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Effects of Dietary Cottonseed and/or Canola Oil Inclusion on the Growth Performance, FA Composition and Organ Histology of the Juvenile Rainbow Trout, *Oncorhynchus mykiss*

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Abstract

This study aimed at demonstrating the effects of total and 50% dietary fish oil replacement by cotton seed and canola oils and a mixture of these vegetable oils (VOs) on growth, tissue fatty acid composition and histology of digestive organs in the juvenile rainbow trout for 84 days. Five iso-nitrogenous and lipidic diets were formulated to replace dietary fish oil (FO) totally by cotton seed oil (CSO), canola oil (CO) and an equal or 50% FO/ 50% VO mixture. Duplicate groups of 50 fish (~15 g) were fed two times daily to apparent satiation. Growth performance, feed efficiency and viscerosomatic index were not influenced by dietary treatments ($P>0.05$). However, hepatosomatic index was significantly higher ($P<0.05$) in fish fed with CSO and CSO50/CO50 diets. Fish fed CSO or CO diets had significantly lower levels of n-3 highly unsaturated FAs and increased levels of 18:2n-6 in the whole body FA composition ($P<0.05$). Furthermore, liver had significantly higher levels of docosahexaenoic acid and arachidonic acid than those in the fish whole body. Results suggest that the 50% replacement of dietary fish oil by cotton seed oil and canola oils in equal amounts could be possible without compromising growth and overall well-being in the juvenile rainbow trout.

Keywords: Rainbow trout, nutrition, vegetable oils, FAs, growth.

Diyetlerde Kullanılan Pamuk Tohumu ve Kanola Yağlarının Jüvenil Gökkuşağı Alabalıklarında (*Oncorhynchus mykiss*) Büyüme Performansı, Yağ Asidi Kompozisyonu ve Organ Histolojisine Etkileri

Özet

Bu araştırmada, diyetlerde balık yağı yerine %50 oranında kullanılan pamuk tohumu yağı (PTY) ve kanola yağı (KY) ya da bu yağların eşit karışımlarının jüvenil gökkuşağı alabalığının büyümesine, yağ asidi kompozisyonuna ve sindirim sistemi histolojisine etkileri araştırılmıştır. Araştırmada, farklı 5 adet izonitrojenik ve izolipidik diyetler hazırlandı. Kontrol diyetinde sadece balık yağı (BY) ve hazırlanan diğer diyetlerde sırasıyla sadece PTY, sadece KY, %50 PTY/%50 KY ve %50 BY/%50 belirtilen bitkisel yağların eşit karışımı kullanılmıştır. Deneyde ortalama ağırlıkları yaklaşık olarak 15 g olan gökkuşağı alabalığı yavruları kullanılmıştır. Deney paralelli ve her deney tankında 50 adet balık olacak şekilde 84 gün süreyle yürütülmüştür. Balıklara günlük olarak doyuncaya kadar ve elle yem verilmiştir. Araştırmanın sonuçlarına göre balıkların büyüme performansı, yem kullanımı ve viserosomatik indeks değerleri kullanılan diyetlerden etkilenmemiştir ($P>0.05$). Ancak PTY ve PTY50/KY50 diyetleriyle beslenen balıkların hepatosomatik indeks değerleri diğer gruplardan daha yüksek bulunmuştur ($P<0.05$). PTY ya da KY içeren diyetlerle beslenen balıkların tüm vücudundaki n-3 serisindeki çok doymamış yağ asitlerinin düzeyi düşük ve 18:2n-6 yağ asitlerinin düzeyi ise yüksek bulunmuştur ($P<0.05$). Ayrıca balıkların karaciğerlerindeki dokosaheksaenoik asit ve araşidonik asit düzeyleri balığın tüm vücudundaki düzeylerden daha yüksek bulunmuştur. Sonuç olarak jüvenil gökkuşağı alabalığı diyetlerinde balık yağı yerine %50 oranında kullanılan pamuk tohumu yağı ile kanola yağının balıkların büyüme performansında herhangi bir olumsuz etki göstermediği görülmüştür.

Anahtar Kelimeler: Gökkuşağı alabalığı, besleme, bitkisel yağlar, yağ asitleri, büyüme.

Introduction

Aquaculture is currently the fastest-growing animal production sector in the world, expanding at an average annual rate of about 8-11% since 1984 (Pike and Barlow, 2002; FAO, 2006). Aquafeeds currently use about 70% of the global supply of fish oil. Fish oil used in aquafeeds is expected to reach about 79-97% of the world supply by the year 2012. This is due mainly to static and/or diminishing global

supplies of wild forage fish destined for reduction into fish meal and oil and increasing market price of these fish as a result of increasing fishing cost and demand for direct human consumption and animal feeding (Tacon and Metian, 2008). Therefore, a substantial amount of research is being conducted on the possibility of replacement of fish oil by increasing amounts of vegetable oils (Pike and Barlow, 2002; FAO, 2007; Tacon, 2008). Over the past few years, significant breakthroughs have occurred in the replacement of FO by plant oils in compounded fish feeds in order to reduce dependence on fish oil as well as reduce costs (Kaushik, 2004; Tacon and Metian, 2008; Wassef *et al.*, 2009).

Fish are the major supplier of polyunsaturated FAs (PUFA) specifically n-3 FAs (FA) including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) in human diets (Bentley, 2006; Pickova, 2009). Since the FA composition of fish fillets is a reflection of dietary FA composition (Robin *et al.*, 2003; Drew *et al.*, 2007), it is important that the dietary lipid source contain balanced levels of PUFA's and HUFA's. Fish oil is considered as an excellent dietary oil source for fish because it is rich in the essential fatty acids (EFAs) such as EPA, DHA and arachidonic acids (ARA, 20:4n-6) that are needed for optimal growth and development. The FAs in fish oil are also needed to maintain the integrity of cell membrane in fish (Sargent *et al.*, 1999; Higgs and Dong, 2000; Tocher, 2010). Vegetable oils rich in linoleic (LA, 18:2n-6) and/or α-linolenic (ALA, 18:3n-3) acids, are considered to be a better substitutes for fish oil in freshwater fish feeds, because freshwater fish are able to convert dietary LA and ALA to highly unsaturated FAs (HUFA), such as ARA, EPA and DHA (Sargent *et al.*, 2002; Tocher, 2003; Tocher, 2010). Several studies conducted on freshwater fish indicated that vegetable oils can successfully replace fish oil in fish feeds without affecting survival and growth (Rosenlund *et al.*, 2001; Sener and Yildiz, 2003; Wonnacott *et al.*, 2004; Grant, 2006; Subhadra *et al.*, 2006). Caballero *et al.* (2002) reported that in rainbow trout up to 80–90% of some vegetable oils (soybean, rapeseed, olive and palm oils) could be used without compromising the growth rates. The replacement of fish oil by vegetable oils has a profound impact on the FA composition of fish tissues with an increase in 18:2n−6 and 18:3n−3 and a decrease in n-3 HUFA (Caballero *et al.*, 2002; Bell *et al.*, 2002).

