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Research Article

Comparison of Muscle Fatty Acids of Three Trout Species (*Salvelinus alpinus, Salmo trutta fario, Oncorhynchus mykiss*) Raised under the Same Conditions

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Abstract: During recent decades, the study of fatty acids (FAs) belonging to the n-3 family in seafood has intensified due to their beneficial effect on cardiovascular disease. This study was undertaken to determine the FA composition of muscle lipids of three different farm raised trouts (*Salvelinus alpinus*, *Salmo trutta fario* and *Oncorhynchus mykiss*) fed the same diet and raised under the same conditions. Palmitic acid (16:0) in total saturated fatty acid (SFA) and oleic acid (18:1 n-9) in monounsaturated fatty acid (MUFA) were the most abundant FAs and significant differences ($P < 0.05$) were observed between fish species. The differences in total SFA among the trouts were significant ($P < 0.01$), and *O. mykiss* had the highest (31.92%) SFA content while *S. trutta fario* showed the lowest in their edible muscle tissue. However, there was a significantly ($P < 0.01$) opposite observation for MUFA among the trouts, in which *S. trutta fario* had the highest content (41.90%) of MUFA in their muscle tissue while *O. mykiss* had the lowest (30.81%). The differences for the polyunsaturated fatty acid (PUFA) content among the species were not significant ($P > 0.05$). *O. mykiss* had the highest n-3 PUFA (22.41%) while *S. trutta fario* showed the greatest n-6 PUFA concentration. Eicosapentaenoic acid (EPA, 20:5 n-3) concentrations were 3.07, 3.03 and 1.78% in *O. mykiss*, *S. alpinus* and *S. trutta fario,* respectively. In addition, *O. mykiss* had a higher content (19.17%) of docosahexaenoic acid (DHA, 22:6 n-3) than *S. alpinus* (15.45%) and *S. trutta fario* (12.74%), and the difference of this FA among the trouts was significant ($P < 0.01$). The ratios of n-3/n-6 PUFA content indicating the availability of n-3 PUFA that are beneficial for human health were 1.58, 1.27 and 0.95 for *O. mykiss*, *S. alpinus* and *S. trutta fario,* respectively. In conclusion, the results of this research for FA analysis suggest the superiority of rainbow trout (*O. mykiss*) for human nutrition in terms of n-3 PUFA contents and the effects on health.

Key Words: Fatty Acid Profile, Food, Trout

Aynı Şartlar Altında Yetiştirilen Farklı Üç Sofralık Alabalık Türünün (*Salvelinus alpinus*, *Salmo trutta fario*, *Oncorhynchus mykiss*) Kas Dokusu Yağ Asidi Profillerinin Karşılaştırılması

Özet: Son yıllarda yağ asitleriyle (FA) ilgili çalışmalar deniz ürünlerindeki n-3 serisinin kalp hastalıklarına karşı olumlu etkileri üzerine yoğunlaşmıştır. Bu çalışma aynı yemi kullanarak yetiştirilen 3 farklı alabalık türünün (*Salvelinus alpinus*, *Salmo trutta fario*, *Oncorhynchus mykiss*) kas dokularındaki yağ asidi kompozisyonlarını belirlemek amacıyla yapılmıştır. Toplam doymuş yağ asitleri (SFA) içerisinde palmitic asit (16:0), tekli doymamış yağ asitleri içerisinde oleik asit (18:1 n-9) en çok bulunan yağ asitleri olup türler arasında önemli farklılıklar ($P < 0.05$) çıkmıştır. Doymuş yağ asitleri bakımından türler arasında önemli farklar ($P < 0.01$) olurken *O. mykiss* en yüksek değere (%31.92), *S. trutta fario* ise en düşük değere sahip olmuştur. Bunun yanı sıra tekli doymamış yağ asitleri (MUFA) bakımından da türler arasında önemli ($P < 0.01$) farklılıklar tespit edilmiştir. Kas dokularında MUFA miktarı en yüksek *S. trutta fario* (%41.90), *O. mykiss* ise (%30.81)'lik değeriyle en düşük değere sahip olmuştur. Türler arasında çoklu doymamış yağ asitleri (PUFA) bakımından önemli farklılıklar ($P > 0.05$) bulunamamıştır. n-3 PUFA bakımından en yüksek *O. mykiss* (%22.41) olurken, n-6 PUFA bakımından ise *S. trutta fario* olmuştur. Eicosapentaenoik asit (20:5 n-3) miktarı sırasıyla *O. mykiss* (%3.07), *S. alpinus* (%3.03) ve *S. trutta fario* (%1.78) çıkmıştır. *O. mykiss* aynı zamanda en yüksek dokosahexaenoik asit (22:6 n-3) miktarına sahip olmuştur (%19.17), *S. alpinus* (%15.48) ve *S. trutta fario* (%12.74) lük değerlerle önemli farklılıklar ($P < 0.01$) göstermişlerdir. Yine n-3/n-6 oranı bakımından ve insan sağlığı için önemli olan n-3 PUFA nın bir kriteri olarak kullanılan değerler sırasıyla balık türlerinde 1.58, 1.27, 0.95 (*O. mykiss*, *S. alpinus* and *S. trutta fario*). Sonuç olarak FA analizleri gökkuşağı (*O. mykiss*)'in n-3 PUFA miktarı bakımından daha zengin olduğu sonucunu desteklemektedir.

