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Comparison of Fatty Acid Profiles of Different Tissues of Mature Trout (*Salmo trutta labrax*, Pallas, 1811) Caught from Kazandere Creek in the Çoruh Region, Erzurum, Turkey

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Abstract: The fatty acid (FA) composition of some tissues of *Salmo trutta labrax*, Pallas, 1811 mature native freshwater wild salmon species caught from Kazandere creek in the Çoruh region in Turkey were investigated. Fatty acids profiles of muscle, liver, gonad, egg and adipose tissue were compared in terms of total and individual saturated and unsaturated fatty acids. The results showed that the highest total saturated fatty acid content was in the muscle ($37.20 \pm 1.13\%$) while the lowest value was in the egg ($27.12 \pm 2.53\%$), and the differences were significant ($P < 0.05$) among the tissues. Monounsaturated fatty acid contents (MUFA) were not significantly different ($P > 0.05$) for the tissues but n-3 polyunsaturated fatty acid (PUFA) contents were significantly ($P < 0.05$) higher in the egg ($48.09 \pm 9.37\%$) compared to in the other tissues. However, the lowest n-3 PUFA content was determined in the gonads ($23.88 \pm 5.41\%$) and statistically significant differences ($P < 0.05$) were observed among the tissues. Similar results were also determined for n-6 PUFA content in the eggs ($6.46 \pm 1.02\%$), and the n-3/n-6 ratio was significant for the tissues tested. The principal fatty acids of the tissues of the trout were 16:0, 18:1n-9c and 22:6n-3, and their ratios in the tissues were significantly different ($P < 0.01$). Finally, it is hypothesized that the high altitude of the catching environment might affect the fatty acid profile of the fish studied, and there is a great deal of potential to produce food fishes rich in n-3 PUFA content.

Key Words: Fish, *Salmo trutta labrax*, Fatty acids, n-PUFA

Çoruh Havzası Kazandere Çayı Olgun Dere Alabalıkları (*Salmo turutta labraks*, Pallas-1811)'nda Farklı Dokuların (Kas, Karaciğer, Gonad, Adipoz) Yağ Asidi Kompozisyonlarının Karşılaştırılması

Özet: Bu çalışmada Çoruh havzası Kazandere çayında yaşayan olgun yabani alabalıkların (*Salmo trutta labrax*, Pallas 1811) bazı dokularında bulunan yağ asitleri (FA) kompozisyonu araştırılmıştır. Karaciğer, gonad, yumurta ile kas ve adipoz dokuları total, doymuş ve doymamış yağ asitleri karşılaştırılmıştır. Toplam doymuş yağ asitleri (SFA), yüzdesi en fazla kasta (37.20 ± 1.13) ve en az yumurtalarında (27.12 ± 2.53) çıkmış ve dokular arası fark önemli ($P < 0.05$) bulunmuştur. Tekli doymamış yağ asitleri (MUFA) bakımından ise dokular arası fark önemsiz ($P < 0.05$) bulunurken, çoklu doymamış yağ asitlerinden n-3 PUFA miktarı yumurtada (48.09 ± 9.37)'lik miktarıyla önemli derecede ($P < 0.05$) diğer dokulardan farklı çıkmıştır. Halbuki en düşük n-3 PUFA miktarı gonadda (23.88 ± 5.41) tespit edilmiş ve dokular arası istatistikî olarak önemli ($P < 0.05$) bulunmuştur. Benzer sonuçlar yumurtanın n-6 PUFA (6.46 ± 1.02) ve n-3/n-6 oranında görülmüştür. Yağ asitleri genel kompozisyonunda ağırlıklı olarak 16:0, 18:1 n 9c, 22:6 n 3 (DHA) yağ asitleri şeklinde yoğunlaşmış olup, dokular arasındaki fark çok önemli ($P < 0.01$) bulunmuştur. Sonuç olarak yüksek rakımda yakalanan bu balıklarda yağ asidi profilleri etkilenmiş olabileceği ve n-3 PUFA bakımından zengin olan bu balığın besinsel olarak zengin bir potansiyele sahip olduğu belirlenmiştir.

Anahtar Sözcükler: Yağ asidi, n PUFA, *Salmo trutta labrax*

Introduction

It is well known that lipids play an important role in human and animal nutrition by supplying both energy and essential fatty acids (FAs) necessary to satisfy the physiological needs of the organism. For example, n-3 polyunsaturated fatty acids (PUFA) like eicosapentaenoic

acid (EPA) and docosahexaenoic acid (DHA) have been recommended for human health and fish fecundity; in particular, DHA has a therapeutic effect on human physiology (1,2). These fatty acids originate from marine phytoplankton commonly consumed by seafood since the FAs from terrestrial sources are mainly saturated or n-6

PUFAs due to their abundance in plant seed oils and animal fats (3-5).

It has been indicated that the composition of fish lipids is generally related to the species of fish, environmental temperature and the nutritional habits of the animal. The effects of water, season, activity, age and some other rearing conditions on the lipid composition and physicochemical properties of fish have been reported (6,7). For example, the degree of lipid unsaturation is usually due to the temperature of the water in which the fish lives, while the proportion of unsaturated FAs increases as the temperature decreases (8,9).

FAs of the n-3 series have been considered essential for normal growth, healthy condition and egg quality for fecundity in most fish species (10-13). For example, Koven et al. (14) reported that the growth properties of rotifer (*Sparus aurata*) larvae were improved by the inclusion of n-3 PUFA in their diets. A number of studies have been conducted to determine the fatty acid profiles of different tissues in many fish species. Investigations focused on the influence of FA composition on reproduction characteristics of fish addressing mainly egg and larval quality and their survival characteristics (15,16).

Since fish species having higher n-3 PUFA in their tissues have received considerable attention worldwide and in Turkey from the standpoint of both management and cultural perspectives, efforts are being expended to investigate them for food fish production (17-19). Hence, in general, to achieve success in fish culture one must delineate the dietary requirements, egg quality, and the effects of environment conditions on FA composition in different tissues. In the present study, the fatty acid compositions of different tissues of a wild trout (*Salmo trutta labrax*) were examined in order to document its general properties for future consultations. Another objective was to document some characteristics of a native wild fish species which may be extinct in the future due to detrimental alterations in the environmental conditions taking place in the region.

Currently, there has been very limited research on this fish species living in a high altitude region, north - east Anatolia. In addition, no report was found in the literature concerning the fatty acid composition of this fish species. Therefore, FA profiles of different tissues of the fish were compared, and n-PUFA contents were

demonstrated for food fish quality, considering it a potential healthy food source.

Materials and Methods

Fish Material

Specimens of the trout *Salmo trutta labrax*, Pallas, 1811, are found only in north - east Anatolia and they live in small creeks, lakes and river basins in the Black Sea region (20). The oldest record for this fish indicates that the origin of this fish is from the Crimean region of the Black Sea, and Slastenenko (21) reported three different ecotypes of this fish that were first described in our region by Aras (22) in 1974. This trout completes all its life stages in a river, and the red spots on their skin never disappear. Their diagnostic properties are as follows: D: III-IV 9-11, A: III-IV 8-9, L. Lateral: 112-125, 58-60 vertebrae, and the number of pyloric caeca is 47-48 (20,22).

Salmo trutta labrax samples were collected from a high altitude, approximately 2000 m, in the Çoruh region, Erzurum, Turkey, during June 2000. The water during the catching time was 14 °C with a 20 °C atmosphere temperature, and it had 7 ppm O₂ with a 7.9 pH. The eggs analyzed in this research were removed from fish that had some unabsorbed oocytes remaining from the last spawning season.

Lipids and the analysis of FAMES

The preparation and analysis of fatty acid methyl esters (FAMES) from these fish tissues were performed according to the method described in the literature (23,24). A piece of tissue was added to 1 ml 1.2 M NaOH in 50% aqueous methanol with five glass beads (3 mm diam) in a screw-cap tube, and then incubated at 100 °C for 30 min in a water bath. The saponified samples were cooled at room temperature for 25 min, they were acidified and methylated by adding 2 ml 54% 6 N HCL in 46% aqueous methanol and incubated at 80 °C for 10 min in a water bath. After rapid cooling, methylated FAs were extracted with 1.25 ml 50% methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 ml 0.3 M NaOH. After mixing for 5 min, the top phase was removed for analysis. Following the base wash step, the FAMES were cleaned in anhydrous sodium sulfate and then transferred into a GC sample vial for analysis.

FAMES were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) with a fused-silica capillary column (25 mm by 0.2 mm) with cross-linked 5% phenylmethyl silicone. The operating parameters for the study were set and controlled automatically by a computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance was achieved through the use of a calibration standard FA mix (Eucary Method 697110) containing nC9-nC30 saturated fatty acids (SFAs). FAs were identified on the basis of equivalent chain length data. FAME profiles of the tissues were identified by comparing the commercial Eucary database with the MIS

software package (MIS ver. no 3.8, Microbial ID, Inc., Newark, Delaware).

Statistical analysis

The data were subjected to ANOVA and the significant means were compared by Tukey's multiple range tests and SAS (25), and the data are presented as mean \pm SEM in the Table.

