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Influence of Dietary Fatty Acids on the Liver Fatty Acid Profile of *Oncorhynchus mykiss* (Walbaum, 1792)

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Abstract: A 28 days feeding experiment was conducted to investigate the effects of dietary fatty acids on the liver fatty acid profile of immature *Oncorhynchus mykiss* (Walbaum, 1792). The two groups of fish were fed with two different diets, diet 1 (commercial diet) [MUFA low, n-3 polyunsaturated fatty acids (n-3 PUFA) high] and diet 2 (MUFA high, n-3 PUFA low). After 28 days, fatty acid profiles of liver total lipid were quite similar. However, it was found that C 20:1, C 20:4 n-6, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3 and total n-3 PUFA decreased while C 14:0, C 16:0, C 18:0, C 18:1n-9, C 18:2 n-6 and C 18:3 n-3 increased in the liver of the fish fed diet 2 compared with fish fed diet 1. These results show that dietary fatty acids are reflected to fish liver fatty acids in *O. mykiss.*

Key words: *Oncorhynchus mykiss*, liver, fatty acids, feeding.

Oncorhynchus mykiss (Walbaum, 1792)' nin Karaciğer Yağ Asidi Profili Üzerine Besinsel Yağ Asitlerinin Etkisi

Özet: Ergin olmayan *Oncorhynchus mykiss* (Walbaum, 1792)' nin karaciğer yağ asidi profili üzerine besinsel yağ asitlerinin etkisini değerlendirmek için 28 günlük beslenme deneyi yapılmıştır. Iki balık grubu, besin 1 (ticari besin) (tekli doymamış yağ asidi (MUFA) düşük, n-3 aşırı doymamış yağ asidleri (n-3 PUFA) yüksek) ve

besin 2 (MUFA yüksek, n-3 PUFA düşük) olmak üzere iki farklı besin ile beslenmiştir. 28 gün sonunda, karaciğer toplam lipidi yağ asidi profilleri oldukça benzerdi. Bununla birlikte, besin 1 ile karşılaştırıldığında, besin 2 ile beslenen balıkların karaciğerlerinde C 14:0, C 16:0, C 18:0, C 18:1n-9, C 18:2 n-6 ve C 18:3 n-3 artarken, C 20:1, C 20:4 n-6, C 20:5 n-3, C 22:5 n-3, C 22:6 n-3 ve toplam n-3 PUFA'nın düştüğü bulunmuştur. Bu sonuçlar besinsel yağ asitlerinin *O. mykiss* karaciğer yağ asitlerine yansıtıldığını göstermektedir.
Anahtar Sözcükler: *Oncorhynchus mykiss*, karaciğer, yağ asitleri, beslenme.

Introduction

Dietary lipids play important roles in fish nutrition, both because of their role as energy providing molecules and as the source of essential fatty acids (EFA) [1, 2]. Recent studies on the composition and significance of fatty acids in fish species have focused on C18 and C20 PUFAs [3, 4, 5, 6]. Pickova et al. [7] and Gelienau et al. [8] showed that long-chain PUFAs are regular components of the examined fish tissues and, in the light of their known nutritional prostaglandinogenic and presumed structural roles, are thus of considerable biological significance. Fish fatty acids, specially n-3 series fatty acids are also known to confer cardiac-health properties to human subjects and increased fish consumption has been recommended [9, 10].

The fatty acid compositions of fish tissue lipids is the momentary net result of complex interrelationship of a number of factors, the details of which are not fully understood. The major factors are dietary fatty acid intakes, rates of oxidative catabolism of the fatty acids, kinetics of desaturation and elongation reactions, competative incorporation and retroconversions among fatty acids [11, 12].

Salmo salar (L.) and *O. mykiss* can elongate and desaturate C18 PUFAs to C20 and C22 PUFAs [3, 13]. Sargent et al. [14] determined that in marine fish the EFA requirement can only be met by C20 or C22 PUFAs. The EFA requirement in rainbow trout can be supplied by n-3 PUFA alone at lower levels than C18:3n-3. These findings indicate that PUFAs (C20:5n-3, C22:6n-3 and C20:4n-6) have essential roles in fish and that the role of C18 EFAs is solely as the metabolic precursors of PUFA as a part of larger survey concerning the effects of dietary lipids on the biochemical properties of tissues in fish.

The effects of dietary fatty acid level on body fatty acid composition have been studied in fish [15, 16]. *O. mykiss* is the most important cultivated fish species in Turkish aquaculture, therefore the aim of the present study was to compare the fatty acid composition of liver in immature fish fed with two different diets.

