). The amount of long chain ω3 PUFAs differs among fishes and can be influenced by a number of factors. The fatty acids composition of fish tissue can be affected by diet, size, age, reproductive cycle, salinity, temperature, season and geographical location (Henderson and Tocher, 1987; Inhamuns and Franco, 2008; Zlatanos and Laskaridis, 2007).

Various environmental conditions and the different diets of wild and reared fish affect their chemical composition, including their fatty acid profile (Suzuki et al., 1986; Jankowska et al., 2010). EPA is the most important essential fatty acid of ω3 series in the human diet because it is the precursor to the 3-series eicosanoids (Chen et al., 1995). Compared with freshwater fish, marine fish have higher levels of PUFAs, especially DHA and EPA. Arachidonic acid (AA, C20:4 ω6), EPA and DHA are important structural components of cell membranes (Innis, 1991).

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Abbreviations: PUFAs, Polyunsaturated fatty acids; **SFAs,** saturated fatty acids; **MUFAs,** monounsaturated fatty acids; **EPA,** eicosapentaenoic acid; **DHA,** docosahexaenoic acid; **ω3,** omega 3; **ω6,** omega 6; **ω9,** omega 9.

Oncorhynchus mykiss as a freshwater fish species have been one of the most widely cultured species all over the world. This species is one of the most abundant freshwater fish in Ivriz Dam Lake. Thus, the fatty acid dynamics of this species is not well known. The main objective of this study was to determine the seasonal fluctuations of fatty acid compositions of muscle and ω3/ω6 fatty acids ratio of this fish species.

MATERIALS AND METHODS

O. mykiss used in this study were obtained from Ivriz Dam Lake, Turkey. In the present study, the seasons chosen for analysis were summer, winter, spring and autumn. The samples were collected in the middle month of each season during 2008 - 2009. Three individuals were sampled in each season. After being caught, they were transported on ice to the laboratories, filleted and frozen. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature, ground and homogenized in chloroform/ methanol mixture (2/1, v/v). Ten gram of muscle sample were extracted.

The total lipids obtained were saponified by refluxing with methanol (50%) containing 6% KOH for 1 h. Samples of fillets were extracted by method of Folch et al. (1957) and lipid samples obtained were transesterified with BF_3 methanol (Moss et al., 1974). The saponifiable lipids were converted to their methyl esters by using the standard boron tri-fluoride-methanol (BF_3) method. FAMEs were analyzed on a HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted to a HP-88 capillary column (100 m, 0.25 mm i.d. and 0.2 µm). Injector and detector temperatures were 240 and 250°C, respectively. The oven was programmed at 160°C initial temperature and 2 min initial time. Thereafter, the temperature was increased up to 185°C at rate of 4°C/min then increased up to 200°C at rate of 1°C/min and held at 200°C for 46.75 min. Total run time was 70 min. Carrier gas used was helium (1 ml/min).

Identification of fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech and Accu standards. Results were expressed as FID response area in relative percentages. Each reported result is given in the average value of three GC analyses. The results are offered as means ± standard deviation (SD). The results were submitted to analysis of varience at 0.05 significant level, using LSD.

RESULTS AND DISCUSSION

The results of fatty acid analyses were shown in Table 1 and 38 fatty acids were identified in muscle lipids of rainbow trout. The highest fatty acids in the fish in all seasons were C18:1 ω9 oleic acid, C18:2 ω6 linoleic acid, C16:0 palmitic acid and DHA, respectively.