Canola oil is less expensive and far more abundant than FO on a global basis (USDA, 2006; Canola Council of Canada, 2007). Low-erucic acid canola oil is rich in 18 carbon FAs most notably the monounsaturated FA (MUFA), oleic acid (OA, 18:1n-9) as well as LA and ALA (about 20-10%). Moreover, canola oil is low in saturated FAs (SFA) (Bell *et al.*, 2002). However, this oil is devoid of n-3 HUFA and its inclusion in trout diets resulted in a significant decrease in the tissue levels of EPA and DHA (Bell *et al.*, 2002). Numerous studies indicated that replacement of fish oil by canola oil could be possible without adversely affecting fish growth performance parameters and whole body and/or fillets chemical composition and FA profiles (Rosenlund *et al.*, 2001; Higgs *et al.*, 2006). Cottonseed oil is rich in 18 carbon FAs most notably LA. Several studies demonstrated that fish oil replacement by cotton seed oil either partially or totally in diets for several fish species including gilthead sea bream (Wassef *et al.*, 2007; Wassef *et al.*, 2009) and rainbow trout (Guler and Yildiz, 2009) did not cause any negative effect either on growth and feed utilization and the main nutrient composition of the fish.

Histological changes in tissues of fish fed diets containing alternative lipid sources to fish oil were previously reported in different fish species by several authors (Bell *et al.*, 1995; Tucker *et al.*, 1997; Caballero *et al.*, 2002; Figueiredo-Silva *et al.*, 2005; Wassef *et al.*, 2007; Wassef *et al.*, 2009). Hepatocytes with large lipid vacuoles and nuclei located at the periphery of the cell and an accumulation of lipid droplets in the enterocytes from the pyloric caeca and midgut were all observed in red drum fed diets containing soybean oil (Tucker *et al.*, 1997) and in Arctic charr fed linseed oil, respectively (Olsen *et al.*, 1999, 2000). Histological studies are scarce but it can not only provide information on the diet quality and metabolism but also indicate the nutritional status of a fish (Segner and Braunbeck, 1988; Caballero *et al.*, 2004). Therefore, the present study was performed to evaluate the effect of fish oil replacement by cottonseed and/or canola oils and their equal mixtures in diets on growth performance, feed utilization, whole body FA content and organ histology of the juvenile rainbow trout, *Oncorhynchus mykiss*.

Materials and Methods

Experimental Conditions and Measurements

Rainbow trout (*Oncorhynchus mykiss*), with a mean initial body weight of 15 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 10 cylindro-conical tanks of 1000 L capacity in the Sapanca Inland Waters Research Center (Adapazari, Turkey). The tanks were supplied with freshwater having an average temperature of 13.6±0.6°C. Dissolved oxygen was maintained around 8.4±0.2 mg L^{-1} and the pH amounted to 7.3±0.6. 12 h light:12 h dark photoperiod regimen was utilized throughout the experimentation. Before starting the experiment, fish were acclimatized to the experimental feeding regimen using a commercial diet for 2 weeks (trout commercial pellet 2 mm in diameter). Fish were fed to apparent satiation by hand twice per day at 0900 and 1700 h throughout the experimentation. The experiment was lasted 12 weeks (84 days). Bulk fish live weight increments

were measured for every 2 weeks and feed intake was recorded daily throughout the experimentation. At the end of the experimentation, fish were individually weighed for determining 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. Growth performance and economic parameters are measured and listed below and the calculations were according to Ricker (1979);

Weight gain (%) = [(final weight-initial weight) / initial weight]×100;

Specific growth rate (SGR) = [(ln final weight - ln initial weight) /days] × 100;

Feed efficiency ratio (FER) = wet weight gain (g) / feed intake (g);

Protein efficiency ratio (PER) = wet weight gain (g) / protein intake (g);

Hepatosomatic index (HSI) = (liver weight / body weight) × 100;

Viscerosomatic index (VSI) = 100 × (viscera weight / body weight);

Economic conversion ratio (kg^{-1}) (ECR) = feed intake (kg) x feed cost (kg^{-1}) / weight gain (kg);

Economic Profit Index [EPI (US$ kg^{-1}) = [final weight (kg $fish^{-1}$) x fish sale price (US$ kg^{-1}) - ECR (US.$ kg^{-1}) x weight increase (kg)]. Rainbow trout sale price is calculated at 3.61 US$ kg^{-1}.

Experimental Diets

Five iso-nitrogenous (approximately 45% crude protein) and iso-lipidic (approximately 17% crude lipid) experimental diets were formulated to contain same ingredients but different lipid sources. The lipids used in diets were fish oil (anchovy oil), cottonseed oil and canola oils. The first diet contained only fish oil (FO, control group). The fish oil was totally replaced by cottonseed oil (CSO) and canola oils (CO) in the second and the third diets. However, the fish oil in the last two diets was replaced by the equal mixture of cotton seed oil (CSO) and canola (CO) oils either totally (CSO50/CO50) or in half (FO50/CSO25/CO25). The dietary ingredients and proximate compositions are given in the Table 1. The FA compositions of diets are presented in Table 2. Experimental diets (2-4 mm diameter) were produced at the Sapanca Inland Waters Research Center (Adapazari, Turkey) of Istanbul University as steam pressured pellets using a laboratory feed mill (KAHL-L, 173). Diets were kept in plastic storage bags at -20°C until used.

