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IMPACT OF FISH MEAL REPLACEMENT WITH FULL-FAT SOYA ON THE MUSCLE AND LIVER FATTY ACID COMPOSITION IN RAINBOW TROUT

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Abstract: The aim of the present study was to determine the impact of partial replacement of fish meal and fish oil with full-fat soya (FFS) on muscle and liver fatty acids composition in rainbow trout (*Oncorhynchus mykiss*). The fatty acid compositions of the rainbow trout muscle and liver were affected by the dietary treatment, and linear relationships between dietary and tissue FA concentrations were observed for some of these. The liver of fish fed with the fish meal and fish oil diet had significantly higher levels of 16:0, 20:5n-3, n-3 polyunsaturated fatty acid than those of fish fed with the full-fat soya diets, whereas increases in 18:1n-9, 18:2n-6, n-6 polyunsaturated fatty acid and polyunsaturated fatty acid /saturated fatty acid were observed in the liver of fish fed the FFS diets. The present results showed that the replacement of fish meal and fish oil with full-fat soya caused significant reductions in the muscle eicosapentaenoic acid, docosahexaenoic acid and n-3/n-6 polyunsaturated fatty acid ratio, and consequently reduced availability of these essential n-3 polyunsaturated fatty acid to consumers.

Keywords: Rainbow trout, *Oncorhynchus mykiss*, Full-fat soya, Fatty acids

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Özet:

Gökkuşağı alabalığı diyetlerinde balık unu yerine tam yağlı soya kullanımının balık eti ve karaciğerinde yağ asitleri kompozisyonuna etkisi

Bu çalışmada gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nda balık unu ve balık yağının bir kısmı yerine tam yağlı soya kullanımının et ve karaciğer üzerinde yağ asitleri kompozisyonuna etkileri belirlendi. Bulgular, gökkuşağı alabalığının et ve karaciğerindeki yağ asitleri kompozisyonun diyetle etkilendiğini ve diyet ile yağ asitleri konsantrasyonları arasında doğrusal bir ilişkinin olduğunu gösterdi. Balık unu ve balık yağı ile beslenen balıklarda karaciğerde bulunan 16:0, 20:5n-3 ve n-3 çoklu doymamış yağ asitlerinin tam yağlı soya ile beslenen balıklardan önemli derecede daha yüksek olduğu belirlendi. Diğer taraftan, tam yağlı soya ile beslenen balıklarda karaciğerde bulunan 18:1n-9, 18:2n-6, n-6 çoklu doymamış yağ asitleri ve çoklu doymamış yağ asitlerinin doymuş yağ asitlerine oranının arttığı gözlendi. Bu çalışmada balık unu ve balık yağı yerine tam yağlı soya kullanımının balık etinde eikosapentaenoik asit, dokosaheksanoik asit ve n3/n-6 çoklu doymamış yağ asitleri oranının önemli düzeyde azaldığını, bu nedenle tüketicilerinin esansiyel n-3 çoklu doymamış yağ asitlerinden faydalanma düzeyini azaltacağını gösterdi.

Anahtar Kelimeler: Gökkuşağı alabalığı, *Oncorhynchus mykiss*, Tam yağlı soya, Yağ asitleri

Introduction

Aquaculture has developed into a highly productive and efficient industry for production of animal protein for human consumption in the world (Cabellero et al. 2002). As aquaculture production continues to enlarge, the need for high-quality and cost-effective protein sources increases (Mundheim et al. 2004). Fish meal and fish oil are main raw materials in the production of fish feeds (Cabellero et al. 2002; Bell et al. 2010). They are also expensive feed ingredients compared to some alternative plant sources (Mundheim et al. 2004). Therefore, many plant protein sources have been used to partially or almost totally replace dietary fish meal in order to reduce cost of feed ingredients (Kaushik et al. 1995; Refstie et al. 2000). However, it is important that the commercial producers realize the influence that dietary fatty acids (FA) have on the composition of storage lipids, particularly because of fish and fish products as a source of bioactive HUFA in human nutrition. Thus, the focus is no longer just on promoting good growth in fish but also on the production of fish with good nutritional qualities (Mourente and Bell 2006).

Soya products have become a widely used protein rich feed ingredient in diets for salmonids and other fish species, which is due to its moderate price, high availability in the market and the relatively well-balanced amino acid profile (Kaushik et al. 1995; Davies and Morris 1997; Ustaoğlu and Rennert 2002; Romarheim et al. 2006; 2008; Bilgüven and Barış 2011). Different soya products are suitable and result in good growth in salmonids and other fish species (Ustaoğlu and Rennert 2002). FFS is also an important alternative feed ingredient amongst soya products. It is included in dietary formulations largely as a protein source; it also contributes to the dietary fat (Morris et al. 2005; Karalazos et al. 2007).

There has been a fast increase in recent years in the aquaculture production in Turkey. The percentage of aquaculture production in total fish production has been rising every year. For example, the ratio of cultured fish production to total fish production was 1.5% in 1990s, 13.57% in 2000 and more than 20% in 2005. Furthermore, aquaculture production constituted 23.55% of the total fishery production in 2008, and exceeded 152.186 tons, showing an 8.1% increase compared to production in 2007. In addition to wild catch, the rainbow trout is the main freshwater species cultured in Turkey. Trout production increased from 43.43 tons in 2004 to about 65.92 tons in 2008 (Anonymous 2008; Harlıoğlu 2011a).

