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Effects of dietary fish oil replacement by cottonseed oil on growth performance and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*)

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Abstract: The aim of the present study was to determine the effects of replacing fish oil (FO) with cottonseed oil (CSO), as an alternative plant lipid source in diets for rainbow trout (89.3 ± 1.1 g mean individual weight), on the growth and fatty acid composition of fish. As a control diet, 5 experimental diets were formulated with pure FO and with partial or complete (25%, 50%, 75%, or 100% CSO, respectively) replacement of FO with CSO. The fish were fed 2% of their body weight per day for 60 days at an average water temperature of 10.2 °C. At the end of the feeding trial, the highest growth and the lowest FCR (1.28) were obtained in fish fed the CSO50 diet. The fillet lipid concentrations and fatty acid composition of the fish were significantly affected by the experimental diets. Fish fed the FO and CSO25 diets contained significantly lower lipid levels (6.8% and 6.5%, respectively) than those fed the 3 other diets. The viscerosomatic index (VSI) and hepatosomatic index (HSI) values increased with increasing cottonseed oil percentages in the diets. Fillet fatty acid composition reflected dietary fatty acid composition. The n-6 polyunsaturated fatty acid (PUFA) concentration increased with increasing cottonseed oil levels in the diets. In contrast, the n-3 PUFA levels decreased with increasing cottonseed oil levels in the diets. The highest level of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations were recorded in fish fed the FO diet and the lowest in those fed the CSO100 diet. Based on the results of growth performance and fatty acid composition of the experimental fish in the present study, it can be concluded that the 50% concentration of cottonseed oil performed best among the diets tested in the experiment.

Key words: Nutrition, cottonseed oil, fatty acids, growth, rainbow trout

Balık yağı yerine pamuk yağı içeren diyetlerin gökkuşağı alabalığının (*Oncorhynchus mykiss*) büyüme performansı ve yağ asidi kompozisyonuna etkisi

Özet: Bu araştırmada, gökkuşağı alabalığı (ortalama başlangıç ağırlığı, 89,3 ± 1,1 g) diyetlerinde balık yağı (BY) yerine alternatif bitkisel yağ kaynağı olarak kullanılan pamuk tohumu yağının (PTY) balıkların büyümesine ve yağ asidi kompozisyonuna etkileri incelenmiştir. Kontrol diyeti olarak sadece BY içeren ve balık yağı yerine kısmen yada tamamen (sırasıyla, % 25, % 50, % 75 ve % 100) PTY içeren beş farklı diyet hazırlandı. Balıklar, 60 günlük deney süresince ve ortalama 10,2 °C su sıcaklığındaki tanklarda günlük olarak canlı ağırlıklarının % 2'si oranında yemlendiler. Deney sonunda, en iyi büyüme ve en düşük yemden yararlanma oranı (1,28) % 50 oranında PTY içeren diyetle beslenen balıklarda görülmüştür. Balık filetolarındaki toplam yağ miktarı ve yağ asidi kompozisyonu deney diyetlerinden önemli

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ölçüde etkilenmiştir. Sadece BY ve % 25 oranında PTY içeren diyetlerle beslenen balıkların filetolarındaki yağ miktarı (sırasıyla, % 6,8 ve % 6,5) diğer üç deney grubundan daha düşük bulunmuştur (P < 0,05). Viserosomatik (VSİ) ve hepatosomatik indeks (HSİ) değerleri diyetlere ilave edilen PTY oranının artışıyla yükselmiştir. Balık filetolarındaki yağ asidi kompozisyonu da diyetlerdeki yağ asidi kompozisyonunu yansıtmıştır. Diyetlerde kullanılan PTY'nın artışıyla birlikte n-6 serisindeki çok doymamış yağ asitleri (PUFA) konsantrasyonu da artmıştır. Buna karşın, diyetlerdeki n-3 PUFA düzeyleri azalmıştır. Sadece BY içeren diyetle beslenen balıkların filetolarındaki eikosapentaenoik asit (EPA) ve dokosaheksaenoik asit (DHA) konsantrasyonları en yüksek oranda bulunmuştur. Balıkların büyüme performansı ve yağ asidi profili ile ilgili elde edilen sonuçlara göre gökkuşağı alabalığı diyetlerinde balık yağı yerine %50 oranında PTY kullanılmasının deneme grupları arasında en iyi sonucu verdiği söylenebilir.

Anahtar sözcükler: Besleme, pamuk tohumu yağı, yağ asitleri, büyüme, gökkuşağı alabalığı

Introduction

Lipids are an important source of energy and essential fatty acids (EFA) in aquaculture diets. The importance of long-chain highly unsaturated fatty acids (HUFA), such as docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6) in fish nutrition is well established (1). These fatty acids are required by fish for optimal growth, development, and reproduction (2). Commercial diets for trout mostly contain fishmeal and fish oil as protein and lipid sources. Fish oil is rich in n-3 HUFA, EPA, and DHA, (3). The fatty acid compositions of trout fed diets containing fish oil are high in n-3 HUFA, which are beneficial in the human diet (4). The demand for fish oil has been increasing and studies show that aquafeeds could consume about 90% of the world's supply by 2010 (5). Therefore, alternative oils have to be investigated for aquafeeds. The only sustainable alternatives are plant oils, which are usually rich in C_{18} polyunsaturated fatty acids (PUFA) such as linoleic acid (LA, 18:2n-6) and linolenic acid (LNA, 18:3n-3), but devoid of HUFA (1). However, many freshwater fish are able to convert dietary LA and LNA to HUFA, such as ARA, EPA, and DHA (6). Therefore, fatty acids of the n-6 series are also required for rainbow trout (7).

Several studies conducted on freshwater fish reported that vegetable oils can replace fish oil in fish feed without affecting growth (8-11). However, vegetable oils in aquaculture diets significantly affect the fatty acid composition of fish flesh with an increase in 18:2n-6 and 18:3n-3 and a decrease in n-3 HUFA (12,13).

Cottonseed oil is an abundantly produced vegetable oil which contains high concentrations of LA. Most CSO is used in diets fed to pigs (14) and broilers (15). Several studies demonstrated that fish oil replacement by cottonseed oil, either partially or totally, in diets for several fish species including gilthead sea bream (16,17), European sea bass (16,18,19) and rainbow trout (11) did not cause any negative effect, either on growth and feed utilization or the main nutrient composition of the fish. However, the flesh of the fish fed the diet with cottonseed oil had a high concentration of n-6 PUFA.

The aim of the present study was to examine the effects of different dietary cottonseed oil supplementation used instead of fish oil on the growth performance and the fatty acid composition of rainbow trout.

Materials and methods

Experimental diets

Experimental diets, a total of 5, were formulated to be isonitrogenous (approximately 45% crude protein) and isolipidic (approximately 15% crude lipid). The lipids used in diets were fish oil (anchovy oil) and cottonseed oil. The first diet contained only fish oil (FO) as the primary lipid source and was named the FO diet. The fish oil was partially or totally replaced by cottonseed oil (CSO) in the diets; 25% (CSO25), 50% (CSO50), 75% (CSO75), and 100% (CSO100), respectively. The dietary ingredients and proximate compositions are given in Table 1. The FA compositions of the diets are presented in Table 2 and Figure 1. The experimental diets were prepared and extruded (3 mm pellet diameter) by a private fish feed factory.

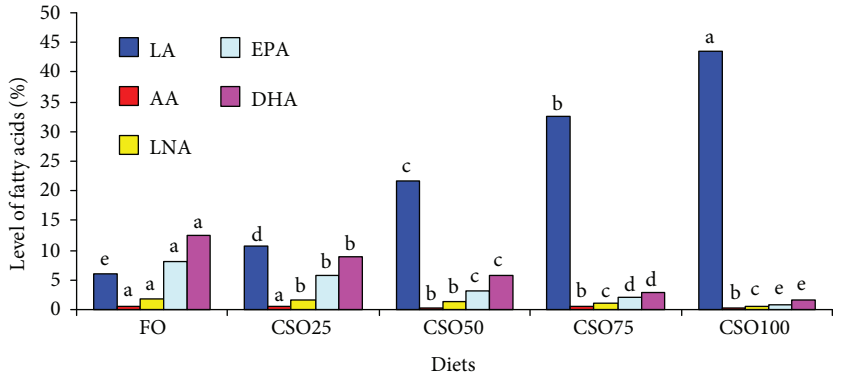

Figure 1. Levels of some essential fatty acids (% of total fatty acids detected) in the experimental diets.

Experimental conditions

Rainbow trout (*Oncorhynchus mykiss*), with a mean initial body weight of 89 g, were obtained from the Sapanca Inland Waters Research Center of the Fisheries Faculty of Istanbul University, Turkey, and stocked randomly (50 fish $tank^{-1}$) into ten 1000 L capacity cylindroconical tanks in the Sapanca Inland Waters Research Center (Adapazarı, Turkey). The tanks were supplied with freshwater and had an average temperature of 10.0 ± 1.2 °C. Dissolved oxygen was maintained at around 8.4 ± 1.0 mg L^{-1}. A photoperiod regimen of 12 h light:12 h dark was utilized throughout the experiment. Before beginning the experiment, the fish were acclimatized to the experimental feeding regimen using a commercial diet for 2 weeks (3 mm pellet diameter). During the experiment, which lasted 60 days, the fish were fed 2 times a day (1000 and 1800 h). The experimental diets were fed by hand, up to 2% of their body weight per day, and feed intake was recorded. Bulk fish live weight increments were measured every 2 weeks and feed intake was recorded daily throughout the experiment. At the end of the experiment, fish were individually weighed to determine growth performance parameters. In addition, 10 fish per tank (20 fish per diet) were collected for analyses of proximate and FA composition. Fish samples were kept at -80 °C until proximate composition and FA profile analysis. The growth performance of the fish was calculated according to Ricker (21).

Proximate analysis of the samples

Feed ingredients, experimental diets, and fish samples were analyzed for proximate composition (protein, lipid, ash, fiber, and moisture) according to the standard methodology of AOAC (22). The moisture content was obtained by weight loss after drying samples in an oven at 105 °C until they reached a constant weight. Crude protein was determined as total nitrogen (N) by using a semi-automatic Kjeldahl (Gerhardt Vapodest, 45s) technique (N × 6.25). Ash content was obtained from the weight loss after incineration of dried samples at 550 °C for about 12 h in a muffle furnace. Crude fiber was determined using sulfuric acid then sodium hydroxide, 12.5% (w/w) for 30 min each, and the final residue was washed with 5% HCl and water, then filtered, dried, and weighed. All samples were analyzed as triplicates.

Lipid extraction and fatty acid analysis

Total lipid was extracted from fish fillets and feed samples by homogenization in chloroform/methanol (2/1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to the method of Folch et al. (23). Fatty acid methyl esters were prepared from total lipid by acid-catalyzed transesterification using 2 mL of 1% H_2SO_4 in methanol, plus 1 mL of toluene, as described by Christie (24), and fatty acids were analyzed by gas liquid chromatography (Auto System XL Perkin Elmer) using a 30 × 0.25 mm capillary column (FID detector CP-2330 Supelco, Bellefonte, USA). The conditions of the method were: carrier gas, helium; flame ionization detection

temperature, 220 °C; split rate: 1/50, oven temperature programmed to rise from 120 °C/2 min to 220 °C/15 min at a rate of 5 °C/1 min; injector temperature, 240 °C. The identification of the individual methyl esters was achieved by comparison of their retention times with commercial standards (Sigma, St. Louis, MO, USA). All analytical determinations were done in triplicate.

Statistical analysis

Results were expressed as mean and standard deviation (means ± SD). For the proximate composition and fatty acids, 3 samples per experimental diet were analyzed and the average value calculated for each diet. With respect to the proximate composition and fatty acid concentrations of fillet, 20 fish per experimental group were analyzed and the average values used for each dietary group. Data were subjected to one-way ANOVA, and a subsequent comparison of means by Tukey's multiple range test was performed. All of the above mentioned statistical analyses were performed using SPSS (Version 10 for Windows). Differences were considered statistically significant at $P < 0.05$ (25).

Results

Proximate and fatty acid compositions of the experimental diets

Proximate composition and gross energy values were found to be similar in the 5 experimental diets (Table 1). The total lipid contents in the experimental diets ranged from 14.9% to 15.8% of dry diet ($P > 0.05$). The fatty acid compositions of the diets were significantly different (Table 2). The FO diet contained high levels of HUFA, especially EPA and DHA (8.2% and 12.4%, respectively), and low levels of total n-6

Table 1. Ingredients and proximate composition of the 5 experimental diets.

	Experimental diets				
	FO	CSO25	CSO50	CSO75	CSO100
Ingredients (%)					
Fish meal	34.8	34.8	34.8	34.8	34.8
Soybean meal	30	30	30	30	30
Wheat	9	9	9	9	9
Corn gluten	5	5	5	5	5
Wheat gluten	4	4	4	4	4
Fish oil (anchovy oil)	16.6	12.4	8.3	4.1	0
Cottonseed oil	0.00	4.1	8.3	12.4	16. 6
Mineral premix[1]	0.5	0.5	0.5	0.5	0.5
Vitamin premix[1]	0.1	0.1	0.1	0.1	0.1
proximate composition[2]					
Dry matter (%)	91.3	92.1	90.4	90.4	91.1
Crude protein (% DM)	44.1	45.5	45.4	45.1	45.1
Lipid (% DM)	15.2	15.8	14.9	15.6	15.4
Ash (% DM)	8.3	8.8	8.5	8.5	8.9
Crude cellulose (% DM)	1.7	1.7	1.5	1.5	1.6
NFE[3] (% DM)	23.0	20.4	19.9	19.8	20.0
Gross energy (kJ g^{-1} DM)	20.2	20.5	20.1	20.2	20.0

[1]Vitamin and mineral requirements of the species were met in accordance with NRC (20).
[2]Data are reported as mean (n = 3).
[3]NFE: nitrogen-free extract.

Table 2. Total lipid and fatty acid composition in the 5 experimental diets[1].