Anahtar Sözcükler: Yağ asidi profili, Yem, Alabalık

Introduction

During recent decades, research on n-3 highly unsaturated fatty acid (n-3 HUFA) in marine foods has intensified due to their beneficial effects on human health. They have been shown to have curative and preventive effects on cardiovascular diseases, mortality and neuro-development in infants (1-4). Although it is generally recognized that HUFA composition may vary among fish species, little attention has been paid to the HUFA profile of different species when selecting fish for diets (5-7).

It is well known that due to differences in the intrinsic biochemical peculiarities among fish species, the lipid metabolism and the composition of FAs may also vary from species to species (8). The composition of fish lipids is generally related to the species of fish, environmental temperature, season, physical and chemical properties of the water, geographic location, age, sex, rearing conditions, physical activity and nutritional habits of the animal (9-12).

As observed in a number of animals such as poultry, equine and fish, the FA profile in muscle can generally be altered by the diet (13-15). Halver (16) noted that the natural diet of fresh and seawater species, which includes a preponderance of marine zooplankton and phytoplanktons, determines the composition of FA of the fish lipids. Demir (17) reported higher n-3 HUFA levels in the muscle and liver of *O. mykiss* when compared to feed with a different FA composition. In research conducted with different diets (two commercial, three capelin roe and two herring roe), the FA profile of salmon (*Salmo salar*) was altered over one month, and the following FAs 14:1 n-5, 16:0, 20:1 n-9, 20:2 n-6 and 22:1 n-11 increased while 18:1 n-9, 18:2 n-3, 20:3 n-6, 20:5 n-3 and 22:6 n-3 decreased (18). Kennish et al. (19) showed that chinook salmon fed with ringa resulted in a lower total lipid level and the n-3/n-6 ratio was 28% higher than that of the control group. From these types of research, it might be concluded that fish farmers are able to alter fish lipid composition a month before marketing. Additionally, it has been reported that salmon parr and trout are capable of elongating and desaturating C:18 PUFA to C:20 and C:22 PUFA, and also the conversion is reduced by feeding the end-products C:20 and C:22 PUFA, i.e., feeding fish oil. Therefore, the farmed salmon fed with fish oil might benefit from increased dietary inputs of 18:3 n-3 and 18:2 n-6 (20).

As mentioned above, the rearing conditions and composition of the diets have a marked influence on the growing conditions of fish and development of the brain, muscle, gonad and liver as well as the chemistry of these tissues (21-23). In order to capitalize on the fate of nutritional and physiological inputs before producing cultured fish, it is important to determine FA composition and n-3 HUFA deposition in fish muscle. Therefore, the objectives of this research were to reveal and compare FA profiles of muscle lipids of three different food salmon breeds (*Salvelinus alpinus*, *Salmo trutta fario* and *Oncorhynchus mykiss*) fed the same commercial diet and reared under the same conditions, and additionally, to give an insight to farmers and consumers when selecting fish either to raise or to consume.

Materials and Methods

Fish Materials

Three different trout species, rainbow trout (*Oncorhynchus mykiss*), arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta fario*), were raised in the same pond in Atatürk University, fishery research center, Erzurum, Turkey. These fish were fed the same commercial feed containing 40% crude protein, 18% crude fat and 3450 kcal energy with a known FA profile (Table). The fish samples were from one-year-old fish randomly removed from the water when they reached 150-200g body weight, and muscles from the dorsal area were subjected to FA analysis.

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 previously (24,25). A piece of tissue was added to 1 ml 1.2 M NaOH in a 50% aqueous methanol solution with 5 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. Then 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

Table. Changes in muscle lipid FA composition of three trout breeds raised in the same conditions.

Fatty acid	Diet	*S. alpinus* X ± SX (n = 5)	*S. trutta fario* X ± SX (n = 5)	*O. mykiss* X ± SX (n = 5)	*F*
14:0	8.51	2.19 ± 0.21	2.20 ± 0.18	2.38 ± 0.18	NS
15:0	0.96	-	-	-	-
16:1 n9	0.57	0.45 ± 0.03	0.43 ± 0.02	-	NS
16:1 n7	8.22	4.32 ± 0.38	4.05 ± 0.33	4.16 ± 0.33	NS
16:0	21.67	20.99 ± 0.76 ab	18.27 ± 0.68 b	22.73 ± 0.68 a	**
17:1n8	0.37	-	-	-	-
17:0	0.54	-	-	-	-
18:3 n6	0.29	0.50 ± 0.32	0.40 ± 0.03	-	NS
18:4 n3	1.62	0.65 ± 0.01 a	0.49 ± 0.01 b	-	*
18:2 n6	12.33	11.49 ± 0.82	12.84 ± 0.73	11.22 ± 0.74	NS
18:1 n9	18.22	28.87 ± 2.14 ab	32.91 ± 1.91 a	24.06 ± 1.91 b	*
18:0	3.72	3.67 ± 0.46 b	4.38 ± 0.41 b	6.79 ± 0.41 a	**
20:4 n6	0.55	1.91 ± 0.23	1.44 ± 0.20	2.16 ± 0.23	NS
20:5 n3	4.48	3.03 ± 0.32 a	1.78 ± 0.28 b	3.07 ± 0.28 a	**
20:3 n6	0.15	1.11 ± 0.77	0.93 ± 0.06	1.18 ± 0.08	NS
20:2 n6	-	0.72 ± 0.06 b	1.18 ± 0.05 a	1.03 ± 0.06 a	**
20:1 n9	0.67	0.99 ± 0.13 b	1.74 ± 0.11 a	1.07 ± 0.15 b	**
22:6 n3	6.74	15.45 ± 1.84	12.74 ± 1.65	19.17 ± 1.65	**
22:5 n3	0.51	0.99 ± 0.09	0.79 ± 0.08	0.87 ± 0.18	NS
18:1 n9t	2.01	2.44 ± 0.09	2.79 ± 0.08	2.43 ± 0.09	NS
22:1 n6	-	0.67 ± 0.04	0.65 ± 0.06	1.02 ± 0.06	NS
ÂSFA	35.4	26.86 ± 1.04 b	25.39 ± 0.93 b	31.92 ± 0.93 a	**
ÂMUFA	32.54	38.37 ± 3.61 ab	41.90 ± 2.29 a	30.81 ± 2.28 b	*
Ân3 PUFA	13.35	19.81 ± 2.12	15.48 ± 1.89	22.41 ± 1.89	NS
Ân6 PUFA	13.32	15.82 ± 0.92	16.57 ± 0.82	14.47 ± 0.82	NS
n3/n6	0.57	1.27 ± 0.22	0.95 ± 0.19	1.58 ± 0.19	NS

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

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 this 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 in SAS (26), and are presented as mean ± SEM in the Table.

Results

The FA analysis showed that there were considerable differences among the fish studied in terms of the FA composition of the muscle fats although they were fed the same diet. For example, the differences in palmitic acid (16:0), stearic acid (18:0), eicasanoic acid (20:1 n-9), eicasadienoic acid (20:2 n-6) and docosahexanoic acid

(DHA 22:6 n-3) were ($P < 0.01$) significant among the trout breeds (Table). The total SFA composition was also significantly ($P < 0.01$) different among the trout breeds probably due to the differences in the amounts of palmitic and stearic acids. The highest total SFA was in rainbow trout muscle (31.92%), compared to the arctic carr (26.86%) and the brown trout muscle (25.39%).

In addition, 78 and 71% of total SFA in muscle fat were composed of palmitic acid in the arctic carr and brown trout, respectively. There were also significant differences in monounsaturated fatty acid (MUFA) among the fish species investigated (Table). In contrast to SFA content, rainbow trout had the lowest level of MUFA in their muscle fat (30.81%) while brown trout muscle contained the highest level (41.90%), and arctic carr had 38.37% . Seventy-five percent of the total MUFA content was oleic acid for rainbow and brown trout while it was 78% for arctic carr muscle.

The long chain n-3-n-6 fatty acid commonly called PUFA and their ratios are also (n-3/n-6) considered important for the FA composition, but their differences in the muscle of the investigated fish were not significant ($P > 0.05$). However, despite their insignificance, the percentage of n-3 PUFA tended to be higher in rainbow trout (22.41%) than in brown trout (15.48%) and arctic carr (19.81%), while n-6 PUFA content was lowest in rainbow trout (14.47%). The (n-3/n-6) ratios were 1.58, 1.27 and 0.95 for rainbow trout, arctic carr and brown trout, respectively.