In the present study, saturated fatty acids (SFAs) were lower than total monounsaturated fatty acids (MUFAs). The ratio of total SFAs ranged between 19.48 and 24.95%. Palmitic acid was the major SFA (12.72 - 16.41%) for rainbow trout in all seasons. Similar results for other fish species have also been reported in literature (Celik et al., 2005; Rahman et al., 1995). According to Akpinar et al. (2009), palmitic acid is the major SFA in liver (19.0 - 19.1%) and muscle (21.2.4 - 21.6%) of male and female *Salmo trutta macrostigma*. The principal fatty acids of both fractions (neutral and phospholipids) were palmitic acid in SFA and oleic acid in MUFAs (Bayir et al., 2010). Palmitic acid was found to be 21.3% in rainbow trout (Haliloğlu et al., 2004). The major fatty acids identified in the rainbow trout are 18:1 ω9, while C16:0 is the third highest fatty acids except for summer. C 10:0, C 11:0, C 12:0 and C 13:0 were found to be of low amounts in the SFA fractions of the muscle investigated. Stearic acid (C18:0) is the second highest SFA (3.28-3.87%). Oleic acid was identified as a primary MUFA in the rainbow trout for all seasons. This fatty acid in muscle tissue of rainbow trout was found to be 24.58, 23.65, 34.06 and 26.80% in spring, summer, autumn and winter, respectively. The highest level of oleic acid was in autumn. According to Akpinar et al. (2009), oleic acid is the major monounsaturated fatty acid in liver (15.6 - 17.6%) and muscle (22.4 - 22.1%) of male and female *S. trutta macrostigma*. Similarly, Guler et al. (2007) found that oleic acid is the major MUFA in muscle in tissue of zander, *Sander lucioperca* living in freshwater in Turkey. Additionally, C18:1 ω9 and 18:2 ω6 were found at higher levels in MUFA in rainbow trout (Haliloğlu et al., 2004). 18:2 ω6 is the second highest fatty acid. . Its ratio range between 14.03% (summer) and 23.56% (autumn). There were differences between summer and autumn in terms of 18:2 ω6 ($p<0.05$). According to Bayir et al. (2010), linoleic acid (18:2) ratio ranged between 10.81% (summer) and 14.56% (autumn) for S*almo trutta caspius*, 8.37% (spring) and 10.34% (autumn) for *S. trutta labrax* and 11.38% (spring) and 14.57% (autumn) for *S. trutta macrostigma*. The high levels of oleic, palmitoleic (C16:1 ω7) and arachidonic acid (AA) had been reported as a charac-teristic property of freshwater fish oils (Andrade et al., 1995). Palmitoleic acid is the second highest MUFA (3.43 - 5.24%) in the present study. C 14:1 ω5 and C 15:1 ω5 were found to be in low amounts in the MUFA fractions of the muscle investigated. On the other hand, MUFA con-tents were higher than the SFA content in spring, summer, winter and autumn. In autumn, a high amount of oleic acid (34.06%) increased the MUFA content and a high amount of linoleic acid and DHA increased the PUFA content in winter. Variations in the fatty acid composition might be related to the changes in nutritional habits of the fish (Norrobin et al., 1990). Fatty acid composition of fish lipids is extremely variable, even within species, depending on different abiotic and biotic factors such as season, the type and amount of feed available, water temperature, pH, salinity and reproduction cycle (Bayir et al., 2010; Shirai et al., 2002). High levels of EPA and DHA were reported by other studies in trout species including *S. trutta macrostigma* (Aras et al., 2003; Akpinar et al., 2009).

Freshwater fish have to elongate and desaturate the short chain fatty acids synthesized by algae or plants, transforming them into long chain fatty acids of the ω3 family, EPA and DHA, and also converting feed of low to high nutritional value (Henderson and Tocher, 1987). The present data showed that DHA was the high level fatty

Table 1. Seasonal variations in total fatty acid composition of fillets of *O. mykiss* from Ivriz Dam Lake * (% of total FA).

Fatty acids	Spring	Summer	Autumn	Winter
C 6:0***	---------	---------	---------	0.01±0.01
C 8:0	---------	0.01±0.01	0.01±0.02	0.01±0.01
C 10:0	---------	0.01±0.01	0.01±0.02	0.01±0.01
C 11:0	---------	0.01±0.01	0.01±0.02	0.02±0.01
C 12:0	0.04±0.00**	0.05±0.00	0.04±0.01	0.03±0.01
C 13:0	0.02±0.00	0.02±0.01	0.01±0.00	0.01±0.01
C 14:0	3.37±0.02a	3.11±0.33b	2.66±0.09bc	2.28±0.22c
C 15:0	0.28±0.00	0.37±0.14	0.18±0.01	0.16±0.01
C 16:0	16.37±0.07	16.41±0.45	12.72±1.59	15.15±1.46
C 17:0	0.52±0.00a	0.65±0.19a	0.18±0.02b	0.23±0.07b
C 18:0	3.38±0.08	3.87±0.06	3.28±0.31	3.86±0.50
C 19:0	---------	0.01±0.01	---------	0.01±0.01
C 20:0	0.02±0.00	0.13±0.04	0.09±0.04	0.10±0.02
C 21:0	0.01±0.00b	0.05±0.01a	0.06±0.04a	0.02±0.01b
C 22:0	0.18±0.09b	0.25±0.06ab	0.23±0.08b	0.35±0.01a
C 24:0	---------	---------	---------	0.01±0.00
Σ SFA	**24.19**	**24.95**	**19.48**	**22.26**
C 14:1 ω5	0.08±0.00b	0.15±0.06a	0.05±0.01b	0.04±0.01b
C 15:1 ω5	0.14±0.01b	0.33±0.04ab	0.05±0.02b	0.51±0.31a
C 16:1 ω7	5.22±0.04a	5.24±0.95a	3.71±0.05b	3.43±0.51b
C 17:1 ω8	0.62±0.03a	0.74±0.09a	0.40±0.01b	0.30±0.10b
C 18:1 ω9	24.58±0.13bc	23.65±1.31c	34.06±1.97a	26.80±1.29b
C 20:1 ω9	2.07±0.23a	0.98±0.24b	0.99±0.17b	0.60±0.18c
C 22:1 ω9	---------	0.63±0.23a	0.02±0.01b	0.01±0.00b
C 24:1 ω9	---------	---------	---------	0.01±0.01
Σ MUFA	**32.71**	**31.72**	**39.28**	**31.70**
C 18:2 ω6	17.12±0.07bc	14.03±2.91c	23.56±0.87a	20.96±1.75ab
C 18:3 ω6	0.06±0.01a	0.03±0.02ab	0.01±0.02bc	0.01±0.01c
C 18:3 ω3	2.06±0.00	2.53±0.11	3.97±0.08	3.24±0.29
C 20:2 ω6	0.62±0.13	0.52±0.01	0.89±0.14	0.77±0.09
C 20:3 ω6	0.01±0.01	0.01±0.00	0.02±0.01	0.01±0.01
C 20:3 ω3	0.01±0.00	0.01±0.00	0.01±0.01	0.01±0.00
C 20:4 ω6	0.87±0.02	0.98±0.02	0.66±0.07	0.98±0.14
C 20:5 ω3	5.52±0.30a	5.17±0.06b	3.11±0.12d	3.88±0.08c
C 22:2 ω6	0.01±0.01	0.03±0.01	0.01±0.00	0.01±0.01
C 22:3 ω3	0.01±0.01	0.01±0.01	0.02±0.01	0.02±0.01
C 22:4 ω6	0.40±0.01	0.26±0.11	0.30±0.03	0.26±0.06
C 22:5 ω6	0.20±0.06	0.34±0.21	0.12±0.02	0.21±0.03
C 22:5 ω3	2.02±0.01	1.86±0.15	1.58±0.08	1.38±0.04
C 22:6 ω3	14.23±0.09b	17.57±0.59a	6.98±0.70c	14.30±1.85b
Σ PUFA	**43.14**	**43.35**	**41.24**	**46.04**
Σ ω3	23.85	27.15	15.67	22.83
Σ ω6	19.29	16.20	25.57	23.21
ω3/ω6	1.24	1.68	0.61	0.98