Total lipid and fatty acids	Experimental diets				
	FO	CSO25	CSO50	CSO75	CSO100
Total lipid (%, DWB)	15.2 ± 0.12	15.8 ± 0.23	14.9 ± 0.18	15.6 ± 0.08	15.4 ± 0.13
n-3 HUFA in diet (%, DWB)	3.1 ± 0.03^{a}	2.3 ± 0.01^{b}	1.3 ± 0.01^{c}	0.78 ± 0.02^{d}	0.35 ± 0.00^{e}
DHA in diet (%, DWB)	1.9 ± 0.04^{a}	1.4 ± 0.01^{b}	0.83 ± 0.02^{c}	0.45 ± 0.03^{d}	0.23 ± 0.01^{e}
EPA in diet (%, DWB)	1.2 ± 0.01^{a}	0.90 ± 0.02^{b}	0.48 ± 0.01^{c}	0.33 ± 0.01^{d}	0.13 ± 0.00^{e}
LNA in diet (%, DWB)	0.29 ± 0.02^{a}	0.25 ± 0.03^{b}	0.21 ± 0.02^{b}	0.17 ± 0.01^{c}	0.11 ± 0.01^{c}
Fatty acids (% of total fatty acids detected)					
14:0	5.7 ± 0.04^{a}	4.3 ± 0.03^{b}	3.1 ± 0.08^{c}	2.8 ± 0.02^{d}	1.7 ± 0.08^{e}
16:0	17.5 ± 0.07^{a}	17.4 ± 0.04^{a}	16.9 ± 0.30^{a}	15.9 ± 0.01^{b}	17.7 ± 0.06^{a}
18:0	3.9 ± 0.05	3.1 ± 0.04	3.2 ± 0.02	3.5 ± 0.04	3.7 ± 0.06
Total saturates[2]	28.1 ± 0.07^{a}	26.9 ± 0.04^{b}	24.6 ± 0.07^{c}	23.8 ± 0.03^{c}	24.8 ± 0.05^{c}
16:1	6.4 ± 0.02^{a}	4.6 ± 0.01^{b}	3.9 ± 0.02^{c}	2.7 ± 0.01^{d}	1.6 ± 0.01^{e}
18:1n-9	17.6 ± 0.07^{a}	15.7 ± 0.08^{bc}	15.2 ± 0.06^{c}	16.4 ± 0.13^{ab}	16.7 ± 0.06^{ab}
20:1n-9	1.2 ± 0.01^{a}	0.94 ± 0.02^{b}	0.78 ± 0.01^{c}	0.56 ± 0.00^{d}	0.69 ± 0.01^{d}
22:1n-9	0.87 ± 0.01^{a}	0.52 ± 0.02^{b}	0.39 ± 0.02^{d}	0.45 ± 0.01^{c}	0.42 ± 0.02^{cd}
24:1n-9	0.67 ± 0.00^{a}	0.18 ± 0.02^{c}	0.23 ± 0.01^{b}	0.25 ± 0.01^{b}	0.28 ± 0.03^{b}
Total monoenes[3]	26.7 ± 0.05^{a}	21.8 ± 0.07^{b}	20.5 ± 0.03^{b}	20.4 ± 0.04^{b}	21.7 ± 0.06^{b}
18:2n-6	6.1 ± 0.03^{e}	10.8 ± 0.04^{d}	21.7 ± 0.09^{c}	32.5 ± 0.12^{b}	43.4 ± 0.26^{a}
18:3n-6	0.01 ± 0.00^{e}	0.02 ± 0.00^{d}	0.04 ± 0.00^{c}	0.08 ± 0.01^{b}	0.12 ± 0.01^{a}
20:4n-6	0.6 ± 0.02^{a}	0.5 ± 0.01^{a}	0.3 ± 0.00^{b}	0.4 ± 0.02^{b}	0.3 ± 0.01^{b}
18:3n-3	1.9 ± 0.02^{a}	1.6 ± 0.03^{b}	1.4 ± 0.01^{b}	1.1 ± 0.02^{c}	0.60 ± 0.01^{c}
20:3n-3	0.15 ± 0.00^{a}	0.11 ± 0.01b^{c}	0.07 ± 0.00^{c}	0.01 ± 0.00^{d}	0.01 ± 0.00^{d}
20:5n-3	8.2 ± 0.05^{a}	5.7 ± 0.06^{b}	3.2 ± 0.04^{c}	2.1 ± 0.07^{d}	0.83 ± 0.10^{e}
22:6n-3	12.4 ± 0.04^{a}	8.8 ± 0.05^{b}	5.6 ± 0.03^{c}	2.9 ± 0.02^{d}	1.5 ± 0.06^{e}
Total polyenes	29.4 ± 0.08^{cd}	27.6 ± 0.23^{d}	32.3 ± 0.35^{c}	39.0 ± 0.42^{b}	46.3 ± 0.19^{a}
Total n-6 PUFA	6.8 ± 0.03^{e}	11.4 ± 0.02^{d}	22.0 ± 0.04^{c}	32.9 ± 0.07^{b}	43.8 ± 0.11^{a}
Total n-3 PUFA	22.6 ± 0.08^{a}	16.2 ± 0.04^{b}	10.3 ± 0.05^{c}	6.1 ± 0.03^{d}	2.9 ± 0.02^{e}
Total n-3 HUFA	20.7 ± 0.06^{a}	14.6 ± 0.05^{b}	8.9 ± 0.01^{c}	5.0 ± 0.02^{d}	2.3 ± 0.02^{e}
Total n-3/Total n-6	3.3 ± 0.03^{a}	1.4 ± 0.01^{b}	0.47 ± 0.02^{c}	0.18 ± 0.01^{d}	0.07 ± 0.00^{e}

DWB: Dry weight basis

[1]Data are reported as mean ± SD (n = 3). Means with different superscript letter in a row are significantly different ($P < 0.05$).

[2]Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0, and 24:0. [3]Includes 14:1, 15:1, and 17:1.

Abbreviations: PUFA, polyunsaturated fatty acid; HUFA, high unsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LNA, linolenic acid (18:3n-3).

PUFA (6.8%). The CSO100 diet had high levels of LA (43.4%; 18:2n-6) and total polyenes (46.3%) and low levels of total n-3 PUFA (2.9%). The total n-6 PUFA percentage gradually increased and the total n-3 HUFA percentage gradually decreased in the experimental diets with the increase of cottonseed oil percentages ($P < 0.05$). In all 5 experimental diets, the most abundant monoenes were 18:1n-9 and 16:1.

Growth performance of rainbow trout

Growth performance values were significantly different among treatments. At the end of the feeding trials, the highest body weights ($P < 0.05$) were recorded in fish fed the CSO50 and CSO25 diets (Table 3). However, fish weights were similar in the other experimental groups ($P > 0.05$). Fish fed the CSO50 diet had the highest SGR and the lowest FCR levels ($P < 0.05$). The highest CF, HSI, and VSI were observed in fish fed the CSO100 diet. However, fish fed the FO diet had the lowest HSI percentages (Table 3; $P < 0.05$).

Proximate composition of fish fillet

Proximate composition of adult rainbow trout fillets was significantly affected by the experimental diets (Table 4; $P < 0.05$). Crude protein was higher in fish fed the FO and CSO25 diets than it was in those fed the other experimental diets. In contrast, the crude lipid levels of fish fed with FO and CSO25 were lower than those of other fish groups ($P < 0.05$). The moisture level was higher in fish fed the CSO25 diet than it was in those fed the other experimental diets ($P < 0.05$). The crude lipid levels of the fish liver increased with increasing cottonseed oil percentages in the diets ($P < 0.05$).

Fatty acid composition of fish fillet

The fatty acid composition of fish fillets is shown in Table 5 and Figure 2. The fatty acid compositions of fillet lipids are closely related to the dietary fatty acids. The concentrations of saturated fatty acids were similar in fish from all dietary groups ($P > 0.05$), except for the fish fed the CSO25 diet. Fish fed the

Table 3. Values of initial and final weights, specific growth rate (SGR), feed conversion ratio (FCR), condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) of rainbow trout fed 5 experimental diets[1].

Growth parameters	Groups of experimental fish				
	FO	CSO25	CSO50	CSO75	CSO100
Initial body weight (g)	90.1 ± 2.56	90.2 ± 2.84	90.1 ± 2.06	88.3 ± 2.52	88.0 ± 2.21
Final body weight (g)	143.4 ± 3.32^{b}	147.1 ± 3.24^{ab}	151.6 ± 3.18^{a}	143.8 ± 3.04^{b}	142.8 ± 2.97^{b}
SGR[2]	0.82 ± 0.05^{c}	0.86 ± 0.04^{b}	0.92 ± 0.05^{a}	0.86 ± 0.06^{b}	0.85 ± 0.05^{b}
FCR[3]	1.4 ± 0.10^{a}	1.4 ± 0.12^{a}	1.3 ± 0.11^{b}	1.4 ± 0.13^{a}	1.4 ± 0.12^{a}
CF[4]	1.1 ± 0.22^{b}	1.2 ± 0.19^{a}	1.1 ± 0.23^{b}	1.2 ± 0.19^{a}	1.2 ± 0.22^{a}
HSI[5]	0.93 ± 0.08^{c}	0.99 ± 0.11^{b}	1.01 ± 0.09^{b}	1.00 ± 0.10^{b}	1.16 ± 0.10^{a}
VSI[6]	11.2 ± 0.19^{c}	11.5 ± 0.14^{c}	11.6 ± 0.13^{c}	13.1 ± 0.17^{b}	14.7 ± 0.19^{a}

[1] Data are reported as mean ± SD. (n=2 except for HSI and VSI where n=10). Means with different superscript letter in a row are significantly different (P<0.05).
[2] SGR = specific growth rate = [(ln final weight-ln initial weight) /days]×100.
[3] FCR = feed conversion ratio = (feed intake/fish weight gain).
[4] CF = condition factor = 100×[(body weight (g) /length (cm)].
[5] HSI = hepatosomatic index = (liver weight /body weight)×100.
[6] VSI = viscerosomatic index = 100×(viscera weight/body weight).

Table 4. Fillet proximate composition and crude lipid in liver of rainbow trout fed 5 experimental diets[1].

	Groups of experimental fish				
	FO	CSO25	CSO50	CSO75	CSO100
Crude protein	18.4 ± 0.05^{a}	18.3 ± 0.02^{a}	17.7 ± 0.04^{b}	17.7 ± 0.03^{b}	17.4 ± 0.03^{b}
Crude lipid	6.8 ± 0.08^{b}	6.5 ± 0.04^{b}	7.7 ± 0.05^{a}	7.5 ± 0.02^{a}	8.2 ± 0.03^{a}
Ash	1.6 ± 0.02^{b}	1.7 ± 0.03^{a}	1.7 ± 0.01^{a}	1.7 ± 0.05^{a}	1.7 ± 0.04^{a}
Moisture	73.1 ± 0.14^{b}	75.2 ± 0.17^{a}	72.9 ± 0.19^{b}	73.1 ± 0.012^{b}	72.4 ± 0.09^{b}
Crude lipid of liver	3.0 ± 0.04^{b}	3.2 ± 0.04^{b}	3.4 ± 0.03^{ab}	3.7 ± 0.05^{a}	4.0 ± 0.02^{a}

[1]Data are reported as mean ± SD (n = 3). Means with different superscript letter in a row are significantly different (P < 0.05).

Table 5. Fatty acid composition (% of total fatty acids detected) in fillet of rainbow trout fed 5 experimental diets[1].

Fatty acids	Groups of experimental fish					
	Initial	FO	CSO25	CSO50	CSO75	CSO100
14:0	3.9 ± 0.02^{a}	4.3 ± 0.03^{a}	2.1 ± 0.08^{cd}	1.9 ± 0.02^{d}	2.9 ± 0.03^{b}	1.7 ± 0.88^{d}
16:0	15.1 ± 0.05^{c}	17.5 ± 0.02^{b}	19.8 ± 0.30^{a}	18.9 ± 0.01^{a}	17.2 ± 0.08^{b}	16.5 ± 0.02^{bc}
18:0	3.9 ± 0.01	4.0 ± 0.05	4.0 ± 0.02	3.9 ± 0.03	4.1 ± 0.01	4.1 ± 0.06
Total saturates[2]	24.8 ± 0.07^{b}	25.5 ± 0.06^{ab}	26.0 ± 0.08^{a}	25.1 ± 0.05^{b}	24.5 ± 0.07^{b}	23.9 ± 0.09^{b}
16:1n-9	4.7 ± 0.01^{a}	5.3 ± 0.02^{a}	3.4 ± 0.03^{b}	2.9 ± 0.02^{c}	1.7 ± 0.02^{d}	1.6 ± 0.01^{d}
18:1n-9	20.2 ± 0.07^{a}	19.6 ± 0.05^{a}	15.2 ± 0.64^{c}	16.4 ± 0.13^{c}	15.7 ± 0.08^{c}	18.3 ± 0.02^{b}
20:1n-9	1.6 ± 0.02^{a}	1.7 ± 0.02^{a}	1.5 ± 0.01^{a}	1.3 ± 0.01^{b}	1.3 ± 0.02^{b}	1.1 ± 0.03^{c}
22:1n-9	0.25 ± 0.01^{a}	0.21 ± 0.01^{a}	0.19 ± 0.00^{ab}	0.14 ± 0.01^{c}	0.18 ± 0.02^{b}	0.17 ± 0.00^{b}
24:1n-9	0.28 ± 0.01^{b}	0.43 ± 0.01^{a}	0.23 ± 0.01^{b}	0.25 ± 0.01^{b}	0.18 ± 0.02^{c}	0.21 ± 0.01^{bc}
Total monoenes[3]	27.4 ± 0.05^{a}	27.6 ± 0.07^{a}	21.1 ± 0.09^{b}	21.4 ± 0.04^{b}	20.0 ± 0.05^{b}	21.7 ± 0.07^{b}
18:2n-6	19.9 ± 0.14^{c}	11.3 ± 0.03^{e}	15.0 ± 0.08^{d}	19.7 ± 0.04^{c}	25.3 ± 0.03^{b}	29.4 ± 0.02^{a}
20:4n-6	0.65 ± 0.01^{ab}	0.78 ± 0.02^{a}	0.71 ± 0.01^{a}	0.61 ± 0.03^{b}	0.52 ± 0.02^{c}	0.55 ± 0.00^{d}
18:3n-3	2.1 ± 0.00^{a}	1.8 ± 0.02^{ab}	1.8 ± 0.02^{ab}	1.7 ± 0.01^{b}	1.8 ± 0.01^{ab}	1.9 ± 0.02^{a}
20:3n-3	0.41 ± 0.00^{a}	0.32 ± 0.00^{b}	0.35 ± 0.02^{b}	0.24 ± 0.00^{d}	0.27 ± 0.01^{c}	0.18 ± 0.00^{e}
20:5n-3	3.8 ± 0.04^{c}	6.4 ± 0.03^{a}	5.9 ± 0.01^{b}	3.6 ± 0.03^{c}	2.7 ± 0.02^{d}	2.1 ± 0.00^{e}
22:6n-3	8.9 ± 0.04^{c}	14.0 ± 0.06^{a}	10.7 ± 0.04^{b}	6.1 ± 0.02^{d}	4.6 ± 0.01^{e}	3.1 ± 0.04^{f}
Total polyenes	35.8 ± 0.05^{a}	34.6 ± 0.03^{b}	34.5 ± 0.06^{b}	31.9 ± 0.09^{c}	35.2 ± 0.15^{b}	37.2 ± 0.07^{a}
Total n-6 PUFA	20.6 ± 0.04^{c}	12.0 ± 0.03^{e}	15.5 ± 0.05^{d}	20.8 ± 0.04^{c}	25.8 ± 0.03^{b}	29.9 ± 0.02^{a}
Total n-3 PUFA	15.2 ± 0.05^{c}	22.6 ± 0.03^{a}	18.8 ± 0.06^{b}	11.7 ± 0.02^{d}	9.4 ± 0.04^{e}	7.3 ± 0.03^{f}
Total n-3 HUFA	13.1 ± 0.03^{c}	20.7 ± 0.06^{a}	16.9 ± 0.04^{b}	10.0 ± 0.05^{d}	7.6 ± 0.04^{e}	5.4 ± 0.04^{f}
n-3/n-6	0.74 ± 0.02^{c}	1.87 ± 0.01^{a}	1.21 ± 0.01^{b}	0.56 ± 0.02^{d}	0.37 ± 0.02^{e}	0.24 ± 0.01^{f}

[1]Data are reported as mean ± SD (n = 3). Means with different superscript letter in a row are significantly different (P < 0.05).
[2]Includes 15:0, 17:0, 20:0, 21:0, 22:0, 23:0, and 24:0. [3]Includes 14:1, 15:1, and 17:1.
Abbreviations: PUFA, polyunsaturated fatty acid; HUFA, high unsaturated fatty acid.

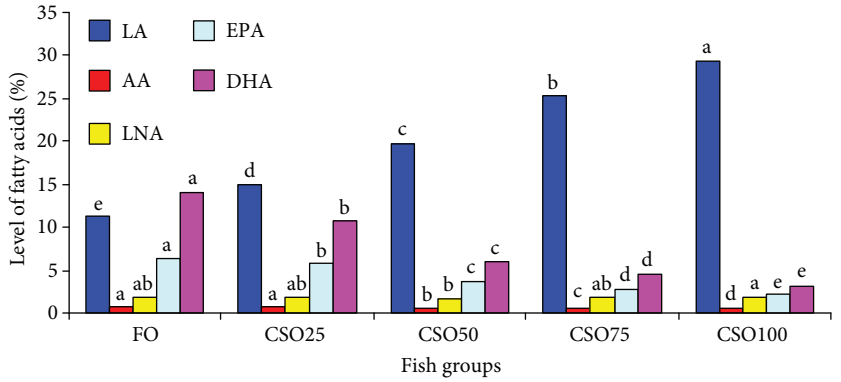

Figure 2. Levels of some essential fatty acids (% of total fatty acids detected) in the fish fillets.

CSO25 and FO diet had the highest concentration of saturates. The palmitic acid (16:0) concentration was the most abundant fatty acid of saturates in fish fillets. The monoenes concentration initially showed higher levels in fish fed the FO diets than in those fed with the other 4 diets. Oleic acid (18:1) was the most abundant fatty acid in fish from all dietary groups ($P < 0.05$). LA concentration was the highest in fish fed the CSO100 diet and the lowest in those fed the FO diet ($P < 0.05$). The amount of total n-3 HUFA differed significantly among the different treatments ($P < 0.05$). The n-3 HUFA concentrations of fish fillets decreased gradually with increasing cottonseed oil levels ($P < 0.05$). While fish fed the FO diet contained the highest concentration of n-3 HUFA, those fed the CSO100 diet contained the lowest level. Similarly, EPA and DHA concentrations were found to be the highest in fish fed the FO diet and the lowest in those fed the CSO100 diet. The ratios of n-3 to n-6 fatty acid decreased with increasing cottonseed oil percentages in diets ($P < 0.05$). Therefore, fish fed the FO diet had the highest ratios of n-3 to n-6 fatty acid. In comparison to the dietary ARA concentrations, the level of this fatty acid increased in the fillet lipids from all of the experimental fish groups. Similarly, ARA concentrations in fish fillets were lower than in the fillet of fish fed with FO or CSO25 and higher than in those fed CSO75 and CSO100 ($P < 0.05$).

Discussion

The present study has shown that it was possible to replace fish oil by cottonseed oil in diets for rainbow trout, either totally or partial, without a negative effect on growth and feed utilization. However, it was observed that the fish fillet composition and liver total lipid level of the fish were significantly influenced by the total (CSO100) or partial (CSO75) replacement of dietary fish oil by cottonseed oil. Fish fed the dietary treatments CSO75 and CSO100 had the highest crude lipid content and the lowest crude protein or moisture content compared to fish fed the other 3 diets. Several studies have been conducted to investigate various vegetable oils as a possible fish oil replacement, either totally or partially, in rainbow trout feed (8-12,26,27). These studies indicated that an up to 80% or 100% replacement of fish oil by several vegetable oils had no significant effect on growth performance or feed utilization efficiency of this species.

It has previously been reported that n-3 PUFA, including both 18:3n-3 and HUFAs, was required for optimal growth and prevention of signs of EFA deficiency in rainbow trout (1,10,26-28). Furthermore, the EFA requirements estimated for juveniles and subadults of the freshwater fish species studied so far indicate that the EFA requirements can generally be satisfied by the C18 PUFA, 18:3n-3, and/or 18:2n-6, at around 1% of the dry diet weight (28). Therefore, it is possible that FAs in diets containing CSO might have been used as an energy source by rainbow trout cultured at a water temperature of 10 °C during this investigation, since there was no significant difference in growth rates obtained on fish fed diets containing CSO compared to that of fish fed a fish oil only diet.

In the present study, the replacement of fish oil with cottonseed oil had no significant effect on fish growth or FCR. Similar studies on rainbow trout and Atlantic salmon (*Salmo salar*) have reported no significant impact on growth, feed intake, or FCR (4,9,12,29). The level of FCR in the present study ranged from 1.3 to 1.4. However, Şener and Yıldız (12) found that the mean level of FCR from juvenile rainbow trout (initial average body weight around 5.8 g) fed diets containing 100% fish oil or vegetable oils (soybean or sunflower) was 1.1 under the experimental conditions of water temperature of 13 ± 1 °C. The FCR level being higher in the present study than it was in the earlier study (12) is thought to be due to the larger size of the experimental fish (initial average body weight around 88 g and 90 g) and lower water temperature (10.0 ± 1.2 °C).