Results

The FA composition and the ratios of FAs in the muscle, egg, gonad, adipose and liver of *Salmo trutta labrax* are shown in the Table. Also indicated are the mean values of total saturated and unsaturated FA percentages with Tukey results revealing the significant

Table. The results of fatty acid composition of different tissues of *Salmo trutta labrax*, and their significance.

Fatty acids	Muscle	Egg	Gonad	Adipose	Liver	P
	X \pm SX (n = 5)	X \pm SX (n = 2)	X \pm SX (n = 3)	X \pm SX (n = 5)	X \pm SX (n = 5)	
12:0	1.09 \pm 1.27	0.45 \pm 1.80	1.09 \pm 1.04	3.71 \pm 0.80	-	NS
14:0	2.99 \pm 0.43b	3.68 \pm 0.96b	3.72 \pm 0.56b	6.44 \pm 0.43a	2.36 \pm 0.43b	**
14:1n-9	-	0.15 \pm 0.19	-	0.63 \pm 0.08	-	NS
16:0	25.39 \pm 1.19a	17.86 \pm 2.66b	19.38 \pm 1.15ab	18.72 \pm 1.19ab	21.09 \pm 1.19ab	*
16:1n-7	5.63 \pm 0.75b	11.33 \pm 1.69a	10.04 \pm 0.09ab	12.93 \pm 0.75a	5.86 \pm 0.75b	**
16:2n-6	-	0.36 \pm 0.21	0.60 \pm 0.12	0.63 \pm 0.09	-	NS
16:1n-6	0.86 \pm 0.20	0.83 \pm 0.29	0.64 \pm 0.29	1.38 \pm 0.13	-	NS
16:1n-5	-	0.37 \pm 0.04	0.29 \pm 0.04	0.30 \pm 0.01	-	NS
18:0	5.91 \pm 0.47a	1.35 \pm 1.06b	2.15 \pm 0.61b	2.70 \pm 0.47b	6.99 \pm 0.47a	**
18:2n-6	2.69 \pm 0.22ab	3.62 \pm 0.49a	3.46 \pm 0.28a	3.78 \pm 0.21a	1.68 \pm 0.21b	**
18:4n-3	3.53 \pm 0.49bc	5.46 \pm 1.10ab	4.59 \pm 0.63abc	7.43 \pm 0.49a	2.02 \pm 0.49c	**
18:3n-3	-	25.82 \pm 5.59	-	30.33 \pm 3.95	-	NS
18:1n-9	20.63 \pm 1.78a	0.52 \pm 3.99b	26.03 \pm 2.31a	29.91 \pm 2.30a	20.46 \pm 1.79a	**
20:0	2.61 \pm 0.97a	3.78 \pm 0.21b	2.82 \pm 0.16a	2.49 \pm 0.09a	2.81 \pm 0.10b	**
20:3n-3	1.08 \pm 0.46ab	-	0.85 \pm 0.26ab	0.74 \pm 0.20b	2.03 \pm 0.26a	*
20:1n-9	-	0.19 \pm 0.25b	0.30 \pm 0.25b	0.56 \pm 0.14b	1.74 \pm 0.12a	**
20:4n-6	1.61 \pm 0.07c	2.48 \pm 0.14b	2.18 \pm 0.143bc	0.52 \pm 0.08d	3.30 \pm 0.14a	**
22:6n-3	21.42 \pm 1.61a	14.88 \pm 3.60a	15.55 \pm 2.08a	2.22 \pm 1.61b	18.04 \pm 1.80a	**
22:5n-3	3.67 \pm 0.26a	2.74 \pm 0.59ba	2.88 \pm 0.34ba	1.32 \pm 0.26b	2.80 \pm 0.29ba	**
22:1n-6	0.76 \pm 0.09	0.30 \pm 0.09	0.67 \pm 0.05	-	-	NS
SFA	37.21 \pm 1.13a	27.12 \pm 2.53c	29.13 \pm 1.46bc	34.29 \pm 1.13ab	32.14 \pm 1.13ab	*
MUFA	26.76 \pm 4.34ab	13.73 \pm 9.71b	37.16 \pm 5.60a	34.00 \pm 4.34ab	27.73 \pm 4.34ab	*
n3 PUFA	28.85 \pm 4.19ab	48.09 \pm 9.37a	23.88 \pm 5.41b	24.92 \pm 4.19ab	26.57 \pm 4.68ab	*
n6 PUFA	3.98 \pm 0.45ab	6.46 \pm 1.02a	4.79 \pm 0.59ab	4.83 \pm 0.45ab	3.07 \pm 0.45b	*
n3/n-6	6.27 \pm 0.99	7.56 \pm 1.98	5.23 \pm 1.14	4.84 \pm 0.88	7.76 \pm 0.99	*