Discussion

Genetic selection in fish breeding may allow a desired FA composition, thus enhancing the position of the fish in the market place. In the case of trout farming, selection of the trout type for desirable traits can only be effective if the breeds differ. To be able to make unbiased comparisons, it is essential to treat the fish breeds similarly in terms of nutritional regimen and environmental or rearing conditions. Unfortunately, most marine food FA composition data in the literature originate from species on diverse diets (16,27) and of varying ages, and involved various tissues (28). This makes it difficult to extrapolate the results and compare breeds or species. In the present study, all the trouts were pure breeds, fed the same diet, reared in identical water conditions and slaughtered at the almost same live weight. As previously mentioned, the notable breed differences in the FA composition of muscle lipid were in the percentage of total SFA found in the trout's muscle. For example, rainbow trout had more total SFA (31.92%) than arctic carr (26.86%) and brown trout (25.39%) in their muscle lipid fraction compared with 35.4% in their diet (Table). Regardless of breed, it is clear that FA composition differs in SFAs and especially in the contents of palmitic (16:0) and stearic acids (18:0). Skuladottir et al. (29) reported similar results for total SFA, and that a large portion of muscle SFA was palmitic acid in Atlantic salmon. This result might be related to the composition of the diet, and identical findings were also reported for some other fish species. For example, the proportion of this FA was about 60% in the diet used in some studies (30,31).

It has been claimed that total body composition reflects the diet or nutrition regimen of the fish (32-34) and the present results partially confirm this thesis in terms of FA profiles of both the diet and muscle lipids for the three trout breeds. For example, arctic charr and brown trout had approximately 8% lower total SFA and 5.8 and 9.3% higher MUFA contents than that of the diet. Nevertheless, rainbow trout had an FA profile more comparable with the diet (Table). This result might be due to the commercial diet manufactured for rainbow trout, and the differences in FA profiles among the species owing to genetic variation is considered to be natural (9,10,16).

The values for the monoene level (18:1 n9) of MUFA in the fish investigated were significantly higher than those of the diet, and similar findings in arctic carr and brown trout were also reported for wild species of brown trout (35). Excluding the n-6 PUFAs that are about the same, generally the n-3 PUFA contents (average 19.2%) of the fish were much higher than those of the diet (8.87%). Since the cultured and wild species of salmonids have efficient n-3 elongation and desaturation activity, this result could be anticipated in the fish investigated (29). This study has shown that cultured trout breeds were generally rich in n-3 PUFAs, which is in line with the findings of Osman et al. (7), despite some contradictory reports in which the levels of n-3 PUFAs were lower than those of n-6 PUFAs in freshwater and cultured fish (35,36). Fish generally need PUFAs to tolerate low water temperatures; therefore, higher PUFA concentrations are expected in fish that live in cold environments (37-39).

EPA and DHA, found only in fish and other seafoods, possess profound beneficial properties for human health (4). In this research, significantly ($P < 0.01$) higher EPA and DHA content were determined in the trouts, especially in rainbow trout and arctic charr when compared to brown trout, and the diet. The amounts of EPA in the trouts studied (*O. mykiss*: 3.07, *S. alpinus*: 3.03 and *S. trutta fario*: 1.78%) are lower than those in farmed rainbow trout in Norway (40), which has a colder water temperature than that in the present research.

A substantial increase in the (n-3)/(n-6) PUFA ratio occurred in the muscle lipids of the breeds studied when compared to the ratio in the diet (0.57). It was doubled in arctic charr (1.27) and brown trout (0.95) and was even higher in rainbow trout (1.58). Bell et al. (41) noted that an increase in the ratio of n-3/n-6 PUFA increases the availability of n-3 PUFAs, which are beneficial for human health. The level of this ratio (0.95-1.58) seems to be much lower than the 5 to 14 values found in their wild counterparts living in marine environments (7,42). In addition, Osman et al. (7) reported that the n-3/n-6 ratio is used as an index to compare relative nutritional value of fish oils from different species.

The variation in the FA composition of the three breeds of food trouts may be explained by the unique features in their genetical inherence. The rainbow trout identified in this research was found to be a good source of n-3 PUFAs and this might enhance its market share in the selection of cultured food fishes. Epidemiological data have suggested that an adequate DHA supply is essential for normal eye and brain development in infants while EPA reportedly decreases cardiovascular risk factors with their biological activity in the elderly. Therefore, PUFA distribution must be considered when fish are suggested as a means of improving health and/or marketing. In conclusion, in the light of the FA composition in the current research, the rainbow trout (*O. mykiss*) is recommended.

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