Proximate Analysis

Feed ingredients, experimental diets, and fish samples were analyzed for proximate composition (protein, lipid, ash, fiber and moisture) according to standard methodology of AOAC (1995). Moisture content was obtained by weight loss after drying samples in an oven at 105°C until constant weight. Crude protein was determined as total nitrogen (N) by

Table 1. Ingredients and proximate composition of the experimental diets

	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Ingredients (g kg^{-1} dry weight)					
Fish meal	450	450	450	450	450
Soybean meal (Defatted)	150	150	150	150	150
Wheat gluten	100	100	100	100	100
Wheat bran	30	30	30	30	30
Corn gluten	50	50	50	50	50
Gelatin	50	50	50	50	50
Fish oil (Anchovy oil)	150			75	
Cottonseed oil		150		37.5	75
Canola oil			150	37.5	75
Mineral premix [a]	10	10	10	10	10
Vitamin premix [a]	10	10	10	10	10
Analyzed proximate composition(g.kg^{-1})					
Moisture	113.8	118.9	121.0	121.2	122.2
Crude protein	454.7	450.3	449.9	454.1	454.9
Lipid	165.3	168.4	168.7	164.5	165.0
Ash	84.5	83.1	83.0	82.5	84.4
Crude cellulose	28.3	27.7	27.5	27.5	28.2
NFE[b]	153.3	151.6	150.0	150.2	145.3
Gross energy (kJ g^{-1})[c]	199.3	199.1	198.8	198.2	197.8

[a] Premix of vitamins and minerals according to NCR (1993) recommendations for fish. [b] NFE: nitrogen-free extract calculated by difference.
[c] Gross energy was calculated as described by Halver (1972).

Table 2. Total lipid (%) and fatty acid composition (% of total fatty acids detected) of the experimental diets containing Cotton Seed Oil (CSO) and Canola Oil (CO) and their combinations[a]

Total lipid and fatty acids	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Total lipid	16.5	16.8	16.9	16.4	16.5
Fatty acids					
14:0	7.3	2.1	1.2	4.4	2.2
16:0	20.5	24.2	9.1	18.9	17.6
18:0	3.2	2.4	1.8	2.8	2.2
20:0	0.5	ND	0.3	0.4	ND
Total saturates[b]	33.5	28.7	12.4	27.6	22.1
16:1n-7	6.5	1.9	1.4	3.9	2.2
18:1n-9	16.5	14.6	49.9	23.2	27.7
20:1n-9	2.7	1.4	2.1	2.3	2.4
22:1n-11	0.8	0.4	0.5	0.6	0.6
Total monoenes[c]	29.2	19.8	56.1	32.3	35.4
18:2n-6	6.9	44.7	21.1	20.7	31.0
20:4n-6	1.7	0.5	0.4	1.1	0.7
Total n-6[d]	8.7	45.2	21.6	21.8	31.7
18:3n-3	1.5	0.5	6.3	2.8	3.5
20:5n-3	9.5	1.5	1.2	5.4	2.2
22:5n-3	0.9	ND	ND	0.5	ND
22:6n-3	15.1	3.7	3.0	9.1	4.7
Total n-3[e]	27.0	5.8	10.4	17.9	10.5
n-3/n-6 ratio	3.1	0.1	0.5	0.8	0.3

[a] Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 10 fish). [b] Includes 14:0, 15:0, 16:0, 17:0, 18:0 and 20:0. [c] Includes 16:1n-7, 18:1n-9, 20:1n-9 and 22:1n-11. [d] Includes 18:2n-6 and 20:4n-6. [e] Includes 18:3n-3, 20:5n-3, 225n-3 and 22:6n-3. ND: Not Detected.

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 ^{o}C for about 12 h in a Muffle Furnace. Crude fiber was determined using sulfuric acid then sodium hydroxide, 12.5% (w/w) for half an hour 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 FA Analysis

Lipid was extracted from individual whole body and feed samples by homogenization in chloroform / methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to the methods of Folch *et al.* (1957). FAs methyl esters (FAME) were prepared by transmethylation using 2M potassium hydroxide (KOH) (Merck, Darmstadt, Germany) in methanol and n-hexane (Sigma-Aldrich, Steinhein, Germany) according to the method described by Ichihara *et al.* (1996) with minor modification; 10 mg of extracted oil were dissolved in 2 ml hexane followed by 4 ml of 2M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4000 rpm for 10 min, the hexane layer was taken for GC analyses. The FA composition was analyzed by a gas chromatograph (Auto System XL Perkin Elmer) using a 30 x 0.25 mm capillary column (FID detector CP-2380 Supelco, Bellefonte, USA). The conditions of the method were: carrier gas, helium; flame ionization detection temperature, 260°C; split rate: 1 / 50, oven temperature programmed to rise from 120^{o}C / 2 min to 220 ^{o}C / 15 min at a rate of 5°C min)1; 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).

Tissue Sampling and Histological Examination

For histological investigations five fish from per tank in each treatment were sampled and systemic necropsy was performed on them. Tissue samples taken from the liver, kidney, stomach, intestines, pancreas and gills were fixed in 10% formaldehyde-saline solution. Tissues were then treated with the series of alcohol and xylene solutions and embedded into parafine blocks before slicing into 3-5 µm thick pieces using rotary microtome. Following slicing, tissues were dyed with hematoxylen-eosin (H&E) and investigated under the light microscopy.

Statistical Analysis

Results were expressed as means ± SD throughout the text. For the proximate composition and FA profile analysis of each diet, three samples per experimental diet were used whereas ten whole fish body for each dietary treatment were analyzed for the same measurements. Data were subjected to one way ANOVA, and subsequent comparison of means was performed using Tukey's multiple range test. All the statistical analyses were made using SPSS statistical software (Version 14.0 for Windows). Differences were considered statistically significant at probability levels below 0.05 (Zar, 1984).

Results

Growth Performance

The survival rate varied between 94-100 % between the treatments (Table 3). The growth and feed utilization efficiency of rainbow trout juveniles fed the experimental diets were not significantly different from fish fed the FO diet (Table 3). At the end of the feeding trial, fish reached a percentage weight gain, FER and PER of 644.0-704.2% and 0.85-0.93, 1.87-2.05, respectively. VSI was not influenced by dietary treatments. However, HSI was significantly higher (1.70 and 1.76, respectively) ($P<0.05$) in fish fed with CSO and CSO50/CO50 diets than in those fed with the other experimental diets.

Economic Analysis

The per unit cost of diets was reduced with the inclusion of vegetable oils (Table 4). The ECR of the FO diet (1.35 US.$ kg^{-1}) was the highest whereas the ECRs of the diets supplemented with cottonseed and/or canola oils (1.18 US.$ kg^{-1}) were the lowest ($P<0.05$). Similarly, the lowest EPI was obtained on fish fed CSO diet (0.27 US.$ fish^{-1}) ($P<0.05$). However, there was no significant difference among the dietary treatments for EPI levels which were ranged from 0.28 to 0.30 US.$ fish^{-1}.