Traditionally, fish meal and fish oil have been the main source of dietary protein for fish in Turkey. In 2003-2004, fish meal and oil were produced by 9 factories, and total fish meal production was 18,000 tons (Yıldırım, 2006; Harlıoğlu 2011b). In 2006, approximately 60,000 tons of fish meal was used in fish food production (Köprücü, 2007). It means that rest of the demand was imported. Therefore, it seems that alternative feed ingredients such as full-fat soya are required to provide the essential nutrients for the growth and quality of aquaculture production in Turkey.

The aim of present study was to determine the impact of changes in rainbow trout muscle and liver fatty acid composition when full-fat soya represented 40% and 55% of the formulation in diets.

Materials and Methods

Experimental diets, fish and management

Three isoenergetic and isonitrogenous diets were formulated, containing approximately 480 g kg^{-1} protein and 140 g kg^{-1} fat. The control diet was prepared as formulated by Cho et al. (1985). The control diet did not contain full-fat soya (FFS) (FFSO), but the experimental diets were formulated to contain 400 and 550 g FFS (FFS40 and FFS55, respectively) per kilogram of diet. Fish meal (FM) was obtained from İzmir Feed Producer Company, İzmir Turkey. Solvent (hexane) extracted soybean meal, FFS, Corn gluten, Wheat Bran were obtained from Özuğur Feed Producer Company, Elazığ Turkey. Proximate composition of the dietary ingredients is given in Table 1. Cristalline amino acid was added to the diets to provide the IAA requirement profile according to the NRC (1993). Fish oil (FO) was purchased from Sinop-Sürsan Company, Sinop Turkey. Vitamin and mineral premixes were obtained from Roche Products. Diet formulation and proximate composition of experimental diets are given in Table 2, and the FA compositions are given in Table 3.

The experiment was carried out in Government Water Management Affairs of IX. Area Directory, Keban, Elazığ from June to August for 10 weeks under natural photoperiod. Each diet having three replicates, nine tanks (200 x 40 x 40 cm) were stocked with 25 rainbow trout of initial average weight of ~40 g each. Fish were weighed initially and at the end of every month. The tanks were supplied with freshwater at constant temperature 9-9.5 ºC. Dissolved oxygen content of water changed between 7.1-7.9 mg L^{-1}, pH content of water changed between 7.8-7.9.

At the end of the experiment, the fish were weighted after 24 h period of starvation. Seven fish were randomly taken from each tank for liver and muscle FA composition analysis. The samples were frozen and stored at -20 ºC until analysis.

Proximate analysis of the diets

Diets samples were analyzed in triplicate for crude protein (CP), crude fat (CF), ash and moisture according to the methods described by the Association of Official Analytical Chemist (AOAC, 1995). Moisture content was measured by drying samples at 105˚C for 24 h to constant weight in an oven. CP (Nitrogen x 6.25) was determined by Kjeldahl method autoanalyzer (Gerhardt VAP 40), CF was estimated using a soxhlet apparatus (Gerhardt) with petroleum ether and ash by heating at 550˚ for 5-6 h in a Shimadzu type ashing furnace.

Fatty Acid Analyses

Lipid extraction of muscle and liver samples were extracted with hexane-isopropanol (3:2 v/v) by the method of Hara and Radin (1978). Each sample was homogenized with 10 ml hexane-isopropanol mixture. The homogenate was centrifuged at 5000 rpm for 5 min at 4˚C and parts of tissue remnants were precipitated. The supernatant part was used fatty acid analysis.

Fatty acids in the lipid extracts were converted into methyl esters including 2% sulfuric acid (v/v) in methanol (Christie, 1992). The mixture was vortexed and then kept at 50˚C for 12 h. Then, after being cooled to room temperature, 5 ml of 5% sodium chloride was added and then it was vortexed. Fatty acid methyl esters were extracted with 2x5 ml hexane. Fatty acid methyl esters were treated with 5ml 2% $KHCO_3$ solution and then the hexane phase was evaporated by the nitrogen flow and then by dissolving in 0.5 ml fresh hexane (Christie, 1992), they were taken to auto sampler vials.

Methyl esters were analyzed with the Shimadzu GC-17 Ver. 3 gas chromatography (Kyoto, Japan). For this analysis, 25 m of long Machery-Nagel (Germany) capillary colon with an inner diameter of 0.25 μm and a thickness of 25 micron film was used. During the analysis, the colon temperature was kept at 120-220 ˚C, injection temperature was kept at 240˚C and the detector temperature was kept at 280 ˚C. The nitrogen carrier gas flow was 1 ml/min. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. After this process, the necessary programming was made and the Class GC 10 software version 2.01 was used to process the data.

Table 1. Proximate composition (% dry matter) of dietary ingredients

Components	Fish meal	Soybean meal	Full-fat soya	Corn gluten	Wheat bran
Crude protein	64.45	48.12	37.61	60.55	15.74
Crude fat	9.81	1.23	18.25	1.41	2.71
Ash	10.24	6.04	5.52	2.48	5.26
Moisture	9.25	12.09	12.04	9.04	12.34

Table 2. Ingredients (Grams per kilogram) and proximate composition (g/100g) of rainbow trout experimental diets