* Average of three lots analyzed.
**Values reported are means± S.D.
*** [abcd] Values for each sample with different superscript letters in the same fraction are significantly different at $p < 0.05$.

acid in muscle lipids of rainbow trout. In summer, a high ratio of DHA (17.57%) increased the in PUFA content. Sargent (1996) reported that ω3 PUFA, principally DHA, has a role in maintaining the structure and functional

integrity of fish cells. The percentages of EPA and DHA were between 3.11-5.52 and 6.98-17.57% in all seasons, respectively. There were significant differences between spring, summer, autumn and winter in terms of EPA and DHA ($p < 0.05$). The percentages of PUFA, such as EPA and DHA, in fish muscle are dependent on diet (Sargent, 1997). The amount of EPA and DHA suggested daily ingestion in the range 200 - 1000 mg (Inhamuns and Franco, 2008; Simopoulos, 1991).

According to Bayir et al. (2010), the highest values for Tls, NLs and PLs were found in winter. The highest ω3/ω6 ratios and EPA+DHA amounts were found in the winter and this coincided with the lowest gonado-somatic index. In this study, data show that the ω3/ω6 ratio was 1.24 in spring, 1.68 in summer, 0.61 in autumn and 0.98 in winter. An increase in the human dietary ω3/ ω6 fatty acid ratio is essential in the diet to help prevent coronary heart disease by reducing plasma lipids and to reduce cancer risk (Kinsella et al., 1990). Studies from Scandinavia, Netherlands and Japan showed that people who eat fish about twice a week (240 g total weekly intake) have lower risks of heart attacks than people who rarely eat fish (Wardlaw et al., 1992). According to Guler et al. (2007), ω3/ω6 fatty acids ratio was 1.49 in spring, 1.45 in autumn, 1.22 in winter and the lowest value 0.72 was in summer in *Sander lucioperca*. A high level of ω6 fatty acids lowered the ω3/ω6 ratio in summer in S. *Lucioperca*. According to Kalyoncu et al. (2009), ω3/ω6 ratio was 1.4 in spring, 1.5 in summer, 1.2 in autumn and 1.4 in winter for vimba from Turkey. Our study has revealed that *O. mykiss* is a freshwater fish species having a high nutritional value for human consumption due to its high ω3/ ω6.

Freshwater fish normally contain ω6 PUFAs, whereas marine fish are rich in ω3 fatty acids, especially DHA and EPA (Wang et al., 1990). In our study, PUFAs levels were found to be 41.24 and 46.04%. EPA, DHA and AA were found to have high ratios in pikeperch (Uysal and Aksoylar, 2005). Arachidonic acid is a precursor for prostaglandin and thromboxane which will influence blood clot formation and its attachment to the endothelial tissue during wound healing (Bowman and Rand, 1980). This fatty acid amount was almost low in the trout muscle lipids. C18:3 ω6 and C20:2 ω6 were found to be at low amounts in the ω6 PUFA fractions of tissues investigated. It was found that C22:4 ω6 (0.26-0.40%) was quite low.

This study has revealed that rainbow trout in the Ivriz Dam Lake of Turkey is a desirable item in the human diet when the levels of EPA, DHA and ω3/ω6 ratio are considered. The fish identified in this study was found to be good source of ω3 fatty acids.

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