Whole Body Proximate Composition

Whole body proximate composition of fish was significantly influenced by dietary treatments ($P<0.05$) (Table 5). Fish fed CSO and CO had the highest crude lipid (13.5% and 12.8%, respectively) and the lowest moisture contents (68.5% and 69.2%, respectively). However, crude lipid level was similar in fish fed FO, FO50/CSO25/CO25 and CSO50/CO50 diet. Whole body protein and ash content of fish fed FO was significantly lower (15.3% and 1.5%, respectively) than those fed the other

Table 3. Growth performance of the rainbow trout (*Oncorhynchus mykiss*) fed diets containing Cotton Seed Oil (CSO) and Canola Oil (CO) and their combinations for 12 weeks[a]

Growth performance	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Initial weight (g fish^{-1})[b]	15.9±0.5	15.4±0.4	15.7±0.3	15.5±0.3	15.9±0.3
Final weight (g fish^{-1})[c]	128.1±21.0^{a}	116.8±20.8^{c}	117.2±22.2^{c}	122.0±21.1^{ab}	120.8±21.8^{ab}
Weight gain (%)[c]	704.2±15.2	676.2±54.4	656.7±26.8	659.7±22.6	644.0±0.4
Survival (%)	98.0±0.0^{a}	94.0±0.7^{c}	96.0±0.0^{b}	100.0±0.0^{a}	98.0±0.0^{a}
SGR[c]	2.5±0.2	2.4±0.4	2.4±0.1	2.5±0.1	2.4±0.3
FER[c]	0.9±0.1	0.8±0.2	0.9±0.1	0.9±0.0	0.8±0.1
PER[c]	2.1±0.3	1.8±0.1	1.9±0.2	2.0±0.0	1.9±0.1
HSI[d]	1.4±0.2^{b}	1.7±0.2^{a}	1.5±0.3^{b}	1.4±0.2^{b}	1.8±0.2^{a}
VSI[d]	13.4±1.9	13.4±2.4	11.5±1.9	12.4±2.5	12.5±2.3

[a]Data are mean ± SD. Means with different superscript letter in a row are significantly different ($P<0.05$). [b]n = 50 x 2. [c]n = 48 x 2. [d]n = 10

Table 4. Results of economic parameters for the rainbow trout (*Oncorhynchus mykiss*) fed diets containing Cotton Seed Oil (CSO) and Canola Oil (CO) and their combinations for 12 weeks [a]

Parameters	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Feed cost (US$ kg^{-1})	1.4	1.2	1.2	1.3	1.2
Feed intake (kg fish^{-1})	0.12±0.1	0.13±0.0	0.12±0.1	0.12±0.1	0.12±0.1
Weight gain (kg fish^{-1})	0.11±0.0	0.10±0.0	0.10±0.0	0.11±0.0	0.11±0.0
ECR[b] (US$ kg^{-1})	1.46±0.1^{a}	1.46±0.1^{a}	1.38±0.1^{c}	1.41±0.1^{b}	1.38±0.1^{c}
EPI[c] (US.$ fish^{-1})	0.30±0.1^{a}	0.27±0.1^{b}	0.28±0.0^{ab}	0.29±0.1^{a}	0.29±0.1^{a}

[a] Data are mean ± SD. Means with different superscript letter in a row are significantly different ($P<0.05$). [b] Economic conversion ratio.
[c] Economic Profit Index.

Table 5. Whole body proximate composition of the rainbow trout (*Oncorhynchus mykiss*) fed diets containing Cotton Seed Oil (CSO) and Canola Oil (CO) and their combinations for 12 weeks[a]

Proximate composition (%)	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Moisture	70.6±0.9^{a}	68.5±0.2^{c}	69.2±0.1^{bc}	70.1±0.4^{ab}	69.7±0.6^{ab}
Crude protein	15.3±0.6^{b}	15.6±0.3^{ab}	15.9±0.2^{a}	16.0±0.4^{a}	16.0±0.3^{a}
Crude lipid	11.9±0.8^{b}	13.5±0.2^{a}	12.8±0.1^{a}	12.0±0.1^{b}	12.0±0.2^{b}
Ash	1.5±0.1^{b}	1.9±0.2^{a}	1.8±0.2^{ab}	1.8±0.2^{ab}	1.8±0.3^{ab}

[a]Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different ($P<0.05$).

dietary treatments.

FA Composition of Whole Body and Liver

The FA composition of whole body reflected the dietary FA composition (Table 6). The total levels of saturates in the whole body was the highest (29.3%) in fish fed with CSO and the lowest (16.8%) in those fed with CO (P<0.05). The livers of fish fed CSO had significantly higher (P<0.05) levels (33.5%) total saturates than those fed the other diets. However, total levels of saturates in the liver of fish fed FO diet (28.5%) were similar to the fish fed with CO (P>0.05) (Table 7). The total levels of monoenes in the whole body samples were the highest (53.7%) in fish fed CO and the lowest (16.8%) in those fed with CSO diet (P<0.05). Nevertheless, total levels of monoenes in the whole body samples of fish fed FO50/CSO25/CO25 (36.1%) and CSO50/CO50 (35.5%) were similar to the fish fed FO (36.6%). In contrast, the total levels of monoenes in the liver of fish fed the FO was significantly higher (P<0.05) compared with fish fed the other diets. Both the whole body and liver samples of fish fed diets in which fish oil was replaced by cotton seed oil (CSO) and/or canola oils (CO) showed an increased levels of 18:2n-6 compared with fish fed the FO diet (P<0.05). The highest level of 18:2n-6 was found in the whole body of fish fed the CSO (P<0.05). The whole body EPA (20:5n-3) and DHA (22:6n-3) levels were the highest in fish fed the FO diet: 4.5% and 15.8%, respectively (P<0.05). Among the fish fed diets containing cotton seed oil (CSO) and canola oils (CO) and their combinations (FO50/CSO25/CO25, CSO50/CO50), EPA and DHA ranged from 0.8% to 2.5% and 4.9% to 10.3% in the whole body, respectively. However, the liver EPA (20:5n-3) level was the highest in fish fed the FO diet (3.6%) and FO50/CSO25/CO25 diet (3.5%) (P<0.05). In contrast, the liver DHA (22:6n-3) level was the highest in fish fed the FO50/CSO25/CO25 diet and the lowest fish fed the CSO diet: 38.3% and 23%, respectively (P<0.05). Nevertheless, DHA levels in the liver of fish fed the CO diet (32.5%) and the CSO50/CO50 diet (33.4%) were similar to the fish fed FO diet (32.1%). Total n-3 PUFA and n-3/n-6 ratios were the highest in the whole body and liver samples of fish fed the FO and FO50/CSO25/CO25 diets (P<0.05). However, the 20:3n-3 lipid class, not detected in diets, was existent in both the whole body and liver samples of fish. Furthermore, it was evident that the level of 22:5n-3 was higher in the whole body samples of fish fed all the dietary treatments even though this fatty acid was detected only in small amounts in FO and FO50/CSO25/CO25 diets. In addition, the level of 22:5n-3 in liver samples of fish fed diets FO and FO50/CSO25/CO25 was found to be twice as much the levels in these diets.

Histological Examinations

The histological examinations of kidney,

Table 6. Whole body total lipid (%) and fatty acid composition (% of total fatty acids detected) of the rainbow trout fed diets containing Cotton Seed Oil (CSO) and Canola Oil (CO) and their combinations for 12 weeks [a]

Total lipid and fatty acids	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Total lipid	15.3±0.6[b]	15.6±0.3[ab]	15.9±0.2[a]	16.0±0.4[a]	16.0±0.3[a]
Fatty acids					
14:0	5.0±0.1[a]	2.2±0.0[d]	1.6±0.1[e]	3.5±0.0[b]	2.3±0.1[c]
16:0	18.3±0.5[c]	21.1±0.6[a]	12.2±0.3[e]	17.2±0.2[d]	19.1±0.2[b]
18:0	3.9±0.2[d]	6.0±0.1[a]	3.1±0.3[e]	4.3±0.1[c]	5.7±0.1[b]
Total saturates[b]	28.5±0.3[b]	29.3±0.4[a]	16.8±0.2[e]	25.8±0.2[d]	27.4±0.2[c]
16:1n-7	6.3±0.1[a]	2.3±0.1[d]	2.0±0.1[e]	3.6±0.1[b]	2.5±0.1[c]
18:1n-9	23.0±0.2[c]	17.2±0.5[d]	45.7±0.7[a]	26.9±0.1[b]	26.7±0.4[b]
20:1n-9	2.6±0.2[b]	1.5±0.1[d]	2.8±0.3[a]	2.2±0.4[c]	2.9±0.1[a]
22:1n-11	0.9±0.1[a]	0.3±0.1[d]	0.4±0.1[c]	0.4±0.1[c]	0.5±0.1[b]
Total monoenes[c]	36.6±0.2[b]	22.9±0.3[c]	53.7±0. 4[a]	36.1±0.2[b]	35.5±0.2[b]
18:2n-6	8.3±0.2[e]	36.7±0.4[a]	18.9±0.2[d]	19.1±0.4[c]	24.4±0.3[b]
20:2n-6	0.4±0.2[e]	1.9±0.3[a]	1.1±0.1[c]	0.9±0.3[d]	1.5±0.1[b]
20:4n-6	0.8±0.1[a]	0.2±0.0[e]	0.5±0.1[c]	0.6±0.0[b]	0.4±0.2[d]
Total n-6[d]	9.5±0.2[d]	38.8±0.3[a]	20.6±0.2[c]	20.6±0.2[c]	26.4±0.2[b]
18:3n-3	1.5±0.1[d]	0.9±0.0[e]	3.8±0.3[a]	2.2±0.1[c]	2.3±0.1[b]
20:3n-3	ND	0.8±0.0[a]	0.5±0.0[c]	0.3±0.0[d]	0.7±0.1[b]
20:5n-3	4.5±0.5[a]	1.0±0.0[c]	0.8±0.0[d]	2.5±0.2[b]	1.1±0.0[c]
22:5n-3	1.8±0.1[a]	0.4±0.1[d]	0.4±0.1[d]	1.0±0.0[b]	0.5±0.0[c]
22:6n-3	15.8±0.6[a]	5.8±0.1[d]	4.9±0.3[e]	10.3±0.4[b]	6.8±0.1[c]
Total n-3[e]	23.6±0.3[a]	8.9±0.2[e]	10.4±0.2[d]	16.3±0.3[b]	11.4±0.1[c]
n-3/n-6 ratio	2.5±0.1[a]	0.2±0.0[e]	0.5±0.0[c]	0.8±0.0[b]	0.4±0.0[d]

[a] Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different (P<0.05). [b] Includes 14:0, 15:0, 16:0, 17:0 and 18:0. [c] Includes 16:1n-7, 18:1n-9, 20:1n-9 and 22:1n-11.
[d] Includes 18:2n-6 and 20:4n-6. [e] Includes 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3.
ND: Not Detected.

Table 7. Liver total lipid (%) and fatty acid composition (% of total fatty acids detected) of the rainbow trout (*Oncorhynchus mykiss*) fed diets containing Cotton Seed Oil (CSO) and Canola Oil (CO) and their combinations for 12 weeks [a]

Total lipid and fatty acids	Diets				
	FO	CSO	CO	FO50/CSO25/CO25	CSO50/CO50
Total lipid	3.2±0.2[a]	3.0±0.3[a]	2.2±0.2[b]	1.4±0.2[c]	1.6±0.2[c]
Fatty acids					
14:0	1.4±0.1[a]	0.9±0.0[b]	ND	0.8±0.1[c]	ND
16:0	14.3±0.0[c]	16.2±0.1[b]	13.9±0.2[d]	16.7±0.3[a]	16.1±0.1[b]
18:0	7.1±0.4[e]	16.4±0.1[a]	8.7±0.5[d]	10.7±0.7[c]	11.7±0.9[b]
Total saturates[b]	22.8±0.3[d]	33.5±0.2[a]	22.6±0.3[d]	26.1±0.5[c]	27.8±0.6[b]
16:1n-7	3.2±0.3[a]	0.9±0.0[b]	ND	0.9±0.0[b]	ND
18:1n-9	21.5±0.5[b]	13.8±0.2[d]	22.8±0.4[a]	13.0±0.3[e]	15.6±0.6[c]
20:1n-9	2.5±0.2[b]	1.2±0.3[e]	3.2±0.6[a]	1.7±0.2[d]	1.8±0.3[c]
22:1n-11	0.7±0.0[c]	0.9±0.0[b]	ND	ND	1.1±0.1[a]
Total monoenes[c]	29.0±0.4[a]	16.8±0.3[e]	26.0±0.5[b]	17.3±0.3[d]	18.5±0.4[c]
18:2n-6	5.5±0.2[e]	17.9±0.4[a]	9.7±0.8[c]	7.9±0.3[d]	10.8±0.8[b]
20:2n-6	0.5±0.1[e]	2.9±0.3[a]	2.2±0.1[c]	1.8±0.5[d]	2.3±0.8[b]
20:4n-6	1.8±0.2[c]	2.5±0.1[b]	3.0±0.1[a]	2.5±0.3[b]	3.1±0.4[a]
Total n-6[d]	7.8±0.2[e]	23.3±0.3[a]	14.8±0.4[c]	12.2±0.4[d]	16.2±0.7[b]
18:3n-3	0.6±0.2	ND	ND	ND	ND
20:3n-3	0.5±0.0[d]	2.4±0.3[b]	2.4±0.4[b]	0.8±0.1[c]	2.6±0.1[a]
20:5n-3	3.6±0.6[a]	1.0±0.1[c]	1.7±0.1[b]	3.5±0.2[a]	1.1±0.1[c]
22:5n-3	1.7±0.2[a]	ND	ND	1.1±0.1[b]	ND
22:6n-3	32.1±0.9[b]	23.0±0.7[c]	32.5±0.4[b]	38.3±0.3[a]	33.4±0.3[b]
Total n-3[e]	38.5±0.5[b]	26.4±0.4[c]	36.6±0.3[b]	43.9±0.3[a]	37.2±0.2[b]
n-3/n-6 ratio	4.9±0.3[a]	1.1±0.1[d]	2.5±0.2[c]	3.6±0.7[b]	2.3±0.2