Dietary treatments	FFS0[4]	FFS40	FFS55
Ingredients			
Fish meal	430	280	130
Soybean meal, (solvent extracted)	250	-	-
Full- fat Soya	-	400	550
Corn gluten	60	170	250
Wheat Bran	113.3	102.1	29.5
Fish oil	125	26	18
Methionine	0.7	0.9	1.5
Antioksidan [1]	1	1	1
Vitamin pre-mix[2]	10	10	10
Mineral pre-mix[3]	10	10	10
Proximate composition			
protein	44.3	44.6	44.1
fat	13.6	13.1	12.7
ash	10.5	8.5	7.5
moisture	7.9	6.8	7.4
total energy (kcal/kg)	4290	4300	4280

1 Butilen Hidroksi Toluen (BHT); 125.000 mg/ kg

2 Vitamin pre-mix (mg kg^{-1}); Riboflavin 4 000, Pyridoxine 3 000, cyanocobalamin 10, Ascorbic acid 50 000, Niasin 10 000, Biotin 150, Thiamin 1 000, Folic acid 1 000, Kolin 1 000, Pantothenik asit 20 000, Myoinositol 300 000, Retinol 2 500 000 IU, Kalsiferol 2 400 000 IU, Tokoferol 50 000 IU.

3 Mineral pre-mix (mg kg^{-1}); Mn 13 000, Fe 60 000, Zn 30 000, Mg 5, K 70, Na 60, Cu 3 000, I 1100, Co 400, Se 300.

4 Control Diet

Table 3. Fatty acid composition (% total fatty acids) of rainbow trout experimental diets.

Dietary treatments	**FFS0** [1]	**FFS40**	**FFS55**
Fatty acid			
14:0	5.5	2.2	1.0
14:1	0.4	0.1	nd
15:0	0.8	0.3	0.2
15:1	nd[5]	0.1	0.2
16:0	18.0	14.7	11.8
16:1n-7	7.1	2.0	1.3
16:1n-9	1.2	1.1	0.5
17:0	0.7	0.2	nd
17:1	0.9	0.4	0.1
18:0	3.6	5.0	5.2
18:1n-9	16.8	26.1	29.0
18:1n-7	2.0	0.1	0.2
18:2n-6	4.7	31.7	36.2
18:3n-6	0.5	2.1	2.3
18:3n-3	1.2	5.1	7.4
18:4n-3	1.8	0.5	0.2
20:0	0.8	0.4	0.5
20:1n-9	0.9	0.5	0.4
20:1n-7	0.3	0.2	0.4
20:2n-6	0.3	0.1	0.1
20:4n-6	0.8	0.1	nd
20:5n-3	11.3	2.6	0.9
22:0	0.5	0.9	0.7
22:1n-9	0.4	0.3	0.2
22:2	0.2	nd	nd
22:5n-6	0.4	0.2	0.1
22:5n-3	1.0	0.2	0.1
22:6n-3	17.1	2.5	0.9
24:1n-9	nd	0.2	nd
∑SFA[2]	29.9	23.7	19.4
∑MUFA[3]	30.0	31.1	32.3
∑PUFA[4]	39.3	45.1	48.2
∑ n-3 PUFA	32.4	10.9	9.5
∑ n-6 PUFA	6.7	34.2	38.7
n-3/n-6 ratio	4.8	0.3	0.2

[1] Control Diet
[2] Saturated fatty acids
[3] Monounsaturated fatty acids
[4]Polyunsaturated fatty acids
[5] not determined

Statistical analyses

Data are presented as means ±standard deviation (SD) (n=3). Significant differences between dietary treatments were determined by one-way analysis of variance (ANOVA) using Duncan's test. The level of significance was chosen at $p<0.05$. ANOVA and regression analyses were performed using SPSS 12 (SPSS Inc. Chicago, Illinois).

Results and Discussion

In the present study final body weight decreased in relation to the increases level of FFS in diets. Mean final weight (g, mean±SD) ranged from 69.8 ±2.1, 64.6 ±2.6 and 60.1 ±2.4 for FFS0, FFS40 and FFS55, respectively.

Diets

The increased inclusion of FFS at the rate of fish meal and fish oil had a direct effect in the FA compositions of the diets (Table 3). In particular, 14:0, 15:0 and 16:0 decreased, whereas 18:0 increased, resulting in a decrease for the total saturated fatty acids (from 29.9% in FFS0, to 19.4% in FFS55). Total monoenes remained stable, but 16:1 n-7, 16:1n-9, 17:1, 20:1n-9 decreased. On the other hand, there was a major increase in the 18:1n-9 from 16.8% to 29%, for FFS0 and FFS55, respectively. Moreover, the high content of 18:2n-6 in FFS resulted in an increase of that FA by almost 7-fold and 18:3n-6 by almost 4-fold, and a subsequent increase in total n-6 polyunsaturated fatty acid (PUFA). 18:3n-3 was increased by 6-fold between FFS0 and FFS55.

In the present study, it was found that 20:5n-3 and 22:6n-3 reduced and total n-3 also reduced from 32.4 to 9.5%, for FFS0 and FFS55, respectively. In addition, the n-3/n-6 ratio decreased from 4.8 in FFS0 to 0.2 in FFS55.

Fatty acid composition of muscle and liver

The fatty acid composition of the rainbow trout muscle was given in Table 4. The inclusion of FFS resulted in a reduction in 14:0, 16:0, 16:1n-7, 20:1n-9, 20:5n-3, 22:1n-9, 22:6n-3, but it caused an increase in 18:1n-9, 18:2n-6 and 18:3n-3 in the fatty acid contents of muscle.