The present study also showed that the HSI and VSI levels of fish fed the CSO100 diet was higher than that of the fish fed other diets. Fillet FA composition of the rainbow trout was significantly influenced by dietary lipid sources. An increased level of 18:2n-6 was observed in the fillet of rainbow trout fed dietary treatment CSO100. Caballero et al. (9) also reported that no significant differences in the HSI and VSI levels were found among rainbow trout fed diets containing vegetable oils (soybean, grapeseed, olive, and palm) compared to those of fish fed a fish oil diet. However, Şener and Yıldız (12) found that the HSI calculated from rainbow trout juveniles fed a diet containing 100% fish oil was significantly lower than that of fish fed diets containing vegetable oils (soybean or sunflower), whereas the VSI in fish fed all dietary treatments was similar, indicating higher selective certain lipid deposition in the livers of fish fed vegetable oils.

The lipid content of fish tissues could be markedly influenced by the dietary lipid source (1) and similar results were obtained from many studies (10,11) where rainbow trout were fed different plant oils. These investigations showed that the high dietary levels of 18:2n-6 or 18:1n-9 was apparently leading to the accumulation of these FAs, particularly in the liver and body of the fish. Biological availability of dietary lipids is directly related to their chemical and physical properties, including chain length and degree of saturation of triglyceride bound FAs (24). In the present study, the replacement of fish oil with cottonseed oil resulted in reduced levels of total n-3 PUFA, and increased the level of total n-6 PUFA in the fillet. In contrast, total saturated FA levels were minimally influenced by dietary treatments despite a wide range of values from 23.9% in the CSO100 diet to 26.0% in the CSO25 diet. The minimal impact of diets on saturated FAs in the fish body was also observed by other studies made on rainbow trout (9,11,12). In line with the findings of the our study, the replacement of fish oil by cottonseed oil was reported to result in significant changes in muscle FA composition in many other marine fish species such as sea bass (13,16,18,19), Atlantic salmon, *Salmo salar* (30), and gilthead sea bream, *Sparus aurata* (16,17).

In general, the replacement of dietary fish oil with vegetable oils resulted in a lower level of n-3 PUFA in the fish flesh. Particularly, EPA and DHA levels in the body of fish were strongly influenced by the dietary levels of EPA and DHA. Feeding rainbow trout plant oil-based diets markedly decreased the levels of these essential FAs in the body. Most vegetable oils are rich in unsaturated 18C FAs (18:1n-9; 18:2n-6; 18:3n-3) but are poor sources of n-3 HUFAs. Many freshwater fish such as rainbow trout are able to convert dietary LA and LNA to HUFA, such as ARA, EPA, and DHA (1,9,28). Therefore, FAs of the n-6 series are also required for rainbow trout (7,9,28). The selective accumulation of 20:3n-3 and 22:5n-3, which were not detected or existed in small amounts in the diets, might have resulted in the activation of Δ6 and Δ5 desaturases when trout were fed diets with low n-3 HUFA, since these are considered as intermediate metabolites of polyunsaturated fatty acid synthesis. Furthermore, in the present study, it also appeared that EPA and DHA were selectively deposited and retained, as fish fillet EPA and DHA levels were always higher than diet levels. This has also been observed in earlier studies in rainbow trout (9,26,27). Moreover, the mechanism of selective FA deposition may include the high specificity of fatty acyltransferase for DHA and the relative resistance of DHA to beta-oxidation because of the complex catabolic pathway required for these FAs (26). The lower concentrations of LA and LNA in the fillet of fish fed the cottonseed oil-based diets as compared to their levels in the corresponding diets may suggest that rainbow trout utilized these FAs for oxidation.

This is in agreement with similar data reported for rainbow trout (9-12), gilthead sea bream (17), and sea bass (13). These studies also demonstrated that total or partial replacement of dietary fish oil by different vegetable oils in the diets of those species did not affect the growth or feed utilization. Although polyunsaturated fatty acid synthesis, namely in the elongation and desaturation of 18C atoms precursors, was found for all diets tested in this study, it seemed that it was more evident in those fish fed lower n-3 HUFA diets (CSO75 and CSO100), thus balancing the DHA level in the fish fillet and suggesting the importance of DHA in physiological processes such as membrane permeability and fluidity (9,27). Caballero et al. (9) and Bell and Dick (31) also reported a reduced percentage of 20:5n-3 compared to 22:6n-3 in the muscle of trout that were fed diets containing vegetable oils, suggesting the possible metabolic competition between 18:2n-6 and 18:3n-3 since both fatty acids are substrates for the same enzymes Δ6-desaturases. Furthermore, it was concluded that a high content of 18:2n-6 in dietary soybean oil might have inhibited the conversion of 18:3n-3 into the longer chain 20:5n-3 and 22:6n-3 essential fatty acids.

In summary, our results indicated that the replacement of fish oil with cottonseed oil in rainbow trout diets did not cause any negative effect on growth performance. However, diets supplemented with a 75% or 100% substitution of fish oil by cottonseed oil increased fillet lipid and reduced protein contents in rainbow trout. Feeding the cottonseed oil diets increased the levels of LA or total n-6 PUFA and decreased EPA or DHA and n-3/n-6 ratio in the fish fillet. In particular, reductions in the levels of n-3 HUFA and increases in the levels of LA fatty acids in the fish fillet arise when feeding with CSO75 or CSO100. Fish fed CSO25 or CSO50 showed a good balance of n-3 HUFA and LA in their fillets when compared with fish fed CSO75 or CSO100. Finally, the results of growth performance, fillet proximate composition, and fatty acid concentration of experimental fish have shown that it is possible to substitute 50% fish oil by cottonseed oil in diets for rainbow trout.

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