[a] Data are reported as mean ± SD (n = 3/diet treatment with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different (P<0.05). [b] Includes 14:0, 16:0 and 18:0. [c] Includes 16:1n-7, 18:1n-9, 20:1n-9, 22:1n-11 and 24:1. [d] Includes 18:2n-6 and 20:4n-6. [e] Includes 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3.
ND: Not Detected.

stomach, pancreas and gill tissue samples of the rainbow trout fed different dietary treatments revealed no differences. However, liver and intestine tissue samples of fish in different dietary treatments were observed to be different to each other. Liver tissue and intestine samples of fish fed FO diet were generally normal but low level of lipid vacuolization was spotted in the cytoplasts of hepatocytes of the liver samples from two fish (Figure 1a and 2a). Low level of lipid vacuolization was generally observed in the cytoplasts of hepatocytes of the rainbow trout fed the CSO diet, but medium level of lipid vacuolization was evident in samples from one fish in this dietary treatment (Figure 1b). As seen in Figure 1c there was a low level of lipid vacuolization in the cytoplasts of hepatocytes of fish fed the CO diet.

It was observed that fish fed dietary treatment FO50/CSO25/CO25 had high degree of lipid vacuolization in the cytoplasts of hepatocytes and the nuclei of hepatocytes was also observed to be pushed towards the periphery of the cells and squeezed as a result of this high degree of vacuolization (Figure 1d). In addition, it was evident that large vacuoles turned into double and triple united smaller lipid vacuoles by tearing their cytoplast membranes. The cross sections of intestine samples of the fish reared in this dietary treatment also revealed that there were many lipid vacuoles at supranuclear position in the cytoplasts of epithelial cells of the villi intestinalis (Figure 2b). On the other hand, the histological examination results of the fish fed CSO50/CO50 diet were similar to that of the fish fed FO50/CSO25/CO25 diet in which large amount of lipid vacuoles in the cytoplasts of hepatocytes and the movements of nuclei of the hepatocytes towards to the periphery of the cells were observed. Furthermore, intestinal cross sections also revealed similar findings to dietary treatment FO50/CSO25/CO25 indicating many lipid vacuoles at the supranuclear position in the cytoplasts of enterocytes.

Discussion

The present study has shown that it was possible to replace fish oil by cotton seed oil (CSO) and canola (CO) oils in diets for the juvenile rainbow trout either totally or in combinations without a negative effect on growth and feed utilization. However it was observed that the whole body composition and liver total lipid concentrations of fish were significantly influenced by the total replacement of dietary fish oil by cotton seed oil and canola oils. Fish fed the dietary treatments CSO and CO had the highest crude lipid and the lowest moisture content compared to fish fed the other three diets. Several studies have been conducted to investigate various vegetable oils as possible fish oil replacer either totally or partially in rainbow trout feeds (Bell *et al.*, 2001; Caballero *et al.*, 2002; Sener and Yildiz, 2003; Ng *et al.*, 2003; Bell and Dick, 2004, 2005; Drew *et al.*, 2007; Rinchard *et al.*, 2007). These studies indicated that replacement of fish oil by several vegetable oils up to 80% or 100% had no significant effect on growth performance or feed utilization efficiency of this species.