- Not detected, (a-b) Means in a row with identical letters are not significantly different, X = mean, SX = standard error, NS = P > 0.05, ** (P < 0.01), * (P < 0.05).

differences. There was a significant amount of palmitic acid (16:0) in all of the tissues compared with other saturated FA like lauric (12:0), miristic (14:0), stearic (18:0) and arachidic acid (20:0). The differences between the tissues were significant ($P < 0.05$), and the highest palmitic acid was in the muscle (25.39%) and the lowest in the egg (17.86%). Similarly, the total SFA contents were significantly different, and the highest and lowest values for total SFA were in the muscle (37.20%) and eggs (27.12%), respectively.

A significantly higher monounsaturated fatty acid (MUFA) content was oleic acid (18:1 n9c) in all of the tissues except eggs. Total MUFA ratio was highest in the gonads and lowest in the eggs while the latter had the highest (48.09%) n-3 polyunsaturated fatty acid (PUFA) content, and the other had the lowest (23.88%) PUFA value. In addition, total n-3 PUFA content was 4.8 to 7.7 - fold higher than that of the n-6 PUFA content in the tissues of the trout.

Discussion

It has been well documented that environmental temperature, as well as dietary precursors, plays a significant role in the FA composition of aquatic animals (26-28). Although the role of n-3 PUFA levels in cold climates is not clear, most researchers believe that these FAs play a special role in membrane fluidity and the temperature adaptation of the fish in cold environments (9,29-31). The results of the present study revealed that the most abundant individual FAs were palmitic, oleic and docosahexaenoic acids (DHA) in most of the tissues. This result was confirmed by several other studies for some tissues of different fish (32,33). Of the PUFAs of the trout, DHA was the major n-3 FA in the tissues despite the relatively lower percentage of the other n-PUFAs. This result was also in agreement with the findings of Sargent et al. (34) and Tanako et al. (35) in these type of fish. Additionally, DHA content was significantly ($P < 0.05$) higher in muscle tissue. This is considered a preferable property in food fishes used for human nutrition (4,36).

In general, SFA was higher in the muscle tissue while the MUFAs were dominant in the gonads. This result might be a special characteristics of *Salmo trutta labrax* because Haliloğlu (19) determined an inverse relationship between the SFA and MUFA in cultured rainbow trouts

having 57.97, 31.54, 37.53 and 48.22% MUFA in adipose, gonad, liver and muscle tissues, respectively. Another notable result was the level of 18:1n-9 FA, approximately 60% of total monoenes in all tissues except eggs. This is also true for most marine species, fish, molluscs and crustaceans (18,37,38).

There was also a significant difference between the PUFA profiles of the tissues. For example, total n-3 PUFAs were 4.8 to 7.7 - fold higher than that of the n-6 PUFAs, and the eggs had the highest n-3 PUFA (48.09 ± 9.38) content while the gonads had the lowest (23.88 ± 5.42) (Table). The difference between the eggs and gonads could be expected, because it is noted that the n-3/n-6 PUFA ratio is usually 1-4 in freshwater species and 5-14 in marine species, Okumuş (18), Steffens, (38) and the present results seem to be closer to those of marine species. However, Saito et al. (2) stated that the n-3/n-6 ratio should be higher in subtropical fish oocytes, especially in wild species. Despite the high DHA (22:6 n-3) content in the tissues, EPA (20:5 n-3) was determined only in the adipose and liver tissue of two samples, and therefore it was not tabulated or discussed. This result might be due to the n-3 elongation and the efficient desaturation system in salmonids, and much of the EPA might be converted to DHA (9). A significant difference was also detected in the DHA content, which was about 1/9.5 - fold between the muscle and adipose tissue, and there is no explanation for this result at present. It might be postulated that localization of this FA is in membranes of the muscle tissue.

Most of the n-3 PUFAs are essential for marine species, but these FAs were plentiful in our samples, *Salmo trutta labrax*. This result may reflect a typical FA profile of a freshwater fish (39), since this fish lives in a high altitude area (about 2000 m), and total lipid and FA profiles may vary according to combined temperature, age and nutrition (6,40,41). In conclusion, the beneficial effects of a diet rich with n-3 PUFA for humans, imply the regular consumption of fish and encourage aquaculturists to find alternatives to increase fish production. The present fish species, *Salmo trutta labrax*, with its high n-3 PUFA content verified in this research, could be a potential healthy food fish for this region in terms of the positive effects of DHA in the diet. In addition, the FA characteristics of this fish indicate a great potential for culturing this wild fish species for future nutrition research and marketing.

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