In muscle, in addition to the increase in linoleic acid (18:2n-6), with graded inclusion of FFS, the concentration of total n-6 PUFA also increased (from 5.8% to 27.7%, for FFS0-FFS55, respectively). However, there were significant reductions in 16:1n-7 (from 4.9% to 2.0%, for FFS0 and FFS55, respectively) ($P<0.05$), but 18:1n-9 increased significantly from 15.0% to 22.0% between the control group and FFS55 ($P<0.05$). The DHA concentration was also decreased from 27.6% in FFS0 (control group) to 15.1% in FFS55. The EPA concentration was also decreased from 8.2% in FFS0 to 4.5% in FFS55 although there were no significant differences between the two groups fed the FFS ($P>0.05$).

The results also showed that the replacement of FFS resulted in a significant reduction in the n-3/n-6 ratio, from 6.7 to 0.8 for the FFS0 and FFS55 groups, respectively, but there were no significant differences in the n-3/n-6 ratio between the two groups fed FFS40 and FFS55 ($P>0.05$). Saturated fatty acid (SFA) and n-3 PUFA were significantly higher in the rainbow trout fed FFS0 while n-6 PUFA was higher in the trout fed diet FFS40 and FFS55. However, there were no significant differences in 20:4n-6, 22:5n-6 and 22:5n-3 between dietary groups ($P>0.05$) (Table 4).

In liver (Table 5), the dietary inclusion of FFS resulted in a reduction in 14:0, 16:0, 16:1n-7, 17:0, 18:1n-7, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-9, 22:5n-3, 22:6n-3 and 24:1n-9 and it caused also increase in 18:0, 18:1n-9, 18:2n-6 and 18:3n-6, 20:3n-6 in the fatty acid contents of liver.

In liver, the dietary inclusion of FFS and the consequent major increase in the concentration of 18:2n-6 resulted in a significant increase in the total n-6 PUFA (from 6.8% FFS0 to 24.3% for FFS40 and 30% for FFS55, respectively) ($P>0.05$). In addition, 18:1n-9, 18:3n-6 and 18:3n-3 were also significantly increased from 18.3%, 0.3% and 1.9% to 25.4%, 1.0% and 3.9%, respectively. In contrast, significant reductions were observed between FFS0 and FFS55 in 14:0 (3.6-1.1%), 16:0 (16.1-10.1%), 17:0 (0.5-0.1%), 16:1n-7 (6.4-3.6%), 18:1n-7 (3.8-1.5%), 20:1n-9 (4.2-1.1%), 20:5n-3 (7.7-1.5%), 22:5n-3 (1.5-0.3%), 22:6n-3 (18.8-11.3%), total SFA (25.1-17.2%), total n-3 PUFA (32.6-18.6%) and the n-3/n-6 ratio (4.7-0.6%) (Table 5).

The concentrations of 18:2n-6, 18:1n-9, 18:3n-3, 20:5n-3, 22:6n-3 and n-3/n-6 ratio in muscle and liver were plotted against the respective dietary FA concentrations. The plots of muscle FA against dietary FA concentrations are shown in Figure 1. The results showed that there was a significant linear correlation between die-

tary and muscle 18:2n-6, 18:1n-9, 18:3n-3, 20:5n-3, 22:6n-3 and n-3/n-6 ratio. Correlation coefficients (r), P values from these plots, slopes of the lines for individual fatty acids are given in Table 6 which establishes that different fatty acids have different slopes.

In addition, the plots of liver FA concentrations against the dietary FA concentrations are shown in Figure 2, and the correlation coefficients (r), P values from these plots, slopes of the lines for individual fatty acids are shown in Table 6. The regression analysis and the plots of the concentrations of 18:2n-6, 18:1n-9, 18:3n-3, 20:5n-3, 22:6n-3 and n-3/n-6 ratio in the diet against their concentrations in the liver showed significant linear correlation for all those correlation coefficients, ranging from 0.94 to 1.00).

Table 4. Fatty acid composition of muscle from rainbow trout fed the experimental diets. (% total fatty acids)

Dietary treatments	**FFS0**[2]	**FFS40**	**FFS55**
Fatty acid [1]			
14:0	3.7±0.3^{a}	1.9±0.2^{b}	0.9±0.1^{c}
16:0	20.8±0.2^{a}	17.4±0.2^{b}	16.5±0.3^{c}
16:1n-7	4.9±0.1^{a}	2.6±0.1^{b}	2.0±0.1^{c}
16:1n-9	1.1±0.2^{a}	1.2±0.1^{a}	0.6±0.0^{b}
18:0	4.2±0.1^{b}	4.5±0.1^{a}	4.8±0.1^{a}
18:1n-7	2.9±0.1^{a}	0.5±0.0^{b}	0.3±0.1^{b}
18:1n-9	15.0±0.7^{c}	18.9±0.5^{b}	22.0±1.5^{a}
18:2n-6	4.1±0.4^{c}	17.9±1.5^{b}	25.9±2.0^{a}
18:3n-6	0.2±0.1^{b}	0.6±0.2^{a}	0.7±0.2^{a}
18:3n-3	1.2±0.2^{c}	1.9±0.1^{b}	2.6±0.3^{a}
18:4n-3	0.9±0.1^{a}	0.8±0.0^{a}	0.6±0.1^{b}
20:1n-9	1.1±0.1^{a}	0.9±0.3^{ab}	0.7±0.1^{b}
20:4n-6	1.1±0.4^{a}	0.9±0.3^{a}	0.8±0.1^{a}
20:5n-3	8.2±1.0^{a}	5.6±0.5^{b}	4.5±0.5^{b}
22:0	0.6±0.0^{a}	0.4±0.0^{b}	0.5±0.0^{a}
22:1n-9	0.6±0.1^{a}	0.4±0.1^{b}	0.2±0.0^{b}
22:5n-6	0.4±0.2^{a}	0.3±0.1^{a}	0.3±0.1^{a}
22:5n-3	1.4±0.3^{a}	1.2±0.3^{a}	1.0±0.2^{a}
22:6n-3	27.6±2.2^{a}	22.1±1.0^{b}	15.1±2.8^{c}
∑SFA[3]	29.3±0.3^{a}	24.2±0.2^{b}	22.7±0.3^{c}
∑MUFA[4]	25.6±0.5^{a}	24.5±0.9^{a}	25.8±1.2^{a}
∑ PUFA[5]	45.1±3.2^{a}	51.3±1.1^{a}	51.5±4.2^{a}
∑ n-3 PUFA	39.3±2.7^{a}	31.6±1.1^{b}	23.8±2.3^{c}
∑ n-6 PUFA	5.8±0.8^{c}	19.7±1.9^{b}	27.7±2.1^{a}
n-3/n-6 ratio	6.7±0.82^{a}	1.6±0.21^{b}	0.8±0.1^{b}
PUFA/SFA	1.5±0.09^{b}	2.1±0.03^{a}	2.2±0.1^{a}

[1] Values are mean±standard deviation; n=3, Means in a line with different superscripts are significantly different each other ($p<0.05$). Standard deviation calculated from residual mean square in the ANOVA

[2] Control Diet

[3] Saturated fatty acids

[4] Monounsaturated fatty acids

[5] Polyunsaturated fatty acids

Table 5. Fatty acid composition of liver from rainbow trout fed the experimental diets. (% total fatty acids)

Dietary treatments	**FFS0**[2]	**FFS40**	**FFS55**
Fatty acid [1]			
14:0	3.6±0.6^{a}	1.5±0.3^{b}	1.1±0.4^{b}
15:0	0.4±0.2^{a}	0.3±0.2^{a}	0.3±0.1^{a}
16:0	16.1±0.3^{a}	13.5±0.8^{b}	10.1±1.0^{c}
16:1n-7	6.4±0.3^{a}	4.7±0.3^{b}	3.6±0.2^{c}
16:1n-9	0.9±0.1^{a}	0.4±0.1^{b}	0.7±0.0^{a}
17:0	0.5±0.2^{a}	0.4±0.1^{ab}	0.1±0.0^{b}
17:1	0.5±0.1^{ab}	0.3±0.0^{b}	0.7±0.1^{a}
18:0	4.5±0.5^{b}	5.9±0.2^{a}	5.6±0.3^{a}
18:1n-7	3.8±0.3^{a}	1.6±0.3^{b}	1.5±0.2^{b}
18:1n-9	18.3±1.7^{b}	23.2±2.0^{a}	25.4±1.7^{a}
18:2n-6	4.9±0.3^{c}	22.2±2.6^{b}	27.3±1.8^{a}
18:3n-6	0.3±0.1^{b}	0.4±0.1^{b}	1.0±0.1^{a}
18:3n-3	1.9±0.1^{c}	2.7±0.2^{b}	3.9±0.5^{a}
18:4n-3	2.7±0.2^{a}	1.8±0.1^{b}	1.6±0.2^{b}
20:1n-9	4.2±0.3^{a}	2.0±0.5^{b}	1.1±0.3^{c}
20:2n-6	0.6±0.1^{ab}	0.5±0.1^{b}	0.7±0.1^{a}
20:3n-6	0.3±0.1^{b}	0.6±0.1^{a}	0.7±0.1^{a}
20:4n-6	0.4±0.0^{a}	0.2±0.1^{b}	0.2±0.0^{b}
20:5n-3	7.7±0.7^{a}	1.8±0.3^{b}	1.5±0.2^{b}
22:1n-9	0.8±0.1^{a}	0.3±0.1^{b}	0.5±0.1^{b}
22:5n-6	0.3±0.1^{a}	0.4±0.0^{a}	0.1±0.0^{b}
22:5n-3	1.5±0.1^{a}	0.7±0.1^{b}	0.3±0.1^{c}
22:6n-3	18.8±0.7^{a}	14.5±0.5^{b}	11.3±1.0^{c}
24:1n-9	0.6±0.1^{a}	0.1±0.0^{b}	0.2±0.0^{b}
∑SFA[3]	25.1±0.4^{a}	21.6±0.5^{b}	17.2±0.8^{c}
∑MUFA[4]	35.5±2.4^{a}	32.6±3.1^{a}	34.2±2.2^{a}
∑PUFA[5]	39.4±2.4^{b}	45.8±4.0^{a}	48.6±2.1^{a}
∑ n-3 PUFA	32.6±1.2^{a}	21.5±0.7^{b}	18.6±0.3^{c}
∑ n-6 PUFA	6.8±0.4^{c}	24.3±2.9^{b}	30.0±2.2^{a}
n-3/n-6 ratio	4.7±0.20^{a}	0.8±0.06^{b}	0.6±0.05^{c}
PUFA/SFA	1.5±0.08^{c}	2.1±0.13^{b}	2.8±0.08^{a}

[1] Values are mean±standard deviation; n=3, Means in a line with different superscripts are significantly different each other ($p<0.05$) Standard deviation calculated from residual mean square in the ANOVA

[2] Control Diet

[3] Saturated fatty acids

[4] Monounsaturated fatty acids

[5] Polyunsaturated fatty acids

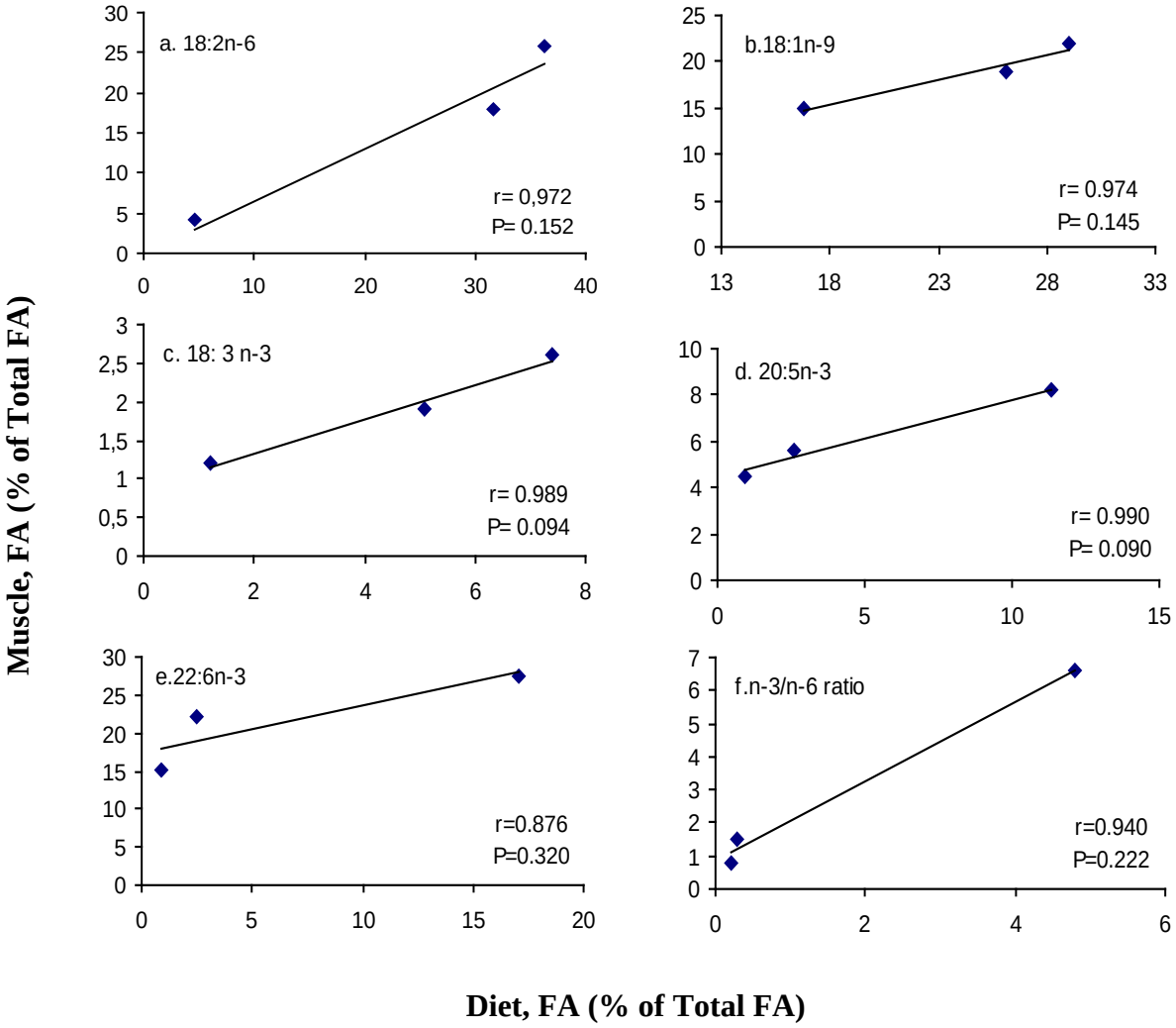

Figure 1. Relationship between dietary fatty acid concentrations and muscle fatty acid concentrations of 18:2n-6 (a), 18:1n-9 (b), 18:3n-3 (c), 20:5n-3 (d), 22:6n-3 (e) and n-3/n-6 (f) in total fatty acids of rainbow trout fed the experimental diets.

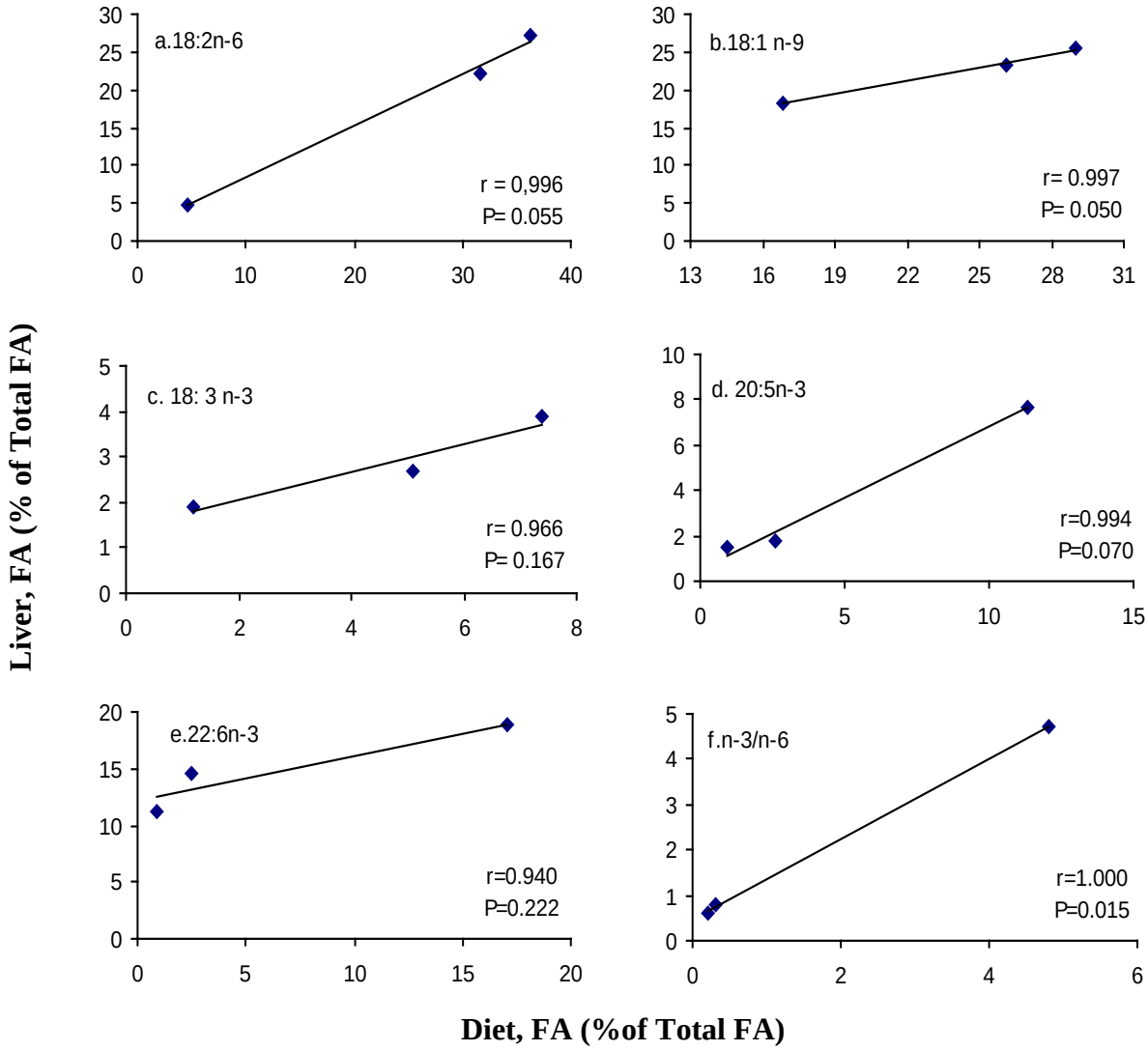

Figure 2. Relationship between dietary fatty acid concentrations and liver fatty acid concentrations of 18:2n-6 (a), 18:1n-9 (b), 18:3n-3 (c), 20:5n-3 (d), 22:6n-3 (e) and n-3/n-6 (f) in total fatty acids of rainbow trout fed the experimental diets.

Table 6. Correlation coefficients (r), P values and slopes of linear plots of fatty acid concentrations (% total fatty acids) in muscle and liver fatty acid against fatty acid concentrations in dietary fatty acid

MUSCLE			
Fatty acid	**Correlation coefficients (r)**	**P**	**Slope**
18:2 n-6	0.972	0.152	0.6289
18:1 n-9	0.974	0.145	0.5361
18:3 n-3	0.989	0.094	0.2209
20:5n-3	0.990	0.090	0.3372
22:6n-3	0.876	0.320	0.6149
n-3/n6 ratio	0.940	0.222	1.1999
LIVER			
Fatty acid	**Correlation coefficients (r)**	**P**	**Slope**
18:2 n-6	0.996	0.055	0.6866
18:1 n-9	0.997	0.050	0.5685
18:3 n-3	0.966	0.167	0.3101
20:5n-3	0.994	0.070	0.6229
22:6n-3	0.940	0.222	0.3961
n-3/n6 ratio	1.00	0.015	0.8795

In general, inclusions of high levels (>25%) of a single plant ingredient in salmonid diets was suggested to have negative effects on fish performance (Ogunkoya et al. 2006; Cheng et al. 2003; Francesco et al. 2007; Drew et al. 2007). The present study also showed that the replacement of fish meal with FFS protein resulted in decrease in trout growth.

In the present study, the replacement of fish meal and fish oil with the FFS resulted in an increase in total PUFA in fish fed FFS40 and FFS55 diets compared to those fed FFS0 diet. This was due to a significant increase in 18:2n-6 and 18:3n-3 which balanced a significant decrease in 20:5n-3, 22:6n-3.

The fatty acid content of fish generally reflects the fatty acid composition of the diet (Steffens, 1997;Izquierdo et al. 2003; Hei-Zhao et al. 2007; Bell et al. 2002; Bell et al. 2010). This was visibly shown in the present study. A linear relationship between dietary and both muscle and liver FA concentrations was observed for 18:2n-6, 18:1n-9, 18:3n-3, 20:5n-3, 22:6n-3. Similarly, several other studies investigating the linear relationship between dietary and muscle FA compositions have been reported for *O. mykiss* by Drew et al. 2007, for *Gadus morhua* by Karalazos et al. 2007 for *Salmo salar* by Bell et al. 2002.

Some of the fatty acids in both muscle and liver reflected significantly by the fatty acid compositions of the feed. This study has also demonstrated that DHA, EPA and total n-3 PUFA fatty acids were reduced in rainbow trout muscle when fish meal and oil were replaced with FFS. The fatty acid profile of the muscle changed to reflect the fatty acid content of the feeds, but without affecting the amount of MUFA and PUFA. Likewise, Wu et al. (2002) found that the amount of MUFA and PUFA did not reflect their dietary origins. The main effect found in the muscle was an increased level of 18:2n-6. In general, replacement of fish oil with plant oil has resulted in a lower level of long chain n-3 fatty acids, EPA and DHA, and higher levels of the 18 C fatty acid oleic acid and linolenic acid in muscle of several salmonid species such as rainbow trout and brown trout (Cabellero et al. 2002).

Most studies have shown that the levels of EPA and DHA are decreased in fish fed diets containing plant oils (Drew et al. 2007; Mourente and Bell 2006). Mourente and Bell (2006) reported that the levels of EPA and DHA found in fish muscle are directly related to their dietary levels in a range of species studied including salmon, rainbow trout, sea bass, sea bream and turbot. The fatty acid composition of the muscle in the present study was significantly influenced by that of the feed. However, in the present study, in trout muscle, EPA and DHA were found in much higher concentrations than in the diet when the fish were fed FFS. On the other hand, the DHA concentration of liver was lower than that of the muscle. According to Izquierdo et al.

(2003) and Hei-Zhao et al. (2007) the differences in utilization of specific FA in muscle and liver are related to the different mechanisms of FA in the two tissues.

Mourente and Bell (2006) stated that while dietary fatty acids are closely related to fatty acids deposited in the muscle, specific fatty acids are selectively retained or utilized. There was selective deposition and retention of DHA, since muscle DHA concentrations were always higher than diet concentrations. This fact has also been stated by Cabellero et al. (2002) and Morris et al. (2005) for *O. mykiss*. Preferential deposition and/or retention of selected fatty acids including linoleic acid, arachidonic acid and DHA has previously been recorded in rainbow trout (Morris et al. 2005). Most freshwater fish can elongate and desaturate 18:2n-6 and 18:3n-3 to into the longer chain n-3 fatty acids (Tocher et al. 2001; Bell et al. 2001).

In the present study, in muscle, EPA and DHA were found in higher concentrations in control diet then the fish fed FFS40 and FFS55 diets. Bell et al. (2001) suggest that the extent to which trout can elongate and desaturate 18:3n-3 to 22:6n-3 may have been exaggerated and that the majority of 22:6n-3 may be obtained from diet. Therefore, Bell et al. (2004, 2005) and Torstensen et al. (2004, 2005) stated that one way to successfully restore total n-3 PUFA is to dilute or wash out the plant oil derived fatty acids using a fish oil finishing diet. Bell et al. (2002) concluded that higher levels of plant oil could, in principle, be used in feed formulations for salmon provided that, at an appropriate time before harvest, fish were placed on a fish oil and fish meal diet so that muscle levels of DHA, EPA, 18:2n-6 were normalized.

In fish, diet composition is mainly affected on muscle and liver fatty acid compositions. Nevertheless, in the present study, in muscle and liver, 18:2n-6, 18:3n-3 and 18:1n-9 was found in lower concentrations than in the diet when the fish were fed FFS. In salmonids, muscle lipids are the main site of body lipid storage. Storage lipids as a reserve of metabolic energy may be used during times of low feed intake and provide fatty acids for energy production (Sargent et al. 2002; Mourente and Bell 2006). For this reason, these FA were selectively utilized for metabolism, probably for energy production (Karalazos et al. 2007; Mourente and Bell 2006). In this study, the concentration of these fatty acids in liver was higher than in the muscle when the fish were fed FFS. This may be due to the different lipid class composition of these two tissues of trout. Yıldız and Şener (2003) found higher 18:2n-6 and 18:3n-3 levels in the liver of the sea bass (*Dicentrarchus labrax*) fed soybean oil than the sea bass fed fish oil, sunflower oil and corn oil diets. Similarly, Hunt and Tekelioğlu (2004) reported that, the levels of 18:2n-6 and 18:3n-3 were increased in the sea bass fed diets containing soybean oil.

As regarding PUFA/SFA, it was found to be 1.5, 2.1 and 2.2 in muscle for FFS0, FFS40 and FFS55 respectively in the present study. According to the nutritional guidelines of the Department of Health (1994) of the UK, a ratio of 0.45 or more is recommended as a balanced fatty acid intake on healthy diet (Wood et al., 2003). Thus, in the present study, PUFA/SFA contents of muscle from *O. mykiss* fed experimental diets presented a higher PUFA/SFA ratio, which is also much higher than the minimum recommended value of 0.45. On the other hand, in the present study inclusion of FFS at levels 40% and 55% resulted in significant reductions in total n-3 PUFA, specifically EPA and DHA in the muscle such that the nutritional benefit to the consumer would be reduced.

Conclusions

In this study it was found that SFA, n-3 PUFA and n-3/n-6 were higher in the muscle of rainbow trout fed fish oil and fish meal than the mussel of rainbow trout fed full-fat soya diets**.** The present results showed that the replacement of full-fat soya instead of fish meal and fish oil did not cause statistically significant changes in the muscle of total PUFA**.** This is important from the perspective of the nutritional quality of the product for human consumer, as long-chain PUFA play important role with regard to human health.

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