Materials and methods

Fish and feeding

Immature rainbow trout (85.25 ± 3.83g) were obtained from a commercial fish farm in Sivas, Turkey. Fish were selected and randomly divided into three groups of 15 fish. Each group was stocked in three tanks (60x80x190 cm) supplied with freshwater (temperature 11.30 ± 0.11 ^{0}C). The oxygen content was maintained stable at around 8.20 ± 0.12 mg l^{-1} and pH was 8.5 ± 0.2. The fish groups were fed by hand twice daily at 09:00 and 17:00 h for 28 days, between September and November 2005. In daily feeding fish, the amount of food (g) to be given was calculated as 2% of average liver weight per fish. Liver from three fish from each tank were sampled to determine fatty acid composition.Table 1 shows the ingradients composition of the experimental two diets. Diet 1 was a commercial pelleted feed for trout and diet 2 was formulated by us. Diet 2 was made into dry pellets using a laboratory pelleting machine. The pellet diameter was 4 mm.

Chemicals

All organic chemicals were obtained from Merck (Darmstadt, Germany), fatty acid standards and other chemicals were bought from Sigma (Deisenhofen, Germany). The capillary column was obtained from Supel co (Bellefonte, USA).

Liver extraction and fatty acid analysis

A sample of liver from each fish was taken and approximately 1 g of each liver sample was homogenised in chloroform:methanol (2/1, v/v) using Ultra-Turrax T25 homogeniser. Autooxidation of PUFAs was minimised by adding 50 µl of butylated hydroxytoluene (2%, w/v in chloroform) to the extraction mixture. The lipids of liver were extracted and purified according to the procedure described by Folch et al. [17]. The samples were stored at -20 ^{0}C until required. Extracts of liver materials were saponified by refluxing with methanol (50%) containing 5% sodium hydroxide for 1 h at 80 ^{0}C. The saponifiable lipids were converted to their methyl esters for 20 min at 85^0 C using the standard Boron triflouride-methanol (BF_3) method [18]. The resultant mixture of fatty acid methyl esters (FAMEs) in hexane:chloroform (4/1, v/v) was injected onto a Shimadzu GCMS-QP 5000 gas chromatograph mass spectrometry equipped with capillary column (30mx0.25mm i.d.) was packed with 100% dimethylpolysiloxane. The carrier gas was helium and injector port and detector temperatures were 240 ^{0}C and 250 ^{0}C, respectively. The temperature program was 180 ^{0}C for 5 min, 180-240 ^{0}C at 2 ^{0}C min^{-1}, and was kept at the final temperature for 5 min. A small quantity of FAMEs solution (1 µl) was introduced onto the column. FAMEs were identified relative to known external standards.

Statistical Analyses

All analytical determinations were performed in triplicate and the mean values were reported. The statistical analysis of percentages of fatty acid were tested by analysis of variance (ANOVA) and comparisons between means performed with Tukey test. Differences between means were evaluated as significant if $p \leq 0.05$.

Results

Table 2 shows the fatty acid compositions of rainbow trout liver and diets. The commercial diet (diet 1) had 10% fish oil and diet 2 had 10% olive oil. Total lipid content of diet 1 and diet 2 were 12% and 15%, respectively. Also total fatty acid contents were 10% for diet 1 and 12% for diet 2 (Table 1). Qualitative analysis of fatty acids revealed the presence of 18 fatty acids in diet 1 and 12 fatty acids in diet 2. The fatty acids data in Table 2 indicate that there were differences between fatty acid profiles of the diets. C20:0 (arachidic acid), C20:1 (eicosenoic acid), C20:4n-6 (arachidonic acid), C20:4n-3, C20:5n-3 (eicosapentaenoic acid) and C22:5n-3 (docosapentaenoic acid) were not detected in diet 2. However, the percentages of the fatty acids were different amoung two diets. Diet 2 had higher percent of total MUFA and n-6 PUFAs than diet 1, while the latter had higher contents of n-3 PUFAs and saturated. The effect of fatty acid compositions of diets on liver fatty acid composition of the fish fed is shown in Table 2.

The fatty acid profiles in liver of the fish fed were quite similar. However, there were variations in the levels of some fatty acids between the groups after 28 days in fed. In *O. mykiss* fed diet 1 and diet 2, C16:0, C16:1, C18:1n-9, C22:6n-3 were the dominant fatty acids, C14:0, C18:0, C18:2n-6, C18:1n-7, C20:4n-6 and C20:5n-3 were invariably the secondary fatty acids (Table 2).