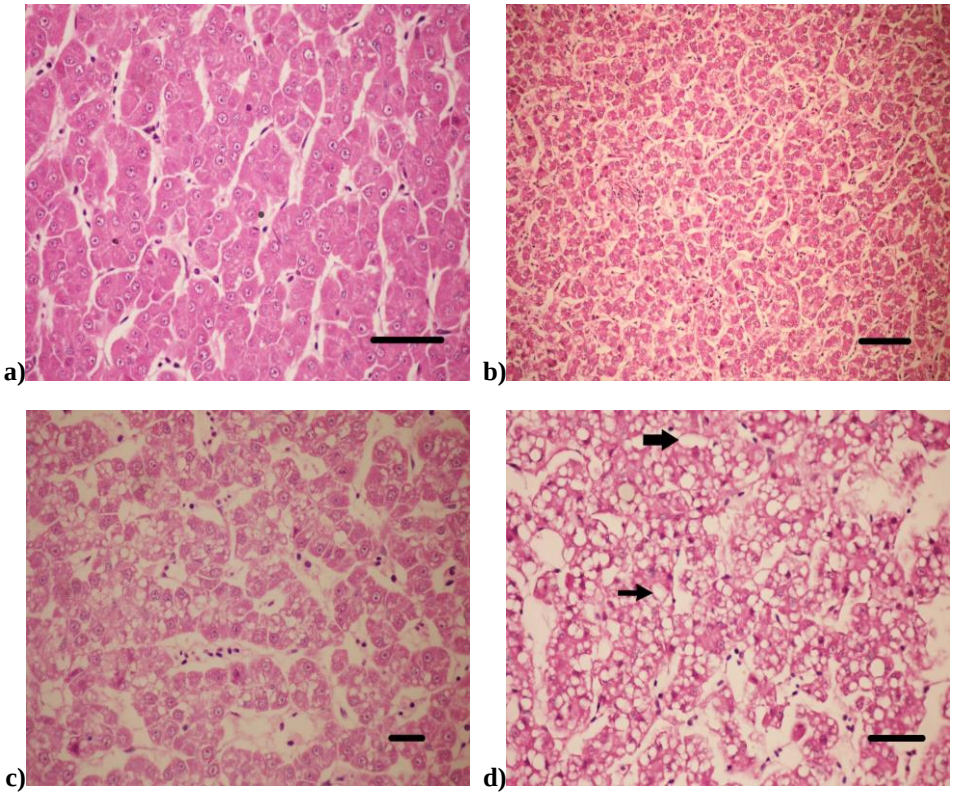

Figure 1. a) Normal liver tissue look (Dietary treatment FO, control group). The position of cytoplasts and nuclei of hepatocytes looks normal (H&E, Bar: 50 µm), **b)** the low level of lipid vacuolization in the cytoplasts of hepatocytes of the liver (Dietary treatment CSO - H&E, Bar: 150 micron), **c)** medium level of lipid vacuolization and parenchyma degeneration in the hepatocytes of the liver (Dietary treatment CO - H&E, Bar: 50 µm), **d)** the high degree of lipid vacuolization in the liver hepatocytes, the nuclei of hepatocytes pushed towards the periphery of the cell and squeezed (thin arrow) and the united lipid vacuoles occurred as a result of tearing of cytoplast membranes (thick arrow) (Dietary treatment FO50/CSO25/CO25 - H&E, Bar: 50 µm).

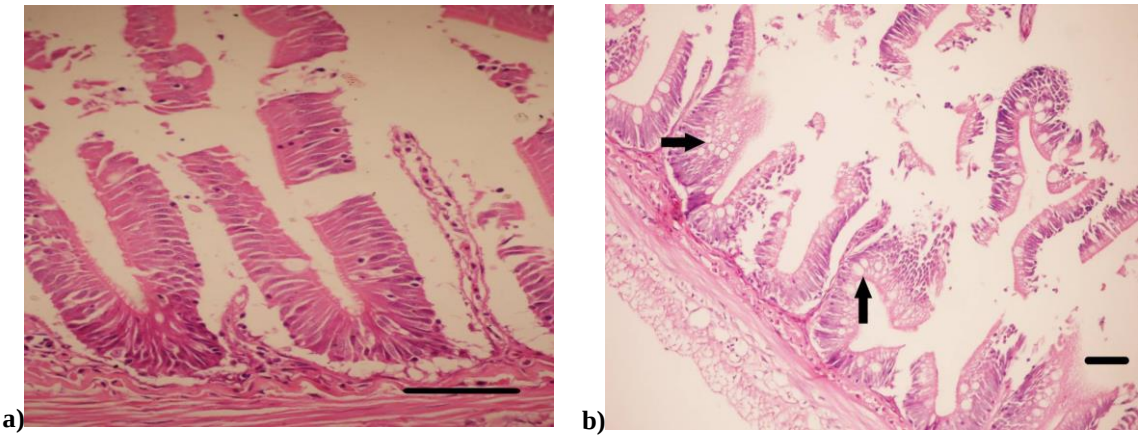

Figure 2. a) normal intestine tissue look (Dietary treatment FO, control group - H&E, Bar: 50 µm), **b)** the high degree of lipid vacuolization spotted in the supranuclear position of the epithelial cells (enterocytes) of the villi intestinalis in the intestines (arrows) (Dietary treatment FO50/CSO25/CO25 - H&E, Bar: 50 µm).

It was previously reported that n-3 PUFA, including both 18:3n-3 and HUFAs, were required for optimal growth and prevention of signs of EFA deficiency in rainbow trout (Bell *et al.*, 2001; Sargent *et al.*, 2002; Bell and Dick, 2004; Rinchard *et al.*, 2007; Tocher, 2010). Furthermore, the EFA requirements estimated for juveniles and sub-adults 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 (Tocher, 2010). Therefore it is possible that FAs in diets containing CSO and CO might have been used as an energy source by rainbow trout cultured at 14°C water temperature during this

investigation since there was no significant difference in growth rates obtained on fish fed diets containing CSO and CO compared to that of fish fed fish oil only diet.

Growth performance indices as well as FER obtained in the present study are considered good since similar findings were recorded earlier for the rainbow trout fed plant oil-supplemented diets (Drew *et al.*, 2007; Oo *et al.*, 2007). Similar studies on Atlantic salmon (*Salmo salar*) have also reported no significant negative impact on growth, feed intake or FER (Bell *et al.*, 2002; Torstensen *et al.*, 2004, 2005). The mean FER obtained in the present study was very close to 1.0 and it could be considered as excellent for rainbow trout under the specified experimental conditions. Similar values have been reported by Drew *et al.*, (2007) (1.0) and Oo *et al.*, (2007) (1.05) for juvenile rainbow trout (initial average mean body weight around 38 and 47 g) when fed diets containing canola, linseed and palm oils either totally or partially replacing fish oil. The present study showed that using single or a mixture of CSO and CO had no apparent negative impact on PER of juvenile rainbow trout. This is in agreement with results previously reported for red sea bream *Pagrus major* (Huang *et al.*, 2007), chinook salmon parr, *Oncorhynchus tshawytscha* (Huang *et al.*, 2008) and gilthead sea bream (Wassef *et al.*, 2009).

The present study also showed that HSI level of fish fed CSO and CSO50/CO50 diets was higher than that of fish fed other diets. Whole body and liver FA compositions of the rainbow trout were significantly influenced by dietary lipid sources. Increased levels of 18:2n-6 and 18:1n-9 was observed in the whole body and liver of rainbow trout fed dietary treatments CSO and CO, respectively. Caballero *et al.* (2002) had also reported that no significant differences in HSI and VSI levels were found among rainbow trout fed diets containing blends of vegetable oils (soybean, rapeseed, olive and palm) compared to those of fish fed fish oil diet. However, Sener and Yildiz (2003) found that HSI calculated from rainbow trout juveniles fed diet containing %100 fish oil was significantly lower than that of fish fed diets containing vegetable oils (soybean or sunflower oil), whereas VSI in the fish fed all dietary treatments was similar indicating relatively higher selective certain lipid deposition in liver of fish fed vegetable oils.

The lipid content of fish tissues could be markedly influenced by the dietary lipid source (Sargent *et al.*, 2002) and similar results were reported by Rinchard *et al.* (2007) and Guler and Yildiz (2009) when 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 in particularly liver and whole body of 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 (Bracco, 1994). In the present study, replacement of fish oil with plant oils resulted in reduced levels of total n-3 PUFA, and increased level of total monoenes and n-6 PUFA in both whole body and liver. In contrast, total saturated FA levels were minimally influenced by dietary treatments despite a wide range of values from 12.4% in the CO diet to 33.5% in the FO diet. The minimal impact of diets on saturated FAs in fish body was also observed by other studies made in rainbow trout (Caballero *et al.*, 2002; Sener and Yildiz, 2003). In line with the findings of the present study, the replacement of fish oil by VOs was reported to result in significant changes in muscle FA composition in many other marine fish species such as sea bass (Yildiz and Sener, 2004), Atlantic salmon, *Salmo salar* (Ng *et al.*, 2007) and gilthead sea bream, *Sparus aurata* (Wassef *et al.*, 2009).

In general, replacement of dietary fish oil with vegetable oils resulted in a lower level of n-3 PUFA in whole body of fish. Particularly, EPA and DHA levels in whole body and only EPA level in liver 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 whole body. Most vegetable oils are rich in unsaturated 18C FAs (OA, 18:1n-9; LA, 18:2n-6; LNA, 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 (Sargent *et al.*, 2002; Caballero *et al.*, 2002; Tocher, 2003; Tocher, 2010). Therefore, FAs of the n-6 series are also required for rainbow trout (Goddard, 1996; Caballero *et al.*, 2002; Tocher, 2010). 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 $\Delta 6$ and $\Delta 5$ desaturases when trout 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 DHA was selectively deposited and retained, as whole body and liver DHA levels were always higher than diet levels. This has also been observed in earlier studies in rainbow trout (Bell *et al.*, 2001; Caballero *et al.*, 2002; Bell and Dick, 2004, 2005). Moreover, the mechanism of selective FA deposition may include the high specificity of fatty acyl transferase for DHA and the relative resistance of DHA to beta-oxidation because of the complex catabolic pathway required for these FAs (Bell *et al.*, 2001). The relatively lower concentrations of LA and LNA in whole body of fish fed the plant oil-based diets as compared to their levels in the corresponding diet (particularly for CSO, CO and CSO50/CO50 diets) may suggest that rainbow trout utilized these FAs for oxidation. This is in agreement with similar data reported for rainbow trout (Caballero *et al.*, 2002; Sener and Yildiz, 2003; Rinchard *et al.*, 2007), gilthead sea bream (Wassef *et al.*, 2009) and sea bass (Yildiz and Sener, 2004). These studies were also

demonstrated that total or partial replacement of dietary fish oil by different VOs in diets of those species did not affect the growth or feed utilization. Although polyunsaturated fatty acid synthesis, namely in the elongation and desaturation of 18°C 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 (CSO, CO and CSO50/CO50 diets) balancing in this way the DHA concentration in both liver and whole body suggesting the importance of DHA in physiological processes such as membrane permeability and fluidity (Caballero *et al.*, 2002; Bell and Dick, 2005). Caballero *et al.* (2002) and Bell and Dick (2005) also reported reduced percentage of 20:5n-3 compared to 22:6n-3 in muscle of trout 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 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.

The economic analysis of diet formulations in the present study indicated that the cost of the diets CSO, CO and CSO50/CO50 were lower (about 13%) when compared to either FO diet or fish oil based diets (FO50/CSO25/CO25). The calculated ECR and EPI in fish fed CO and FO50/CSO25/CO25 and CSO50/CO50 diets could be used economically. A recent study indicated that both nutritionally and economically sound diet formulation is possible even when the farmed fish have been fed diets with high inclusions of vegetable oils (Wassef *et al.*, 2009).

This study demonstrated that rainbow trout fed dietary treatments FO, CSO and CO had all similar liver and intestinal histology results with low and medium level of lipid vacuolization in the cytoplasts of hepatocytes and no visible lipid vacuolization in the enterocytes. However, fish fed the dietary treatments FO50/CSO25/CO25 and CSO50/CO50 had high degree of lipid vacuolization both in the cytoplasts of hepatocytes and enterocytes at supranuclear position. In addition, no correlation was detected between the liver lipid levels and lipid vacuolization in the cytoplasts of hepatocytes of the fish in different experimental treatments. However, it was evident that fish fed the dietary treatments FO50/CSO25/CO25 and CSO50/CO50 had high degree of lipid vacuolization in the hepatocytes even though they had the lowest liver lipid levels compared to that of fish fed the other dietary treatments indicating the negative effects of combination of different oil sources in diets for fatty acid absorption and metabolism in the rainbow trout. High levels of vegetable oil inclusion in aqua feeds have also been previously demonstrated to create degenerations in tissue histological structure of fish (Alexis, 1997), resulting in an accumulation of large lipid vacuoles in the enterocytes and hepatocytes (Tucker *et al.*, 1997; Olsen *et al.*, 1999, 2000; Caballero *et al.*, 2002; Figueiredo-Silva *et al.*, 2005; Wassef *et al.*, 2009). For example, total replacement of dietary fish oil by soybean oil resulted hepatocytes with large lipid vacuoles and nuclei located at the peripheral of the cell in livers of red drum (*Sciaenops ocellatus*) (Tucker *et al.*, 1997). In contrast to our findings, Bell *et al.* (1995) reported a high degree of vacuolisation due to lipid deposition in livers of turbot (*Scophthalmus maximus*) fed marine fish oil indicating the differences in the capacity of freshwater and marine fish species to absorb, metabolize and utilize the fatty acids of marine origin. However, Wassef *et al.* (2009) indicated that lipid accumulation in hepatocytes of gilthead sea bream fed diets replacing dietary fish oil by mixture of cotton seed oil, sunflower oil, linseed oil and soybean oil on an equal ratio (20CSO:20SFO:20LO or 20CSO:20SFO:20SO) had no significant effect on liver histology. Similarly, Figueiredo-Silva *et al.* (2005) reported that soybean oil replacing dietary fish oil up to 50 % had no significant effect on liver histology of the rainbow trout.

In conclusion, results of this study indicated that cotton seed and canola oil partially or totally substitute for fish oil can be used in diets for rainbow trout without affecting growth performance and feed utilization. Moreover, results of ECR and EPI in rainbow trout fed CO and FO50/CSO25/CO25 and CSO50/CO50 diets indicated that these diets would be used for rainbow trout nutrition as economically. The 50% replacement of dietary fish oil by cotton seed and canola oil in equal amounts can be used in diets for rainbow trout without compromising in major nutrient composition and whole body or liver fatty acid compositions of fish compared to fish oil only diet. However it was observed that the dietary treatments FO50/CSO25/CO25 and CSO50/CSO50 had a negative impact on the liver and intestinal histology of the juvenile rainbow trout. Therefore, further investigations targeting the effects of the replacement of fish oil by the combinations of different oil sources on long term growth, fatty acid metabolism and utilization and organ histology would be highly useful in the rainbow trout nutrition.

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