The fatty acid compositions of fed fish were well reflected by fatty acid compositions in dietary lipid. The fatty acid compositions of liver in fish fed diet 1 and diet 2 were very similar, but in the fish fed diet 2, the fatty acid levels were different than that of the diet 1.

Saturated fatty acids, C14:0 and C18:0 (stearic acid) contents in the fish fed diet 2 were higher than in the fish fed diet 1 ($p<0.05$). There were no significant differences in C16:0 and C20:0 content between those two groups ($p>0.05$). The content of total saturated fatty acids in fish fed with diet 1 and 2 was quite similar ($p>0.05$).

Significantly higher contents of C18:1n-9 and total MUFAs were observed in the fish fed diet 2 than in the fish fed diet 1 ($p<0.05$). C18:2n-6 was significantly increased, whilst C20:1 and C20:4n-6 decreased in the fish fed diet 2 according to fish fed diet 1. Total n-6 PUFA content was similar in the fed two groups ($p>0.05$), whilst total n-3 PUFA was

significantly decreased in only fish livers ($p<0.05$) fed diet 2. C18:3n-3 content in the fish fed diet 2 was higher ($p<0.05$) than in the fish fed diet 1. Hovewer, C20:5n 3, C22:5n-3 and C22:6n -3 contents were definitely decreased in the fish fed diet 2. Also, the contents of these fatty acids present in the fish fed diet 2 were affected by diet 2. These increases and decreases occured in both fish which were fed with diet 1 and diet 2 as a result of different dietary fatty acids content in diets.

Discussion and Conclusion

The fatty acid changes in relation to starvation and feeding have been investigated in many fish species, such as *Scophthalmus maximus* (L.) [19], *Coregonus muksun* (Pallas) [20], *Sparus aurata* (L.) [21], *S. salar* [22] and *Cyprinion macrostomus* (Heckel) [23]. The decrease in body protein and lipid content in starved fish is indicative of the fact that both protein and lipid are utilised as energy resources, during starvation. The lipid content of fish is highly variable between and within species [24, 25]. In this study, the amounts of C 16:0, C 20:0, C 16:1, C 18:3n-6, C 20:2n-6, C 20:3n-6 and C 20:4n-3 in the liver of the fish fed diet 1 and diet 2 were very similar in spite of differences in diets. Hovewer, the percentages of C 18:1n-9, C 18:2n-6 and C 18:3n-3 (abundant in diet 2) were higher in the fish fed diet 2 than in the fish fed diet 1. This suggest that liver may have a high lipogenic capacity. These results are in agreement with other similar studies showing fatty acid composition [11, 26].

Although apparent physical dietary fatty acids were deficient, signs such as fin erosion and mortality or any disease [27] were not observed in any of the groups during the feeding (especially, in the fish fed diet 2) in this study. Although the fatty acid compositions of diet 1 and diet 2 were different, there was no significant difference in any of the fatty acid profiles after 28 days in fish fed, but there were variations in the content of some indivudial fatty acids between the groups.

Many reports have described the effect of dietary n-3 PUFA deficiency on fatty acid compositions and development in freshwater fish [28, 29]. It has also been shown that livers of fish fed diets low in n-3 PUFA developed fatty livers, and similar results have been reported for livers of some fish [3, 30, 31]. In our study, there was a reduction in n-3 PUFA of livers of the fish fed diet 2, compared with the fish fed diet 1. Similar findings obtained also showed a decreasing trend in n-6 PUFA. Total MUFAs were significantly increased in the fish fed diet 2. These results suggest that the fatty acid contents in the fish fed are related to dietary lipid levels.

In this study, the level of C20:5n-3, C22:5n-3 and C22:6n-3 in the fish fed diet 2 decreased with decreasing dietary level. The reduction in n-3 PUFA level in liver in the fish

fed diet 2 was due to non-detection of C20:4n-3, C20:5n-3 and C22:5n-3 fatty acids in diet 2. Comparison between the metabolism of n-6 and n-3 PUFA showed that the Δ6 desaturase activity was not significantly influenced by the series of PUFA but the level of C18:2n-6 was increased, whilst C20:4n-6 and C22:4n-6 were decreased by diet 2. When compared with C 18:2 n-6, this suggests that C18:3 n-3 can be more readily transformed to their C 20 and C 22 homologues [3].

The n-3 PUFA are essential fatty acids for fish and must be included in the diet. C20:5 and C22:6 are the most important n-3 PUFA for fish and play important roles as components of membrane lipids [3, 32]. Dietary deficiencies of these fatty acids have been reported to produce a wide variety of effects on different species including decreased growth and survival, metabolic alterations [33, 34] and modifications of tissue biochemical composition (35).

The results suggest that the variations in the amount of MUFA, total n-6 PUFA, total n-3 PUFA in the fish fed different diets are the consequence of fatty acids in diets. These results suggest that dietary fatty acids may be implicated in the influencing on the level of fatty acids in fish liver. Further experiments will be necessary to determine the importance of dietary fatty acids on cultured fish quality for human food.

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Table 1. Ingredients composition of the experimental diets (weight %)

Ingredient	Diet 1	Diet 2
Liver +Spleen meal (of cattle)	–	10
Fish meal	43	40
Bone meal	–	15
Corn meal	–	7
Soybean meal	10	10
Crude fiber	15	–
Olive oil	–	10
Fish oil	10	–
Rock salt	2.5	2
Vitamin premix	10	5
Mineral premix	1.5	1
Crude ash	8	–
Total lipid %	12	15
Total fatty acid %	10	12

Vitamin premix contained the following ingredients, Vit A, Vit D_3, Vit E, Vit C, Vit B_2 Vit B_{12}, Inositol, Choline.

Mineral premix contained the following ingredients, $ZnSO_4$, MgO, $CuSO_4$, $CaCO_3$ $Ca_3(PO_4)_2$, $MnSO_4$, KCl.

Table 2. Influence of dietary fatty acid on the fatty acid composition (% of total fatty acids) of rainbow trout liver

Fatty Acids	Diet 1 (Mean* ±S.E.)	Diet 2 (Mean* ±S.E.)	Liver Fatty Acids of Fed Groups	
			Diet 1 (Mean* ±S.E.)	Diet 2 (Mean* ±S.E.)
C14:0[t]	7.25±007d	2.24±0.02c	3.93±0.04a	4.58±0.16b
C16:0	26.25±0.20c	16.83±1.41a	23.49±0.27b	24.51±0.17b
C180	3.01±0.10c	4.08±0.05a	4.26±0.13a	4.90±0.12b
C20:0	1.10±0.06a	-	0.64±0.08b	0.53±0.07b
Σ SFA	37.54±0.37a	23.15±0.35b	32.33±0.36c	34.53±0.15c
C16:1	7.03±0.05c	1.81±0.02	10.69±0.27b	10.83±0.48b
C18:1n-9	20.53±1.28a	45.34±0.20b	19.85±0.35a	28.13±0.14c
C18:1n-7	2.33±0.32a	3.32±0.18b	2.57±0.23ab	3.39±0.29b
C20:1	1.50±0.21c	-	2.33±0.16b	1.14±0.18c
Σ MUFA	31.44±0.33b	50.48±1.18d	35.51±0.21a	43.50±0.44c
C18:2n-6	4.98±0.06a	8.61±0.13b	4.06±0.007a	5.32±0.01c
C18:3n-6	0.32±0.02c	2.14±0.06b	0.72±0.13a	1.10±0.13a
C20:2n-6	0.79±0.01a	0.81±0.05a	0.81±0.25a	0.84±0.04a
C20:3n-6	3.15±0.08b	3.31±0.13b	1.13±0.09a	1.53±0.13a
C20:4n-6	0.92±0.05b	-	3.08±0.36a	1.15±0.02b
C22:4n-6	-	-	0.31±0.02	-
Σ n-6 PUFA	10.17±0.15b	14.85±0.36a	10.11±0.73b	9.94±0.26b
C18:3n-3	2.73±0.29b	10.60±0.25a	1.96±0.08c	2.61±0.06b
C20:4n-3	1.07±0.07a	-	1.38±0.20a	2.06±0.06a
C20:5n-3	8.68±0.33c	-	5.80±0.17a	1.41±0.07b
C22:5n-3	0.79±0.03a	-	1.66±0.11c	0.88±0.03a
C22:6n-3	7.57±0.32d	0.89±0.08c	11.40±0.20a	5.07±0.04b
Σ n-3 PUFA	20.85±0.19a	11.51±0.22b	22.18±0.29a	12.03±0.11b
Σ PUFA	31.01±0.34b	26.40±0.27a	32.29±0.44b	21.97±0.31c

*, Each value represents the mean of three experiments; t , Means with the same letter in each row do not significantly differ at 0.05 level; –, not detectable; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid