



The effects of fish oil replacement by vegetable oils on growth performance and fatty acid profile of rainbow trout: Re-feeding with fish oil finishing diet improved the fatty acid composition

Mustafa Yıldız^{a,*}, Tufan O. Eroldoğan^b, Samuel Ofori-Mensah^c, Kenan Engin^d, M. Ali Baltacı^e

^a Istanbul University, Faculty of Aquatic Sciences, Department of Aquaculture, Ordu Cad. No: 200, Laleli, 34470 Istanbul, Turkey

^b Cukurova University, Faculty of Fisheries, Department of Aquaculture, Balcalı, 01330 Adana, Turkey

^c Istanbul University, Institute of Graduate Studies in Science and Technology, Department of Aquaculture, Istanbul, Turkey

^d Mersin University, Faculty of Fisheries, Department of Aquaculture, Yenişehir Kampüsü, 33169 Mersin, Turkey

^e Istanbul University, Faculty of Aquatic Sciences, Sapanca Inland Waters Research Center, Kurtköy, Sapanca, Adapazari, Turkey

ARTICLE INFO

Keywords:

Rainbow trout
Nutrition
Fish oil
Vegetable oil
Fatty acid
Highly unsaturated fatty acid

ABSTRACT

The present study aimed to demonstrate the effects of feeding vegetable oil (VO)-based diets and their blends on growth, feed utilization and fatty acid (FA) profile in rainbow trout, *Oncorhynchus mykiss*. Juveniles were fed five experimental diets in which dietary fish oil (FO diet containing anchovy oil) was totally or partially replaced by cottonseed oil (CSO), canola oil (CO), MIX1 (50% FO, 25% CSO and 25% CO) and MIX2 (50% CSO and 50% CO) in a grow-out period for 12 weeks. Afterwards, all fish were switched to a diet containing 100% FO diet for a further 12 weeks to determine the progressive recovery of fatty acid (FA) profile of rainbow trout. Results showed that total and/or partial replacement of FO did not negatively affect growth and feed utilization. Feeding VOs significantly reduced body contents of arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to a lower degree than their reduction in the diet. Re-feeding with FO-diet for 12 weeks led to significant ($P < 0.05$) improvements in highly unsaturated fatty acid (HUFA), with a full recovery in ARA contents of rainbow trout. Finally, FA profile in whole body of MIX1 fed fish had the closest resemblance to that of FO fed fish.

1. Introduction

Over several decades, aquatic food production has been transformed from capture fisheries to culture of increasing numbers of farmed fish species (FAO, 2016). This success has been due to the ability of aquaculture to convert low value fish meal and oil into high value food for humans (Tidwell and Allan, 2001). Therefore aquaculture continues to be one of the fastest-growing food producing sectors (FAO, 2014), providing an increasing proportion of high quality protein source destined for human consumption. Due to the continuous increase in aquaculture production, its contribution to the supply of fish for human consumption surpassed that of wild-caught fish for the first time in 2014 (FAO, 2016). As known, fish meal and oil are the basic protein and lipid sources in aquafeeds. Although aquaculture production has been expanding at 8–10% annually, global fish oil (FO) production has been stagnating. It is obvious global FO supplies cannot meet the increasing demand of FO in aquafeeds, especially for carnivorous fishes (NRC, 2011). There is the need for alternative lipid sources to guarantee

the future growth of the industry. Over the last two decades, considerable effort has been given to identify the alternatives. Increased production, price stability and sustainability make vegetable oils (VOs) good candidates for fish oil replacement in aquafeeds (Hardy et al., 2001; Mourente and Bell, 2006; Turchini et al., 2009). Numerous investigations have shown that FO can be replaced by a variety of VOs such as soybean, linseed, rapeseed, olive oil, palm oil and corn oils in salmonids and freshwater fish feeds (Torstensen et al., 2000; Bell et al., 2001; Rosenlund et al., 2001; Mourente and Bell, 2006). Although this has not had negative effects on growth, survival and feed utilization, substitution with VO has been shown to modify flesh fatty acid (FA) profile to reflect that of the diet (Bell et al., 2003a, 2004; Bransden et al., 2003; Fonseca-Madrugal et al., 2005; Miller et al., 2007; Thanuthong et al., 2011b; Yıldız et al., 2013, 2015). Different VOs have different fatty acid composition with most having $n-6$ and $n-9$ polyunsaturated fatty acids (PUFA) in abundance. Irrespective of the FA composition of VOs, they have no $n-3$ highly unsaturated fatty acid (HUFA, $C \geq 20$). Therefore, increased replacement of FO with VO has

* Corresponding author.

E-mail address: mstar@istanbul.edu.tr (M. Yıldız).

led to the reduction in the $n-3$ HUFA and the overall nutritional qualities of the final product (Rosenlund et al., 2010). Since fish is a rich source of $n-3$ HUFA with health benefits (Sargent et al., 2001), a reduction in these important fatty acids as a result of feeding with VO-based diets may have a negative effect on human nutrition. Irrespective of this, the use of VOs is thought to present an opportunity in understanding the effects of the different fatty acid classes within the different VOs on the lipid utilization and metabolism in farmed fish species (Thanuthong et al., 2011a). Most VOs are known to contain PUFA, which are capable of being bioconverted into HUFA in freshwater fish species. Unlike marine fish species, substitutions with VO should allow more effective utilization, minimizing the negative impact of fish oil replacement in freshwater species (Thanuthong et al., 2011a).

In order to increase the nutritional value and to ensure high levels of HUFA in fish whole body and fillet, finishing diet strategies have been adopted where fish initially fed FO-deprived diets are fed diets containing FO as the only dietary lipid source in the latter stages of their rearing cycle (Jobling, 2003; Turchini et al., 2006, 2007; Stone et al., 2011a, 2011b). In a previous study, Bell et al. (2003b) fed Atlantic salmon post-smolts with diets in which FO was increasingly replaced by rapeseed oil for 16 weeks. Re-feeding with FO diet for 12 weeks in the study restored muscle content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In another study by Bell et al. (2004), it was demonstrated that flesh DHA and EPA concentrations were partially restored using a finishing diet containing FO for 16 weeks in Atlantic salmon that were initially fed increasing levels of linseed oil-based diet for 40 weeks. Therefore, the effects of dietary FO replacement with VOs and the overall efficacy of a fish oil finishing strategy remains unclear. This is because the responses of salmonids to the different VOs are as varied as the fatty acid profile of the VOs. In addition, FA recovery could also differ appreciably even within the same species and size of fish. The main aim of the present study was to determine the effect of feeding CSO (Cotton Seed Oil) and CO (Canola Oil) based diets and their blends on growth, feed utilization and fatty acid (FA) profile of whole body of rainbow trout. In addition, the study also aimed at demonstrating the progressive recovery of fatty acid (FA) profile of rainbow trout initially fed diets containing VOs and/or their mixtures during grow-out period through subsequent re-feeding with a FO-based diet for the final part of the rearing cycle.

2. Materials and methods

2.1. Experimental diets

Five iso-nitrogenous (approximately 45% crude protein) and iso-lipidic (approximately 17% crude lipid) experimental diets (2–4 mm diameter) were formulated with different lipid sources at the Sapanca Inland Waters Research Center of the Faculty of Aquatic Sciences, Istanbul University, Turkey, as steam pressured pellets using a laboratory feed mill (KAHL-L, 173). The lipids used were fish oil (anchovy oil), cottonseed oil and canola oil. The first diet (FO, control group) contained only fish oil which was totally replaced by cottonseed oil and canola oil in the CSO and CO diets respectively. In the last two diets, FO was partially replaced (50% FO, 25% CSO and 25% CO) in MIX1 whereas dietary FO was totally substituted with an equal mixture of cotton seed and canola oils (50% CSO and 50% CO) in MIX2 diets. The dietary ingredients and proximate compositions are given in Table 1 whereas FA compositions of diets are presented in Table 2. Diets were kept in plastic storage bags at -20°C until used.

2.2. Experimental conditions and measurements

Rainbow trout (*Oncorhynchus mykiss*), of weight 15.7 ± 0.5 g (mean \pm SD) were randomly stocked at 50 fish tank⁻¹ into 10 cylindrical-conical tanks of 1000 L capacity at the Sapanca Inland Waters Research Center of the Fisheries Faculty of Istanbul University, Turkey.

The tanks were supplied with freshwater of an average temperature of $13.6 \pm 0.6^{\circ}\text{C}$ originating from a well. Dissolved oxygen (DO) and pH were maintained at 8.4 ± 0.2 mg L⁻¹ and 7.3 ± 0.6 respectively. A photoperiod regimen of 12 h light:12 h dark was utilized throughout the experiment. Using a commercial diet (trout commercial pellet 2 mm in diameter), fish were acclimatized to the experimental feeding regimen for 2 weeks before starting the experiment. Fish were fed experimental diets to apparent satiation by hand twice per day at 0900 and 1700 h for 12 weeks (84 days) from July to October. Fish were bulk weighed every 2 weeks and feed intake recorded daily. At the end of the experimental feeding, fish were individually weighed for determining growth and survival performance. Further, samples of experimental diet and 5 fish per tank (10 fish per diet) were collected for analyses of proximate and FA composition and kept at -80°C until proximate composition and FA profile analysis. Growth performance was evaluated according to Ricker (1979), with parameters listed below;

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

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

Viscerosomatic index (VSI) = 100 \times (viscera weight / body weight).

At the end of the grow-out period (feeding with experimental test diets), fish were fed a FO-based diet in a re-feeding (finishing) phase. Thus, after evaluation of growth and survival performance and sampling for fatty acid composition of whole body, all the remaining fish previously fed FO, CSO, CO, MIX1 and MIX2 diets were fed finishing diet (100% FO-based diet, anchovy oil) for 12 weeks from October to the end of December. During this period, 5 fish per tank were sampled once every 3 weeks (triweekly) to monitor the recovery of whole body fatty acid profile. Fish samples were kept at -80°C until FA profile analysis.

2.3. Proximate analysis

All analyses followed standard methodology of AOAC (2006) and were run in triplicates. Dietary ingredients, experimental diets, and fish samples were analyzed for proximate composition (protein, lipid, ash, moisture and fiber). Crude protein (N \times 6.25) was determined using a semi-automatic Kjeldahl (Gerhardt Vapodest, 45s) technique. Crude lipid was ether extracted by means of a Soxtherm Multistat/SX PC (Gerhardt, Germany). Moisture content was determined by drying samples in an oven at 105°C for 12 h and then weighing at hourly intervals until constant weight was obtained. Dried samples were incinerated at 550°C for about 12 h in a Muffle Furnace for the determination of ash content after weight loss. Using sulfuric acid and then sodium hydroxide, 12.5% (w/w) for half an hour each, with the final residue washed with 5% HCl and water, filtered, dried, and weighed, the crude fiber was determined.

2.4. Lipid extraction and FA analysis

Lipid was extracted from whole body of fish and feed samples by homogenization in chloroform/methanol (2:1, v/v) according to the methods of Folch et al. (1957). FAs methyl esters (FAME) were trans-methylated with 2 M potassium hydroxide (KOH) (Merck, Darmstadt, Germany) in methanol and *n*-hexane (Sigma-Aldrich, Steinheim, Germany) according to Ichihara et al. (1996) with minor modification; 10 mg of extracted oil was dissolved in 2 mL hexane followed by 4 mL of 2 M methanolic KOH. By vortexing the tube for 2 min at room

Table 1
Ingredients and proximate composition of the experimental diets.

	Diets				
	FO	CSO	CO	MIX1	MIX2
<i>Ingredients (g kg⁻¹ dry weight)</i>					
Fish meal	450	450	450	450	450
Soybean meal	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
<i>Analyzed proximate composition (% DM)</i>					
Moisture	11.38 ± 0.19	11.88 ± 0.39	12.10 ± 0.38	12.12 ± 0.12	12.22 ± 0.07
Crude protein	45.47 ± 0.74	45.03 ± 0.66	44.99 ± 0.84	45.41 ± 0.58	45.49 ± 0.10
Lipid	16.53 ± 0.04	16.84 ± 0.05	16.87 ± 0.12	16.45 ± 0.05	16.50 ± 0.16
Ash	8.45 ± 0.06	8.31 ± 0.05	8.30 ± 0.04	8.25 ± 0.03	8.44 ± 0.04
Crude cellulose (% DM)	2.83 ± 0.15	2.77 ± 0.14	2.75 ± 0.09	2.75 ± 0.06	2.82 ± 0.14
NFE ^b	15.33 ± 0.81	15.16 ± 0.44	15.00 ± 0.30	15.02 ± 0.51	14.53 ± 0.32
Gross energy (kJ g ⁻¹)	19.93 ± 0.05	19.91 ± 0.09	19.88 ± 0.08	19.82 ± 0.04	19.78 ± 0.06

^a Premix of vitamins and minerals according to NRC (1993) recommendations for fish.

^b NFE: nitrogen-free extract calculated by difference.

temperature and a centrifugation at 4000 rpm for 10 min, the resulting hexane layer was taken for GC analyses. By means of a gas chromatograph (Auto System XL Perkin Elmer) using a 30 × 0.25 mm capillary column (FID detector CP-2380 Supelco, Bellefonte, USA) the FA composition was analyzed. The conditions of the method were: carrier gas, helium; flame ionization detection temperature, 260 °C; split rate: 1/0, oven temperature programmed to rise from 120 °C/2 min to 220 °C/15 min at a rate of 5 °C min⁻¹; injector temperature, 240 °C. The individual methyl esters were identified in comparison with commercial

standards (Sigma, St. Louis, MO, USA). All FA analyses were performed in triplicate.

2.5. Statistical analysis

Results were expressed as means ± SD. All the statistical analyses were made using SPSS statistical software (Version 14.0 for Windows). Percentage data were arcsine-transformed prior to analysis. After confirmation of normality and homogeneity of variance, data were

Table 2
Fatty acid composition of the experimental diets containing FO, CSO, CO and their mixtures (g/100 g fatty acids).^a

	Diets				
	FO	CSO	CO	MIX1	MIX2
14:0	7.3 ± 0.08 ^a	2.1 ± 0.02 ^d	1.2 ± 0.01 ^e	4.4 ± 0.01 ^b	2.2 ± 0.01 ^c
15:0	0.9 ± 0.02 ^a	ND	ND	0.5 ± 0.02 ^b	ND
16:0	20.5 ± 0.06 ^b	24.2 ± 0.10 ^a	9.1 ± 0.03 ^e	18.9 ± 0.08 ^c	17.6 ± 0.02 ^d
17:0	1.1 ± 0.00 ^a	ND	ND	0.7 ± 0.01 ^b	ND
18:0	3.2 ± 0.02 ^a	2.4 ± 0.04 ^c	1.8 ± 0.01 ^e	2.8 ± 0.02 ^b	2.2 ± 0.00 ^d
20:0	0.5 ± 0.01 ^a	ND	0.3 ± 0.00 ^c	0.4 ± 0.01 ^b	ND
16:1n-7	6.5 ± 0.02 ^a	1.9 ± 0.03 ^d	1.4 ± 0.01 ^e	3.9 ± 0.01 ^b	2.2 ± 0.05 ^c
18:1n-9	16.5 ± 0.02 ^d	14.6 ± 0.05 ^e	49.9 ± 0.02 ^a	23.2 ± 0.11 ^c	27.7 ± 0.15 ^b
20:1n-9	2.7 ± 0.02 ^a	1.4 ± 0.01 ^e	2.1 ± 0.01 ^d	2.3 ± 0.01 ^c	2.4 ± 0.01 ^b
22:1n-11	0.8 ± 0.03 ^a	0.4 ± 0.01 ^d	0.5 ± 0.01 ^c	0.6 ± 0.01 ^b	0.6 ± 0.00 ^b
18:2n-6	6.9 ± 0.05 ^c	44.7 ± 0.03 ^a	21.1 ± 0.03 ^c	20.7 ± 0.01 ^d	31.0 ± 0.05 ^b
20:4n-6	1.7 ± 0.01 ^a	0.5 ± 0.00 ^d	0.4 ± 0.01 ^c	1.1 ± 0.01 ^b	0.7 ± 0.01 ^c
18:3n-3	1.5 ± 0.01 ^d	0.5 ± 0.01 ^e	6.3 ± 0.01 ^a	2.8 ± 0.01 ^c	3.5 ± 0.00 ^b
20:5n-3	9.5 ± 0.01 ^a	1.5 ± 0.03 ^d	1.2 ± 0.02 ^e	5.4 ± 0.06 ^b	2.2 ± 0.01 ^c
22:5n-3	0.9 ± 0.03 ^a	ND	ND	0.5 ± 0.04 ^b	ND
22:6n-3	15.1 ± 0.06 ^a	3.7 ± 0.04 ^d	3.0 ± 0.01 ^e	9.1 ± 0.09 ^b	4.7 ± 0.01 ^c
Σ saturates	33.5 ± 0.14 ^a	28.7 ± 0.05 ^b	12.4 ± 0.04 ^e	27.7 ± 0.05 ^c	22.0 ± 0.03 ^d
Σ monoenes	29.2 ± 0.04 ^d	19.8 ± 0.09 ^e	56.1 ± 0.03 ^a	32.3 ± 0.13 ^c	35.4 ± 0.09 ^b
Σ n-3	27.0 ± 0.13 ^a	5.8 ± 0.06 ^d	10.4 ± 0.02 ^c	17.9 ± 0.13 ^b	10.5 ± 0.02 ^c
Σ n-6	8.7 ± 0.06 ^c	45.2 ± 0.03 ^a	21.6 ± 0.04 ^d	21.8 ± 0.01 ^c	31.7 ± 0.02 ^b
Σ n-9	19.2 ± 0.04 ^d	16.1 ± 0.06 ^e	52.0 ± 0.03 ^a	27.2 ± 0.11 ^c	30.1 ± 0.14 ^b
Σ n-3 HUFA	25.5 ± 0.12 ^a	5.2 ± 0.06 ^d	4.2 ± 0.03 ^e	15.0 ± 0.14 ^b	7.0 ± 0.02 ^c
AA/EPA	0.18 ± 0.00 ^d	0.33 ± 0.01 ^b	0.38 ± 0.02 ^a	0.20 ± 0.00 ^c	0.32 ± 0.00 ^b
EPA/DHA	0.63 ± 0.01 ^a	0.41 ± 0.00 ^d	0.40 ± 0.01 ^e	0.59 ± 0.00 ^b	0.47 ± 0.00 ^c
n-3/n-6 ratio	3.1 ± 0.04 ^a	0.1 ± 0.00 ^e	0.5 ± 0.00 ^c	0.8 ± 0.01 ^b	0.3 ± 0.00 ^d
Lipid (%)	16.53 ± 0.04 ^a	16.84 ± 0.05 ^a	16.87 ± 0.12 ^a	16.45 ± 0.05 ^a	16.50 ± 0.16 ^a

ND: not detected.

^a Data are reported as mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P < 0.05).

subjected to one way ANOVA, and subsequent comparison of means was performed using Tukey's ($P < 0.05$) multiple range test.

3. Results

3.1. Dietary FA composition

Dietary fatty acid profiles are presented in Table 2 and were reflective of the various lipid sources used in their formulation. Total saturates (SFA) ranged from 12.4 to 33.5 g/100 g total fatty acids and was highest in the FO diet, mainly made up of palmitic acid (16:0) level. Although FO diet had the highest SFA, CSO diet had the highest level of palmitic acid. Monoenes were highest in the VO-based diets, from 19.8 g/100 g in CSO to 56.1 g/100 g in CO diets, and oleic acid (OA, 18:1n – 9) formed the main constituent. Linoleic acid (LA, 18:2n – 6) was highest in CSO diet (44.7 g/100 g) while FO (6.9 g/100 g) had the lowest content. CO diet had the highest level of linolenic acid (ALA, 18:3n – 3), 6.3 g/100 g, whereas CSO diet had the lowest of 0.5 g/100 g. Eicosapentaenoic acid (EPA, 20:5n – 3) ranged from 1.2 g/100 g in CO diet to 9.5 g/100 g in FO while docosahexaenoic acid (DHA, 22:6n – 3) was from 3.0 g/100 g in CO to 15.1 g/100 g in FO. Arachidonic acid (ARA, 20:4n – 6) was highest in FO whereas CO had the least. Thus CO diet had the lowest level of ARA, EPA and DHA. Compared to FO diet, ARA, EPA and DHA were reduced by 35.3%, 43.2% and 64.2% when FO was partially replaced (50% FO, 25% CSO and 25% CO) in MIX1 diet. For total replacement with a VO or their mixture (CSO, CO and MIX2 diets), ARA, EPA and DHA were reduced by 58.8–76.5%, 76.8–87.4% and 69.3–80.7% compared to their respective values in FO-diet.

3.2. Growth performance

Growth and survival parameters measured at the end of each rearing phase are presented in Table 3 whereas proximate composition of fish shown in Table 4. Although no difference was found in feed intake and FERs, final weight of trout fed CSO and CO diets was slightly but significantly lower ($P < 0.05$) than that of fish fed other dietary

treatments for the grow-out period. However, no significant differences in SGRs were recorded among fish. PER of fish fed CSO diet was also found to be significantly lower ($P < 0.05$) than that of fish fed the other dietary treatments. At the end of the 84 days of grow-out period, final weight of fish ranged from 116 to 125 g and a survival rate of 97% to 100% was achieved (Table 3). In addition, no significant differences in HSI and VSI values among dietary treatments were found after the initial grow-out period. At the end of the finishing phase, fish weight (ranged from 298 to 318 g) was almost tripled with no significant differences among dietary treatments (Table 3). However, fish fed FO diet attained the lowest ($P < 0.05$) SGR. FER ranged from 0.8 to 0.9 among dietary treatments, with trout previously fed CO diet recording the highest value. Similarly, PER was highest in CO fed fish (Table 3). Re-feeding with FO-diet led to 100% survival in all groups. HSI was highest in fish previously fed MIX2 diet while fish fed FO diet had the highest VSI (Table 3).

3.3. Whole body FA profile

FA profile of whole body before and after the grow-out period is shown in Table 5. Total lipid and fatty acid profile showed the effects of the respective dietary treatments. The levels of SFA were significantly reduced ($P < 0.05$) in fish fed the dietary VO-based diets CO, MIX1 and MIX2 whereas the CSO fed fish had the highest content (29.3 g/100 g total FA). Among the FA classes, body contents of monoenes were highest in all groups, except CSO fed fish. Although CO fed fish had the highest body monoenes content, which was predominantly made up of OA, SFA was lowest in this group. LA content increased ($P < 0.05$) with the increased inclusion of VO (Fig. 1) with CSO fed fish having the highest value (36.7 g/100 g, Table 5). Likewise, the inclusion of VO in diet led to increased ALA content ($P < 0.05$) in all groups fed a VO-based diet apart from CSO group (Fig. 2). ARA, EPA and DHA were significantly high ($P < 0.05$) in control group in comparison to the other groups (Figs. 3, 4, 5). ARA was reduced by 71.4, 42.9, 28.6 and 14.3% their initial values in CSO, MIX2, CO and MIX1 fed fish respectively. Total or partial replacement of dietary FO reduced whole body EPA levels in rainbow trout by 46.8, 76.6, 78.7 and 83% in MIX1,

Table 3
Growth performance of the rainbow trout for different phases of the experiment.^a

Growth performance	Diets				
	FO	CSO	CO	MIX1	MIX2
<i>Grow-out period (feeding with experimental diets) for 12 weeks</i>					
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	124.9 ± 19.8 ^a	116.6 ± 20.2 ^c	116.1 ± 22.4 ^c	120.9 ± 20.3 ^{ab}	122.3 ± 21.8 ^{ab}
Weight gain (%) ^c	685.6 ± 32.0	657.3 ± 7.0	639.8 ± 11.3	680.1 ± 30.2	669.6 ± 27.9
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 ± 0.05	2.4 ± 0.01	2.4 ± 0.02	2.4 ± 0.05	2.4 ± 0.04
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
<i>Finishing phase (feeding with 100% FO-based diet) for 12 weeks</i>					
Initial weight (g fish ⁻¹) ^e	124.9 ± 19.8 ^a	116.6 ± 20.2 ^c	116.1 ± 22.4 ^c	120.9 ± 20.3 ^{ab}	122.3 ± 21.8 ^{ab}
Final weight (g fish ⁻¹) ^c	309.4 ± 28.0	298.2 ± 36.7	318.2 ± 41.0	310.1 ± 42.5	315.9 ± 40.2
Weight gain (%) ^c	148.6 ± 2.9 ^c	155.6 ± 0.1 ^{bc}	174.0 ± 7.1 ^a	156.5 ± 1.9 ^b	158.2 ± 0.8 ^b
Survival (%)	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
SGR ^c	1.08 ± 0.01 ^c	1.12 ± 0.01 ^{bc}	1.20 ± 0.03 ^a	1.12 ± 0.01 ^{bc}	1.13 ± 0.01 ^b
FER ^c	0.8 ± 0.02 ^b	0.8 ± 0.00 ^b	0.9 ± 0.04 ^a	0.8 ± 0.01 ^b	0.8 ± 0.01 ^b
PER ^c	1.8 ± 0.03 ^c	1.9 ± 0.01 ^b	2.1 ± 0.08 ^a	1.9 ± 0.02 ^b	1.9 ± 0.01 ^b
HSI ^d	1.4 ± 0.22 ^b	1.4 ± 0.24 ^b	1.4 ± 0.23 ^b	1.4 ± 0.15 ^b	1.6 ± 0.23 ^a
VSI ^d	14.6 ± 1.4 ^a	14.1 ± 1.6 ^a	13.7 ± 1.9 ^{ab}	13.5 ± 0.9 ^{ab}	12.6 ± 1.1 ^b

^a Data are mean ± SD. Means with different superscript letter in a row are significantly different ($P < 0.05$).

^b n = 50 × 2.

^c n = 20 × 2.

^d n = 10.

^e n = 35 × 2.

Table 4
Whole body proximate composition of the rainbow trout for different phases of the experiment.^a

Proximate composition (%)	Fish groups				
	FO	CSO	CO	MIX1	MIX2
<i>Grow-out period (feeding with experimental diets)</i>					
Dry matter	29.4 ± 0.90 ^a	31.5 ± 0.18 ^c	30.8 ± 0.11 ^{bc}	29.9 ± 0.36 ^{ab}	30.3 ± 0.59 ^{ab}
Crude protein	15.3 ± 0.63 ^b	15.6 ± 0.28 ^{ab}	15.9 ± 0.20 ^a	16.0 ± 0.37 ^a	16.0 ± 0.29 ^a
Crude lipid	11.9 ± 0.81 ^b	13.5 ± 0.16 ^a	12.8 ± 0.09 ^a	12.0 ± 0.05 ^b	12.0 ± 0.24 ^b
Ash	1.5 ± 0.05 ^b	1.9 ± 0.21 ^a	1.8 ± 0.20 ^{ab}	1.8 ± 0.22 ^{ab}	1.8 ± 0.26 ^{ab}
<i>Finishing phase (feeding with 100% FO-based diet)</i>					
Dry matter	29.1 ± 0.17 ^a	28.8 ± 0.13 ^{ab}	28.0 ± 0.34 ^b	27.9 ± 0.91 ^b	28.5 ± 0.56 ^{ab}
Crude protein	16.8 ± 0.58	16.9 ± 0.04	17.2 ± 0.31	17.1 ± 0.79	17.5 ± 0.54
Crude lipid	11.1 ± 0.36	11.1 ± 0.52	10.9 ± 0.09	10.3 ± 0.82	10.3 ± 0.19
Ash	2.1 ± 0.35	2.1 ± 0.57	1.7 ± 0.22	2.0 ± 0.26	1.8 ± 0.21

^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).

Table 5
Fatty acid composition of whole body of fish fed experimental diet containing FO, CSO, CO, MIX 1 and MIX 2 for 12 weeks (g/100 g fatty acids).^a

	Fish groups					
	Initial	FO	CSO	CO	MIX1	MIX2
14:0	4.9 ± 0.05	5.0 ± 0.04 ^a	2.2 ± 0.01 ^d	1.6 ± 0.03 ^e	3.5 ± 0.02 ^b	2.3 ± 0.03 ^c
16:0	18.5 ± 0.03	18.3 ± 0.47 ^c	21.1 ± 0.06 ^a	12.2 ± 0.05 ^e	17.2 ± 0.02 ^d	19.1 ± 0.02 ^b
18:0	3.8 ± 0.05	3.9 ± 0.02 ^d	6.0 ± 0.01 ^a	3.1 ± 0.03 ^e	4.3 ± 0.01 ^c	5.7 ± 0.01 ^b
16:1n-7	6.5 ± 0.03	6.3 ± 0.01 ^a	2.3 ± 0.01 ^d	2.0 ± 0.01 ^e	3.6 ± 0.01 ^b	2.5 ± 0.01 ^c
18:1n-9	24.5 ± 0.1	23.0 ± 0.12 ^c	17.2 ± 0.05 ^d	45.7 ± 0.07 ^a	26.9 ± 0.11 ^b	26.7 ± 0.04 ^b
20:1n-9	2.6 ± 0.08	2.6 ± 0.21 ^b	1.5 ± 0.05 ^d	2.8 ± 0.03 ^a	2.2 ± 0.04 ^c	2.9 ± 0.01 ^a
22:1n-11	0.8 ± 0.02	0.9 ± 0.01 ^a	0.3 ± 0.01 ^d	0.4 ± 0.01 ^c	0.4 ± 0.01 ^c	0.5 ± 0.01 ^b
20:2n-9	0.3 ± 0.01	0.4 ± 0.02 ^c	1.9 ± 0.02 ^a	1.1 ± 0.01 ^c	1.0 ± 0.02 ^d	1.5 ± 0.01 ^b
18:2n-6	11.2 ± 0.01	8.3 ± 0.02 ^e	36.7 ± 0.04 ^a	18.9 ± 0.02 ^d	19.1 ± 0.04 ^c	24.4 ± 0.03 ^b
18:3n-6	ND	ND	0.4 ± 0.02 ^a	ND	ND	0.3 ± 0.01 ^b
20:2n-6	0.4 ± 0.01	0.4 ± 0.02 ^c	1.9 ± 0.03 ^a	1.1 ± 0.01 ^c	0.9 ± 0.03 ^d	1.5 ± 0.01 ^b
20:4n-6	0.7 ± 0.01	0.8 ± 0.00 ^a	0.2 ± 0.01 ^e	0.5 ± 0.02 ^c	0.6 ± 0.02 ^b	0.4 ± 0.02 ^d
18:3n-3	1.5 ± 0.03	1.5 ± 0.01 ^d	0.9 ± 0.03 ^e	3.8 ± 0.03 ^a	2.2 ± 0.01 ^c	2.3 ± 0.01 ^b
20:3n-3	ND	ND	0.8 ± 0.03 ^a	0.5 ± 0.02 ^c	0.3 ± 0.07 ^d	0.7 ± 0.01 ^b
20:5n-3	4.7 ± 0.02	4.5 ± 0.05 ^a	1.0 ± 0.03 ^c	0.8 ± 0.02 ^d	2.5 ± 0.02 ^b	1.1 ± 0.01 ^c
22:5n-3	1.6 ± 0.03	1.8 ± 0.01 ^a	0.4 ± 0.01 ^d	0.4 ± 0.01 ^d	1.0 ± 0.03 ^b	0.5 ± 0.02 ^c
22:6n-3	14.9 ± 0.02	15.8 ± 0.06 ^a	5.8 ± 0.01 ^d	4.9 ± 0.03 ^e	10.3 ± 0.04 ^b	6.8 ± 0.01 ^c
Σ saturates	27.2 ± 0.03	28.5 ± 0.47 ^b	29.3 ± 0.06 ^a	16.8 ± 0.11 ^e	25.8 ± 0.09 ^d	27.4 ± 0.01 ^c
Σ monoenes	31.4 ± 0.05	36.6 ± 0.40 ^b	22.9 ± 0.05 ^c	53.7 ± 0.14 ^a	36.1 ± 0.04 ^b	35.5 ± 0.04 ^b
Σ n-3	22.7 ± 0.04	23.6 ± 0.10 ^a	8.9 ± 0.06 ^e	10.4 ± 0.02 ^d	16.3 ± 0.08 ^b	11.4 ± 0.03 ^c
Σ n-6	12.3 ± 0.01	9.5 ± 0.04 ^d	38.8 ± 0.03 ^a	20.6 ± 0.04 ^c	20.6 ± 0.08 ^c	26.4 ± 0.03 ^b
Σ n-9	24.4 ± 0.03	26.2 ± 0.20 ^c	20.7 ± 0.03 ^d	49.6 ± 0.10 ^a	30.0 ± 0.06 ^b	31.2 ± 0.03 ^b
Σ n-3 HUFA	21.2 ± 0.01	23.9 ± 0.07 ^a	8.0 ± 0.07 ^d	6.6 ± 0.02 ^e	14.1 ± 0.09 ^b	9.1 ± 0.03 ^c
AA/EPA	0.15 ± 0.06	0.18 ± 0.00 ^d	0.26 ± 0.01 ^c	0.63 ± 0.03 ^a	0.23 ± 0.01 ^{cd}	0.40 ± 0.02 ^b
EPA/DHA	0.32 ± 0.01	0.29 ± 0.00 ^a	0.17 ± 0.01 ^c	0.17 ± 0.01 ^c	0.24 ± 0.00 ^b	0.16 ± 0.00 ^c
n-3/n-6 ratio	1.85 ± 0.03	2.5 ± 0.01 ^a	0.2 ± 0.00 ^e	0.5 ± 0.00 ^c	0.8 ± 0.01 ^b	0.4 ± 0.00 ^d
Lipid (%)	14.3 ± 0.05	15.3 ± 0.63 ^b	15.6 ± 0.28 ^{ab}	15.9 ± 0.20 ^a	16.0 ± 0.37 ^a	16.0 ± 0.29 ^a

ND: not detected.

^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).

MIX2, CSO and CO dietary groups respectively. At the end of the grow-out period, body content of DHA was reduced to 32.9, 38.9, 45.6 and 69.1% of their initial content in CO, CSO, MIX2 and MIX1 fed fish respectively. FO fed fish had the highest (P < 0.05) n-3 HUFA content, EPA/DHA ratio and n-3/n-6 ratio.

A re-feeding experiment (finishing phase) was conducted with a 100% FO-based diet for 12 weeks (84 days) to monitor the recovery of FAs that are important to human nutrition with intermediate sampling every 3 weeks. Generally, SFA was the predominant class of FA in all groups except CO fed fish for the entire finishing phase. Levels of SFA reduced gradually from the end of the grow-out period until the week 9 of re-feeding period (Tables 5, 6, 7, 8) and increased thereafter (Table 9). The levels of monoenes decreased with increasing re-feeding period in groups initially fed FO-deprived diets. Total n-3 showed an increasing trend from the beginning of the re-feeding until the week 9

in all groups whereas total n-6 showed an opposite trend (Tables 6, 7, 8, 9). n-3 HUFA increased with re-feeding period until the week 9.

Although LA content of rainbow trout showed a decreasing trend during the finishing phase, total or partial replacement of FO resulted in high (P < 0.05) LA levels in comparison to FO diet (Fig. 1). ALA body content decreased in CO, MIX1 and MIX2 groups in the refeeding period while it increased in FO and CSO fed groups (Fig. 2). Further, CSO group had the lowest ALA content (P < 0.05). ARA content (Fig. 2) increased in all groups fed VO-based diet from the beginning of the re-feeding period until week 9 and was higher than ARA content during the grow-out period. ARA levels in fish fed partially substituted FO-diet (MIX1) reached values of FO fish from week 6 to week 9. EPA and DHA levels in fish initially fed VOs progressively increased along re-feeding with FO diet, but not reaching the values of FO fed fish (Figs. 4 and 5). Compared to the end of grow-out period, using a FO finishing diet

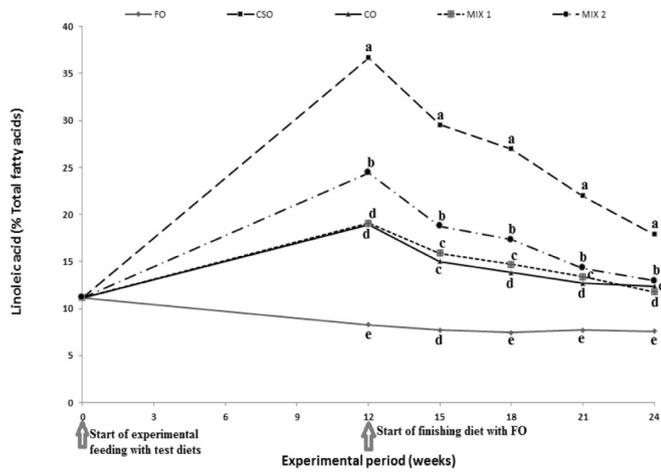


Fig. 1. Variation in linoleic acid content of whole body rainbow trout during the different phases of experimental feeding. Different letters at a particular time denote significant differences.

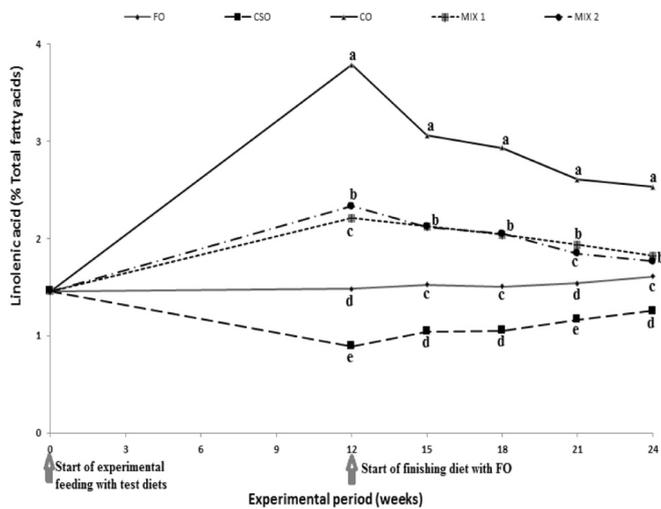


Fig. 2. Variation in linolenic acid content of whole body rainbow trout during the different phases of experimental feeding. Different letters at a particular time denote significant differences.

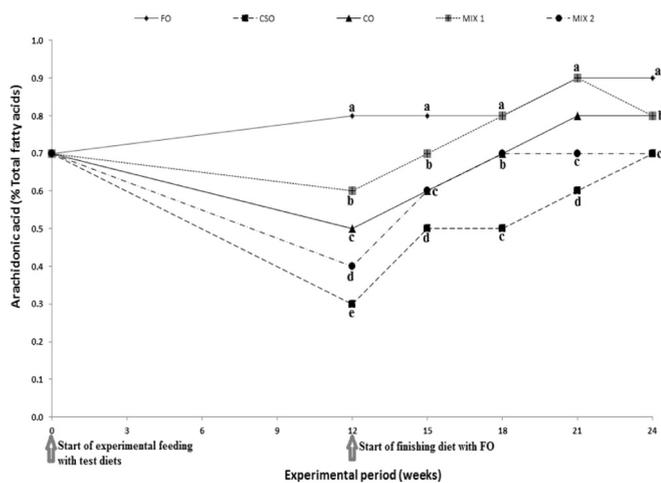


Fig. 3. Variation in arachidonic acid content of whole body rainbow trout during the different phases of experimental feeding. Different letters at a particular time denote significant differences.

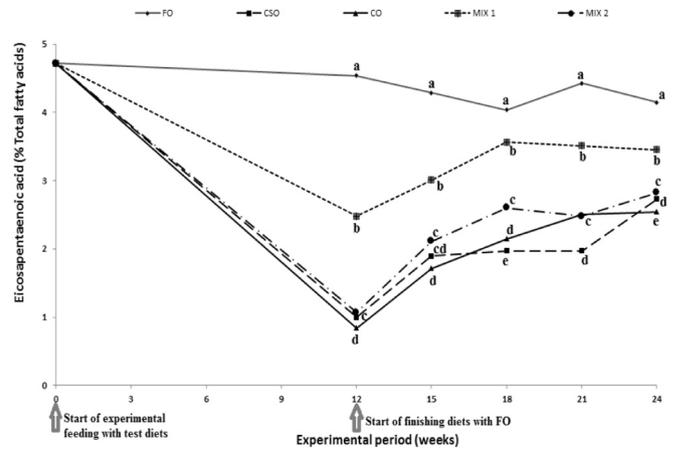


Fig. 4. Variation in eicosapentaenoic acid content of whole body rainbow trout during the different phases of experimental feeding. Different letters at a particular time denote significant differences.

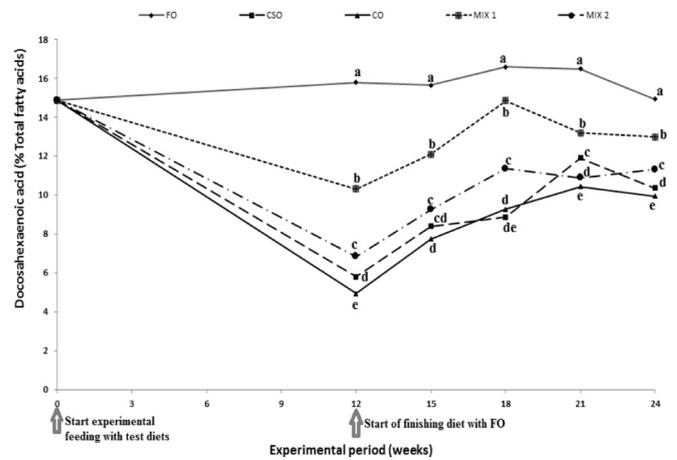


Fig. 5. Variation in docosahexaenoic acid content of whole body rainbow trout during the different phases of experimental feeding. Different letters at a particular time denote significant differences.

resulted in 133.3–350%, 140–312.5% and 125–204.1% increase in body contents of ARA, EPA and DHA respectively.

4. Discussion

After feeding VO-based diet in the grow-out period, fish weight increased over seven (7) folds in 12 weeks. The growth performance recorded in the present study has shown that it is possible to totally replace FO with VO in diets for rainbow trout without significantly affecting growth and feed utilization, in agreement with the results of previous studies (Fonseca-Madrigal et al., 2005; Guler and Yildiz, 2011; Yildiz et al., 2013). However, lower final weight of fish fed CSO and CO diets along with significantly lower PER obtained from fish fed CSO diet compared to that of other dietary treatments might have resulted from palatability problem and/or the reduced ability of trout of this size (~15 g) to utilize high amounts of plant oils in diets. However, this was not reflected in final SGR values. There were no significant differences in final weight among rainbow trout fed any of the dietary treatments at the end of the re-feeding period with FO finishing diet. This has probably indicated that fish initially fed the CSO diet did catch up with the growth rates, as observed in fish fed other dietary treatments.

After the grow-out period, fatty acid profiles of diets containing VOs were reflected in the FA profiles of whole body of fish, characterized by high LA and OA and low HUFA contents. Several studies have shown that salmonids respond differently to different VOs, making it difficult

Table 6
Fatty acid composition of whole body of fish fed finishing diet containing only FO as lipid source for 3 weeks (g/100 g fatty acids).^a

	Fish groups				
	FO	CSO	CO	MIX1	MIX2
14:0	5.0 ± 0.25 ^a	3.0 ± 0.03 ^c	2.6 ± 0.01 ^d	4.4 ± 0.04 ^b	3.3 ± 0.19 ^c
15:0	0.6 ± 0.03 ^a	0.3 ± 0.01 ^d	0.3 ± 0.01 ^d	0.5 ± 0.01 ^b	0.4 ± 0.02 ^c
16:0	18.3 ± 0.88 ^b	20.7 ± 0.08 ^a	14.4 ± 0.05 ^c	19.5 ± 0.18 ^{ab}	18.8 ± 0.95 ^b
17:0	0.8 ± 0.04 ^a	0.4 ± 0.01 ^d	0.4 ± 0.01 ^d	0.7 ± 0.02 ^b	0.5 ± 0.03 ^c
18:0	4.0 ± 0.17 ^d	5.6 ± 0.03 ^a	3.6 ± 0.02 ^e	4.8 ± 0.02 ^c	5.2 ± 0.24 ^b
20:0	0.2 ± 0.01 ^b	0.2 ± 0.01 ^b	0.2 ± 0.00 ^b	0.3 ± 0.00 ^a	0.2 ± 0.01 ^b
16:1n-7	6.3 ± 0.38 ^a	3.2 ± 0.01 ^d	3.3 ± 0.01 ^d	4.7 ± 0.04 ^b	3.7 ± 0.19 ^c
18:1n-9	23.0 ± 1.01 ^{bc}	15.8 ± 0.05 ^d	38.6 ± 0.06 ^a	20.5 ± 0.10 ^c	24.5 ± 3.48 ^b
20:1n-9	2.8 ± 0.12 ^b	2.2 ± 0.02 ^a	2.8 ± 0.03 ^b	2.9 ± 0.01 ^{ab}	3.0 ± 0.16 ^a
22:1n-11	0.8 ± 0.03 ^b	0.7 ± 0.07 ^c	0.7 ± 0.07 ^c	0.8 ± 0.02 ^b	0.9 ± 0.05 ^a
20:2n-9	0.5 ± 0.02 ^d	1.7 ± 0.01 ^a	1.0 ± 0.01 ^c	1.0 ± 0.01 ^c	1.3 ± 0.08 ^b
18:2n-6	7.7 ± 0.08 ^d	29.6 ± 0.07 ^a	15.0 ± 0.02 ^c	15.9 ± 0.06 ^c	18.8 ± 0.94 ^b
18:3n-6	ND	0.3 ± 0.01 ^a	0.2 ± 0.01 ^b	ND	0.2 ± 0.01 ^b
20:4n-6	0.8 ± 0.04 ^a	0.5 ± 0.01 ^d	0.6 ± 0.01 ^c	0.7 ± 0.01 ^b	0.6 ± 0.03 ^c
18:3n-3	1.5 ± 0.08 ^c	1.0 ± 0.01 ^d	3.1 ± 0.01 ^a	2.1 ± 0.01 ^b	2.1 ± 0.10 ^b
20:3n-3	0.2 ± 0.02 ^e	0.7 ± 0.01 ^a	0.4 ± 0.00 ^c	0.3 ± 0.00 ^d	0.5 ± 0.03 ^b
20:5n-3	4.3 ± 0.20 ^a	1.9 ± 0.01 ^{cd}	1.7 ± 0.01 ^d	3.0 ± 0.01 ^b	2.1 ± 0.10 ^c
22:5n-3	1.7 ± 0.07 ^a	0.8 ± 0.00 ^d	0.7 ± 0.01 ^d	1.2 ± 0.01 ^b	0.9 ± 0.03 ^c
22:6n-3	15.6 ± 0.67 ^a	8.4 ± 0.04 ^{cd}	7.7 ± 0.03 ^d	12.1 ± 0.03 ^b	9.2 ± 0.46 ^c
Σ saturates	28.9 ± 1.38 ^{ab}	30.2 ± 0.13 ^a	21.6 ± 0.05 ^c	30.1 ± 0.27 ^a	28.4 ± 1.41 ^b
Σ monoenes	25.8 ± 1.13 ^{bc}	18.0 ± 0.06 ^d	41.4 ± 0.04 ^a	23.3 ± 0.09 ^c	27.5 ± 3.33 ^b
Σ n-3	23.4 ± 1.03 ^a	12.8 ± 0.05 ^d	13.6 ± 0.03 ^{cd}	18.7 ± 0.03 ^b	14.9 ± 0.70 ^c
Σ n-6	8.5 ± 0.06 ^e	30.4 ± 0.07 ^a	15.7 ± 0.03 ^d	16.6 ± 0.07 ^c	19.5 ± 0.97 ^b
Σ n-9	26.3 ± 1.15 ^{bc}	19.7 ± 0.06 ^d	42.3 ± 0.04 ^a	24.3 ± 0.09 ^c	28.8 ± 3.3 ^b
Σ n-3 HUFA	21.8 ± 0.96 ^a	11.8 ± 0.05 ^{cd}	10.6 ± 0.03 ^d	16.6 ± 0.04 ^b	12.7 ± 0.61 ^c
AA/EPA	0.19 ± 0.00 ^e	0.25 ± 0.00 ^c	0.33 ± 0.00 ^a	0.22 ± 0.00 ^d	0.27 ± 0.00 ^b
EPA/DHA	0.27 ± 0.00 ^a	0.23 ± 0.00 ^c	0.22 ± 0.00 ^d	0.25 ± 0.00 ^b	0.23 ± 0.00 ^c
n-3/n-6 ratio	2.5 ± 0.02 ^a	0.4 ± 0.00 ^e	0.9 ± 0.00 ^c	1.1 ± 0.01 ^b	0.8 ± 0.00 ^d
Lipid (%)	15.1 ± 0.21 ^a	15.2 ± 0.15 ^a	15.5 ± 0.13 ^a	15.7 ± 0.10 ^a	15.6 ± 0.09 ^a

ND: not detected.

^a Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 5 fish). Means with different superscript letter in a row are significantly different (P < 0.05).

Table 7
Fatty acid composition of whole body of fish fed finishing diet containing only FO as lipid source for 6 weeks (g/100 g fatty acids).^a

	Fish groups				
	FO	CSO	CO	MIX1	MIX2
14:0	4.7 ± 0.08 ^a	2.9 ± 0.09 ^d	2.8 ± 0.03 ^d	4.2 ± 0.11 ^b	3.6 ± 0.02 ^c
15:0	0.6 ± 0.02 ^a	0.3 ± 0.02 ^c	0.3 ± 0.01 ^c	0.4 ± 0.01 ^b	0.4 ± 0.01 ^b
16:0	16.9 ± 0.05 ^c	19.7 ± 0.69 ^a	14.2 ± 0.08 ^d	18.8 ± 0.24 ^b	19.4 ± 0.04 ^a
17:0	0.8 ± 0.00 ^a	0.4 ± 0.01 ^c	0.4 ± 0.00 ^c	0.6 ± 0.01 ^b	0.6 ± 0.01 ^b
18:0	3.6 ± 0.00 ^c	5.6 ± 0.20 ^a	3.4 ± 0.01 ^c	5.1 ± 0.03 ^b	5.3 ± 0.02 ^b
20:0	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.01 ^a	0.2 ± 0.02 ^a	0.2 ± 0.00 ^a
16:1n-7	6.1 ± 0.02 ^a	3.1 ± 0.12 ^c	3.6 ± 0.01 ^d	4.9 ± 0.11 ^b	4.2 ± 0.01 ^c
18:1n-9	21.1 ± 0.14 ^b	18.2 ± 1.90 ^c	36.6 ± 0.02 ^a	20.7 ± 0.01 ^b	21.4 ± 0.09 ^b
20:1n-9	2.8 ± 0.02 ^b	2.3 ± 0.08 ^c	2.9 ± 0.01 ^a	2.8 ± 0.03 ^b	3.0 ± 0.04 ^a
24:1n-9	0.9 ± 0.01 ^a	0.6 ± 0.03 ^d	0.7 ± 0.06 ^c	0.8 ± 0.00 ^b	0.8 ± 0.01 ^b
20:2n-9	0.5 ± 0.00 ^c	1.7 ± 0.06 ^a	0.9 ± 0.01 ^b	0.9 ± 0.02 ^b	1.1 ± 0.01 ^b
18:2n-6	7.4 ± 0.01 ^e	27.0 ± 1.03 ^a	13.8 ± 0.02 ^d	14.7 ± 0.03 ^c	17.3 ± 0.05 ^b
18:3n-6	ND	0.3 ± 0.02 ^a	0.2 ± 0.01 ^b	ND	0.2 ± 0.01 ^b
20:4n-6	0.8 ± 0.01 ^a	0.5 ± 0.01 ^c	0.7 ± 0.00 ^b	0.8 ± 0.01 ^a	0.7 ± 0.00 ^b
18:3n-3	1.5 ± 0.01 ^c	1.1 ± 0.03 ^d	2.9 ± 0.01 ^a	2.0 ± 0.01 ^b	2.1 ± 0.01 ^b
20:3n-3	0.2 ± 0.01 ^d	0.7 ± 0.02 ^a	0.4 ± 0.01 ^c	0.2 ± 0.00 ^d	0.5 ± 0.01 ^b
20:5n-3	4.0 ± 0.02 ^a	2.0 ± 0.08 ^c	2.1 ± 0.01 ^d	3.6 ± 0.05 ^b	2.6 ± 0.02 ^c
22:5n-3	1.7 ± 0.02 ^a	0.8 ± 0.04 ^d	0.8 ± 0.01 ^d	1.4 ± 0.02 ^b	1.0 ± 0.02 ^c
22:6n-3	16.6 ± 0.09 ^a	8.9 ± 0.32 ^{de}	9.3 ± 0.04 ^d	14.9 ± 0.17 ^b	11.3 ± 0.14 ^c
Σ saturates	26.5 ± 0.09 ^b	29.0 ± 1.01 ^a	21.4 ± 0.10 ^c	29.3 ± 0.28 ^a	29.5 ± 0.03 ^a
Σ monoenes	23.9 ± 0.15 ^b	20.5 ± 1.81 ^c	39.5 ± 0.04 ^a	23.5 ± 0.02 ^b	24.4 ± 0.08 ^b
Σ n-3	24.1 ± 0.11 ^a	13.3 ± 0.48 ^c	15.6 ± 0.03 ^d	21.9 ± 0.38 ^b	17.5 ± 0.18 ^c
Σ n-6	8.2 ± 0.01 ^d	27.7 ± 1.06 ^a	14.7 ± 0.03 ^c	15.5 ± 0.03 ^c	18.2 ± 0.06 ^b
Σ n-9	25.3 ± 0.16 ^{cd}	22.8 ± 1.73 ^d	41.2 ± 0.04 ^a	25.2 ± 0.03 ^{cd}	26.3 ± 0.08 ^b
Σ n-3 HUFA	22.6 ± 0.12 ^a	12.3 ± 0.45 ^d	12.7 ± 0.03 ^d	19.9 ± 0.36 ^b	15.4 ± 0.17 ^c
AA/EPA	0.20 ± 0.00 ^e	0.24 ± 0.00 ^c	0.31 ± 0.00 ^a	0.21 ± 0.00 ^d	0.25 ± 0.00 ^b
EPA/DHA	0.24 ± 0.00 ^a	0.22 ± 0.00 ^a	0.23 ± 0.00 ^a	0.24 ± 0.00 ^a	0.23 ± 0.00 ^a
n-3/n-6 ratio	2.9 ± 0.01 ^a	0.5 ± 0.00 ^c	1.1 ± 0.00 ^c	1.4 ± 0.03 ^b	1.0 ± 0.01 ^d
Lipid (%)	15.3 ± 0.32 ^a	14.9 ± 0.19 ^a	15.1 ± 0.12 ^a	15.9 ± 0.09 ^a	15.8 ± 0.10 ^a

ND: not detected.

^a Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 5 fish). Means with different superscript letter in a row are significantly different (P < 0.05).

Table 8

Fatty acid composition of whole body of fish fed finishing diet containing only FO as lipid source for 9 weeks (g/100 g fatty acids).^a

	Fish groups				
	FO	CSO	CO	MIX1	MIX2
14:0	4.6 ± 0.03 ^a	3.3 ± 0.02 ^d	3.2 ± 0.02 ^c	4.1 ± 0.01 ^b	3.5 ± 0.02 ^c
15:0	0.6 ± 0.01 ^a	0.4 ± 0.01 ^c	0.4 ± 0.01 ^c	0.5 ± 0.01 ^b	0.4 ± 0.01 ^c
16:0	16.6 ± 0.10 ^c	18.8 ± 0.05 ^a	14.7 ± 0.03 ^d	16.5 ± 0.02 ^c	18.2 ± 0.10 ^b
17:0	0.8 ± 0.01 ^a	0.4 ± 0.02 ^c	0.4 ± 0.00 ^c	0.6 ± 0.01 ^b	0.4 ± 0.03 ^c
18:0	3.6 ± 0.00 ^d	5.3 ± 0.01 ^a	3.4 ± 0.01 ^e	3.9 ± 0.01 ^c	5.1 ± 0.01 ^b
20:0	0.3 ± 0.00 ^a	0.2 ± 0.01 ^b	0.3 ± 0.00 ^a	0.3 ± 0.01 ^a	0.3 ± 0.01 ^a
21:0	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a	0.1 ± 0.01 ^a
22:0	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a
14:1n	0.1 ± 0.01 ^a	ND	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a	0.1 ± 0.01 ^a
16:1n-7	5.9 ± 0.04 ^a	3.6 ± 0.02 ^c	4.0 ± 0.02 ^d	4.6 ± 0.01 ^b	4.2 ± 0.02 ^c
18:1n-9	20.8 ± 0.04 ^d	18.7 ± 0.02 ^c	31.0 ± 0.03 ^a	22.5 ± 0.03 ^c	24.4 ± 0.05 ^b
20:1n-9	2.7 ± 0.02 ^c	2.2 ± 0.01 ^e	2.9 ± 0.01 ^a	2.6 ± 0.02 ^d	2.8 ± 0.01 ^b
22:1n-9	0.2 ± 0.01 ^a	0.1 ± 0.01 ^b	0.1 ± 0.01 ^b	0.1 ± 0.02 ^b	0.1 ± 0.05 ^b
24:1n-9	0.8 ± 0.04 ^a	0.6 ± 0.01 ^c	0.7 ± 0.03 ^b	0.7 ± 0.02 ^b	0.7 ± 0.01 ^b
20:2n-9	0.6 ± 0.00 ^e	1.4 ± 0.02 ^a	0.9 ± 0.01 ^c	0.8 ± 0.01 ^d	1.1 ± 0.00 ^b
22:2n	0.5 ± 0.01 ^a	0.2 ± 0.00 ^b	0.2 ± 0.01 ^b	0.2 ± 0.01 ^b	0.2 ± 0.00 ^b
18:2n-6	7.7 ± 0.05 ^e	22.0 ± 0.01 ^a	12.7 ± 0.01 ^d	13.4 ± 0.01 ^c	14.3 ± 0.02 ^b
18:3n-6	0.1 ± 0.00 ^c	0.3 ± 0.01 ^a	0.2 ± 0.01 ^b	0.1 ± 0.01 ^c	0.2 ± 0.00 ^b
20:4n-6	0.9 ± 0.01 ^a	0.6 ± 0.02 ^d	0.8 ± 0.00 ^b	0.9 ± 0.00 ^a	0.7 ± 0.01 ^c
18:3n-3	1.5 ± 0.01 ^d	1.2 ± 0.01 ^c	2.6 ± 0.01 ^a	1.9 ± 0.01 ^b	1.8 ± 0.01 ^c
20:3n-3	0.2 ± 0.00 ^d	0.6 ± 0.01 ^a	0.4 ± 0.00 ^b	0.3 ± 0.01 ^c	0.4 ± 0.01 ^b
20:5n-3	4.4 ± 0.02 ^a	2.0 ± 0.58 ^d	2.5 ± 0.01 ^c	3.5 ± 0.02 ^b	2.5 ± 0.01 ^c
22:5n-3	1.7 ± 0.02 ^a	0.9 ± 0.01 ^c	0.9 ± 0.01 ^c	1.3 ± 0.01 ^b	0.9 ± 0.01 ^c
22:6n-3	16.5 ± 0.15 ^a	11.9 ± 0.09 ^c	10.4 ± 0.02 ^e	13.2 ± 0.09 ^b	10.9 ± 0.11 ^d
Σ saturates	26.1 ± 0.14 ^c	28.5 ± 0.07 ^a	22.6 ± 0.04 ^d	26.1 ± 0.04 ^c	28.0 ± 0.12 ^b
Σ monoenes	23.5 ± 0.02 ^d	21.0 ± 0.02 ^e	34.0 ± 0.04 ^a	25.1 ± 0.03 ^c	27.2 ± 0.04 ^b
Σ n-3	24.3 ± 0.18 ^a	16.5 ± 0.58 ^c	16.9 ± 0.04 ^c	20.2 ± 0.12 ^b	16.5 ± 0.12 ^c
Σ n-6	8.8 ± 0.05 ^e	22.9 ± 0.03 ^a	13.7 ± 0.01 ^d	14.4 ± 0.02 ^c	15.19 ± 0.03 ^b
Σ n-9	25.0 ± 0.03 ^c	23.1 ± 0.04 ^c	35.6 ± 0.04 ^a	26.7 ± 0.02 ^b	29.1 ± 0.09 ^b
Σ n-3 HUFA	22.8 ± 0.18 ^a	15.3 ± 0.58 ^c	14.3 ± 0.03 ^d	18.3 ± 0.11 ^b	14.7 ± 0.12 ^{cd}
AA/EPA	0.21 ± 0.00 ^b	0.34 ± 0.13 ^a	0.32 ± 0.00 ^{ab}	0.24 ± 0.00 ^{ab}	0.28 ± 0.00 ^{ab}
EPA/DHA	0.27 ± 0.00 ^a	0.17 ± 0.05 ^b	0.24 ± 0.00 ^a	0.27 ± 0.00 ^a	0.23 ± 0.00 ^a
n-3/n-6 ratio	2.8 ± 0.04 ^a	0.7 ± 0.03 ^c	1.2 ± 0.00 ^c	1.4 ± 0.01 ^b	1.1 ± 0.01 ^d
Lipid (%)	14.5 ± 0.20 ^a	14.1 ± 0.25 ^a	15.2 ± 0.09 ^a	14.7 ± 0.04 ^a	15.1 ± 0.09 ^a

ND: not detected.

^a Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 5 fish). Means with different superscript letter in a row are significantly different (P < 0.05).

to make direct comparison between studies. In Atlantic salmon, flesh fatty acid profile showed significant increases in OA and LA as well as ALA with increasing inclusion of rapeseed oil (Bell et al., 2003b). Tocher et al. (2003) reported increased levels of OA and LA with increasing inclusion of rapeseed oil whereas ALA increased with increasing inclusion of linseed oil in Atlantic salmon. Increasing levels of dietary sunflower oil led to increased levels of muscle LA content in Atlantic salmon (Miller et al., 2007). Bell et al. (2001) reported selective utilization of OA, LA and ALA when present at high concentrations in the diet in Atlantic salmon. Thus, the reduced accumulation of dietary monoenes and LA coupled with good growth, survival and feed utilization in the current study is suggestive of the ability of rainbow trout to meet their essential fatty acid (EFA) requirement by the use of monoenes and LA. It may also be explained that residual fish oil in the fishmeal of the diets containing VOs was perhaps enough to provide essential HUFAs for normal growth and development. Further research targeting detailed fatty acid metabolism using diets containing fat free fishmeal or purified diets may help to understand the fate of predominant fatty acid classes in cottonseed (CSO) and canola oils (CO) in the rainbow trout.

Fatty acid metabolism in living organisms is complex involving uptake of free FA that may not be on a ratio of 1:1 (dietary amount: uptake) and re-esterification in the body (Tocher, 2003). However, in the present study, total or partial substitution of dietary FO (as in CSO, CO and MIX2 or MIX1) led to a reduction in the body HUFAs (EPA, DHA and ARA). This is in agreement with previous studies in which rainbow trout have been fed diets that included plant oils (Caballero et al., 2002; Bell et al., 2004; Hixson et al., 2014a, 2014b; Yıldız et al., 2015). HUFA

retention in rainbow trout in the present study followed the pattern, DHA > ARA > EPA. FA, especially HUFA, are known to be very important to the optimal growth of rainbow trout (Bell et al., 2001; Sargent et al., 2002; Bell and Dick, 2005; Rinchard et al., 2007; Tocher, 2010) in culture and are highly retained when their dietary levels are low. Results of the current study showed a higher retention of DHA in fish fed VO-based diet (Table 5). Previous studies in which fish were fed VO-based diets reported high DHA retention in muscles of salmonids (Bell et al., 2001, 2003a; Caballero et al., 2002; Thanuthong et al., 2011a). The preferred retention of DHA over EPA could be EPA being β-oxidised readily (Madsen et al., 1998; Izquierdo et al., 2005), and may also indicate a selective utilization of EPA over DHA when dietary levels decrease, as a means of meeting the requirements for tissue membrane integrity and function (Fountoulaki et al., 2009). There was also the production and selective retention of 20:3n-3 and 22:5n-3 (Tables 5, 6, 7, 8, 9), intermediate metabolites in the DHA synthesis from precursor ALA, which were not detected in dietary FA profiles. The detection of these intermediate metabolites confirms the ability of fresh water species such as rainbow trout to bio-convert PUFA such as ALA to DHA (Sargent et al., 2002; Tocher, 2003, 2010) through the activation of Δ6 and Δ5 desaturases when fed diets low in n-3 HUFA (Guler and Yıldız, 2011; Yıldız et al., 2013). Aside from providing vitamins, minerals and being a rich source of easily digested high quality proteins, fish consumption is associated with health benefits due to its high HUFA content when eaten whole (FAO, 2016). These EFA provide protection against cardiovascular and neurological diseases (Simopoulos, 2005; Von Schacky, 2006; Ruxton et al., 2007) while aiding fetal and infant brain and nervous system development (FAO,

Table 9

Fatty acid composition of whole body of fish fed finishing diet containing only FO as lipid source for 12 weeks (g/100 g fatty acids).^a

	Fish groups				
	FO	CSO	CO	MIX1	MIX2
14:0	4.7 ± 0.03 ^a	3.8 ± 0.03 ^c	3.3 ± 0.02 ^d	4.1 ± 0.01 ^b	3.8 ± 0.00 ^c
15:0	0.6 ± 0.01 ^a	0.5 ± 0.01 ^b	0.4 ± 0.01 ^c	0.5 ± 0.01 ^b	0.1 ± 0.01 ^d
16:0	16.9 ± 0.05 ^c	19.0 ± 0.07 ^a	14.9 ± 0.04 ^d	16.8 ± 0.00 ^c	17.5 ± 0.03 ^b
17:0	0.7 ± 0.01 ^a	0.6 ± 0.01 ^b	0.5 ± 0.01 ^c	0.7 ± 0.01 ^a	0.2 ± 0.01 ^d
18:0	3.6 ± 0.01 ^d	5.1 ± 0.01 ^a	3.4 ± 0.01 ^e	4.3 ± 0.01 ^c	4.6 ± 0.01 ^b
20:0	0.2 ± 0.01 ^b	0.2 ± 0.01 ^b	0.3 ± 0.01 ^a	0.3 ± 0.00 ^a	0.2 ± 0.01 ^b
21:0	0.1 ± 0.01 ^a	0.1 ± 0.02 ^a	0.1 ± 0.01 ^a	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a
22:0	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.00 ^a	0.1 ± 0.00 ^a
23:00	ND	ND	ND	ND	0.8 ± 0.01
14:1n	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.01 ^a	0.1 ± 0.00 ^a	0.1 ± 0.01 ^a
16:1n-7	5.9 ± 0.04 ^a	4.4 ± 0.03 ^d	4.2 ± 0.02 ^e	4.9 ± 0.01 ^b	4.6 ± 0.01 ^c
18:1n-9	20.8 ± 0.04 ^d	19.7 ± 0.01 ^e	30.7 ± 0.02 ^a	22.6 ± 0.04 ^c	24.0 ± 0.03 ^b
20:1n-9	2.7 ± 0.02 ^b	2.4 ± 0.01 ^c	2.9 ± 0.01 ^a	2.7 ± 0.01 ^b	0.2 ± 0.01 ^d
22:1n-9	0.2 ± 0.01 ^b	0.2 ± 0.01 ^b	0.3 ± 0.01 ^a	0.2 ± 0.01 ^b	0.2 ± 0.00 ^b
24:1n-9	0.8 ± 0.04 ^a	0.7 ± 0.01 ^b			
20:2n-9	0.6 ± 0.00 ^e	1.1 ± 0.01 ^a	0.9 ± 0.01 ^c	0.8 ± 0.01 ^d	1.0 ± 0.01 ^b
22:2n	0.5 ± 0.01 ^a	0.4 ± 0.01 ^b	0.4 ± 0.01 ^b	0.4 ± 0.01 ^b	0.2 ± 0.01 ^c
18:2n-6	7.7 ± 0.05 ^c	17.9 ± 0.01 ^a	12.4 ± 0.01 ^c	11.8 ± 0.02 ^d	12.9 ± 0.01 ^b
18:3n-6	0.1 ± 0.00 ^c	0.3 ± 0.01 ^a	0.2 ± 0.01 ^b	0.1 ± 0.01 ^c	0.3 ± 0.00 ^a
20:4n-6	0.9 ± 0.01 ^a	0.7 ± 0.01 ^c	0.8 ± 0.01 ^b	0.8 ± 0.01 ^b	0.7 ± 0.01 ^c
18:3n-3	1.5 ± 0.01 ^c	1.3 ± 0.01 ^d	2.5 ± 0.00 ^a	1.8 ± 0.00 ^b	1.8 ± 0.06 ^b
20:3n-3	0.2 ± 0.00 ^d	0.5 ± 0.01 ^a	0.4 ± 0.01 ^b	0.3 ± 0.01 ^c	0.4 ± 0.06 ^b
20:5n-3	4.4 ± 0.02 ^a	2.7 ± 0.01 ^d	2.5 ± 0.02 ^e	3.5 ± 0.02 ^b	2.8 ± 0.02 ^c
22:5n-3	1.7 ± 0.02 ^a	1.1 ± 0.01 ^c	1.0 ± 0.03 ^d	1.3 ± 0.01 ^b	1.1 ± 0.01 ^c
22:6n-3	16.5 ± 0.15 ^a	10.4 ± 0.04 ^d	10.0 ± 0.03 ^e	13.0 ± 0.04 ^b	11.3 ± 0.10 ^c
Σ saturates	26.1 ± 0.14 ^c	29.3 ± 0.12 ^a	23.1 ± 0.07 ^d	26.8 ± 0.01 ^b	26.4 ± 0.03 ^c
Σ monoenes	24.5 ± 0.01 ^c	22.1 ± 0.01 ^e	33.6 ± 0.02 ^a	25.3 ± 0.04 ^b	24.1 ± 0.03 ^d
Σ n-3	22.7 ± 0.12 ^a	15.9 ± 0.06 ^e	16.4 ± 0.08 ^d	19.9 ± 0.06 ^b	17.4 ± 0.04 ^c
Σ n-6	8.5 ± 0.02 ^c	18.6 ± 0.01 ^a	13.4 ± 0.02 ^c	12.7 ± 0.04 ^d	14.0 ± 0.02 ^b
Σ n-9	26.1 ± 0.02 ^{bc}	24.1 ± 0.02 ^c	35.4 ± 0.03 ^a	27.1 ± 0.04 ^b	26.1 ± 0.03 ^{bc}
Σ n-3 HUFA	21.1 ± 0.11 ^a	14.7 ± 0.05 ^d	13.9 ± 0.08 ^e	18.1 ± 0.06 ^b	15.6 ± 0.05 ^c
AA/EPA	0.21 ± 0.00 ^d	0.25 ± 0.00 ^b	0.31 ± 0.00 ^a	0.23 ± 0.00 ^c	0.26 ± 0.00 ^b
EPA/DHA	0.28 ± 0.00 ^a	0.26 ± 0.00 ^c	0.26 ± 0.00 ^c	0.27 ± 0.00 ^b	0.25 ± 0.00 ^d
n-3/n-6 ratio	2.7 ± 0.01 ^a	0.8 ± 0.01 ^d	1.2 ± 0.01 ^c	1.6 ± 0.01 ^b	1.2 ± 0.01 ^c
Lipid (%)	14.8 ± 0.36 ^a	15.3 ± 0.52 ^a	15.1 ± 0.09 ^a	14.4 ± 0.82 ^a	14.2 ± 0.19 ^a

ND: not detected.

^a Values are mean ± SD (n = 3/diet treatment with each mean based on the analysis of 5 fish). Means with different superscript letter in a row are significantly different (P < 0.05).

2016). According to EFSA (2010), recommended daily intake (RDI) of EPA + DHA for healthy human individuals is estimated to be at least 0.25 g per day. At the end of grow-out period, rainbow trout in the present study attained a final weight of approximately 125 g. The body content of EPA + DHA ranged from 0.91–1.26 g/100 g in rainbow trout fed 100% VO-diets (CSO, CO, MIX2). Although feeding a VO-diet may negatively affect their nutritional value for human health due to the reduced body contents of HUFA (Izquierdo et al., 2005), n-3 HUFA levels in rainbow trout after the grow-out denoted good nutritional quality.

Re-feeding with FO-diet as a finishing strategy (wash out) for 84 days partially restored alterations in body fatty acids make-up by the 12 week grow-out period in which FO was totally and/or partially replaced with CSO, CO and their mixtures. In a 91 day growth-out period in which fish were fed alternative oil treatments with increasing ALA content, Thanuthong et al. (2011a) obtained similar results after 35 day finishing period. The levels of individual fatty acids at the beginning of a finishing period directly influence the time required to influence the final composition (Turchini et al., 2009). With LA and OA being the predominant fatty acids at the beginning of the finishing phase, feeding FO diet reduced body contents of these, but could not restore the levels of these in fish previously fed a VO-based diet in the present study (Table 9, Figs. 1 and 2). After 12 weeks of feeding exclusively FO diet, Bell et al. (2003b) reported significantly high levels of OA and LA in fish previously fed increasing levels of rapeseed oil. A longer period (24 weeks) finishing phase only partially reduced LA and ALA levels in Atlantic salmon previously fed diets in which linseed oil totally or

partially replaced FO for 40 weeks (Bell et al., 2004). Similarly, Thanuthong et al. (2011a) reported partial reductions in ALA, LA and OA respectively in rainbow trout following finishing period of 35 days. This implies some levels of OA, LA and ALA continue to be retained in tissues throughout the finishing phase in fish initially fed VOs high in OA, LA and ALA. The rationale of the finishing strategy was to monitor the restoration of HUFA contents of fish previously fed VO-based diet. The re-feeding with FO diet improved body contents of ARA, EPA and DHA in fish previously fed VO-based diets (Table 9). This further enhanced the nutritional value of rainbow trout fed FO-deprived diets for human nutrition. Although body content of ARA was fully recovered in all dietary groups, the 84 days re-feeding with FO-diet could not completely restore the levels of EPA and DHA in fish previously fed diets devoid of FO. It also appeared that progressive increment in growth during finishing phase had no direct relationship to the HUFAs (measured triweekly), specifically EPA and DHA recovery rates of fish in this study. Fountoulaki et al. (2009) suggested that the low contents of these HUFA were due to slower rates in restoration of HUFA (ARA, EPA and DHA) in tissues with higher phospholipid content.

There was a partial restoration in muscle HUFA contents in rainbow trout fed a FO finishing diet for 35 days after an initial 91 days of feeding diets containing varying mixtures of beef tallow, sunflower and linseed oils (Thanuthong et al., 2011a). Following an 18-week grow-out period on a VO-based diet, rainbow trout fillet DHA and EPA were partially restored in an 8-week finishing phase (Thanuthong et al., 2012). 24 weeks of FO feeding restored 83% flesh concentrations of DHA and EPA in salmon initially fed 100% linseed oil for 40 weeks (Bell

et al., 2004). Bell et al. (2003a) found that Atlantic salmon initially fed diets containing 100% linseed and/or rapeseed oils restored the concentrations of EPA and DHA up to 80% of the values in fish fed 100% FO following 20 week FO finishing diet phase. In another study by Bell et al. (2003b), flesh concentrations of EPA and DHA in 200 g post-smolt salmon previously fed increasing rapeseed oil for 16 weeks took 4 and 12 weeks to recover whereas LA remained significantly higher in fish fed RO diets even after the 12 week wash out period. Similar results were also reported for European sea bass (Mourente and Bell, 2006) and gilthead seabream (Fountoulaki et al., 2009) after refeeding with FO diet for 20 weeks and 120 days respectively. ARA and/or DHA but not EPA could be restored in long feeding trials (trials \geq 5 months) and subsequent re-feeding with FO-diet for 104 days in sea bream (Izquierdo et al., 2005), 150 days in European sea bass (Montero et al., 2005) and 56 days in turbot (Regost et al., 2003). Interestingly, a similar study by Reis et al. (2014), evaluating the long term (5 months) effects of feeding VO-based diets to Senegalese sole completely restored all HUFA (ARA, EPA and DHA) after re-feeding with FO-diet for just 26 days. It can therefore be inferred from above that the rate of restoration of HUFA in fish previously fed a VO-based employing 100% FO finishing diet to maintain nutritional value for human consumption is dependent on the species, size of fish, inclusion level, duration of feeding with VO-based diet and the subsequent re-feeding period. Of all the groups fed the VO-based diets, MIX1 fish had the FA profile that resembles closely that of FO fed fish (Table 9). This group were fed a diet in which FO was partially (50% FO) replaced with a mixture of VO (CSO and CO in equal parts) during the grow-out phase. There was a 74.5 and 87.2% recovery of body content of EPA and DHA, whereas ARA was fully recovered in this dietary group. Therefore modification of body FA profile was not as severe as those fed FO-deprived (CSO, CO and MIX2) diets. Perhaps, a week or two of re-feeding with FO-diet in MIX1 group could have fully restored FA profile to that of FO group. At the end of the re-feeding, fish attained body weights above 300 g in the current study and consumption of this size of rainbow trout would be enough to provide the daily RDI of EPA and DHA for humans.

In conclusion, results of this study suggest that it is possible to replace FO totally with VO-based diets in rainbow trout without significantly affecting growth and feed utilization. FA profile of fish reflected dietary FA composition, characterized by reduced contents of ARA, EPA and DHA, when fed VO-based diets. The subsequent feeding with FO diet effectively restored body contents of ARA although EPA and DHA were not fully recovered. In spite of this, rainbow trout produced from the present study remained highly nutritious for human health. Among all groups fed VO-based diets, MIX1 fed fish had FA profile close to FO group.

Acknowledgement

The authors express their sincere gratitude to the management and staff of Sapanca Inland Waters Research Center of the Faculty of Aquatic Sciences, Istanbul University, Turkey for the technical help given throughout the investigation. This work was funded by the Research Fund of Istanbul University (grant number 2977).

References

- AOAC (Ed.), 2006. Official Methods of Analysis of AOAC International, eighteenth ed. AOAC International, Maryland, USA.
- Bell, M.V., Dick, J.R., 2005. Distribution of 22:6n-3 newly synthesized from 18:3n-3 into glycerolipid classes from tissues of rainbow trout (*Oncorhynchus mykiss*). *Lipids* 40 (7), 703–708.
- Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P.J., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J. Nutr.* 131, 1535–1543.
- Bell, J.G., Tocher, D.R., Henderson, R.J., Dick, J.R., Crampton, V.O., 2003a. Altered fatty acid compositions in Atlantic salmon (*Salmo salar*) fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J. Nutr.* 133, 2793–2801.
- Bell, J.G., McGhee, F., Campbell, P.J., Sargent, J.R., 2003b. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil “wash out”. *Aquaculture* 218, 515–528.
- Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R., 2004. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet. *Lipids* 39 (3), 223–232.
- Bransden, M.P., Carter, C.G., Nichols, P.D., 2003. Replacement of fish oil with sunflower oil in feeds for Atlantic salmon (*Salmo salar* L.): effect on growth performance, tissue fatty acid composition and disease resistance. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* 135 (4), 611–625.
- Caballero, M.J., Obach, A., Roselund, G., Montero, D., Gisvold, M., Izquierdo, M.S., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214, 253–271.
- EFSA Panel on Dietetic Products, Nutrition, and Allergies, 2010. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J.* 8 (3), 1461. <http://dx.doi.org/10.2903/j.efsa.2010.1461>.
- FAO, 2014. The State of World Fisheries and Aquaculture: Opportunities and Challenges. (Rome).
- FAO, 2016. The State of World Fisheries and Aquaculture: Contributing to Food Security and Nutrition for All. (Rome).
- Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226, 497–509.
- Fonseca-Madrugal, J., Karalazos, V., Campbell, P.J., Bell, J.G., Tocher, D.R., 2005. Influence of dietary palm oil on growth, tissue fatty acid composition, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.* 11, 241–250.
- Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B., Alexis, M.N., 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.): effects on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. *Aquaculture* 289, 317–326.
- Guler, M., Yıldız, M., 2011. Effects of dietary fish oil replacement by cottonseed oil on growth performance and fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Turk. J. Vet. Anim. Sci.* 35 (3), 157–167.
- Hardy, R.W., Higgs, D.A., Lalla, S.P., Tacon, A.G.J., 2001. Alternative Dietary Protein and Lipid Sources for Sustainable Production of Salmonids. *Fisken Og Havet NR*, pp. 8.
- Hixson, S.M., Parrish, C.C., Anderson, D.M., 2014a. Changes in tissue lipid and fatty acid composition of farmed rainbow trout in response to dietary camelina oil as a replacement of fish oil. *Lipids* 49, 97–111. <http://dx.doi.org/10.1007/s11745-013-3862-7>.
- Hixson, S.M., Parrish, C.C., Anderson, D.M., 2014b. Use of camelina oil to replace fish oil in diets for farmed salmonids and Atlantic cod. *Aquaculture* 431, 44–52.
- Ichihara, K., Shibahara, A., Yamamoto, K., Nakayama, T., 1996. An improved method for rapid 304 analysis of the fatty acids of glycerolipids. *Lipids* 31, 535–539.
- Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Roselund, G., Ginés, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* 250, 431–444.
- Jobling, M., 2003. Do changes in Atlantic salmon, *Salmo salar* L., fillet fatty acids following a dietary switch represent wash-out or dilution? Test of a dilution model and its application. *Aquac. Res.* 34, 1215–1221.
- Madsen, L., Froyland, L., Dyroy, E., Helland, K., Berge, R.K., 1998. Docosahexaenoic and eicosapentaenoic acids are differently metabolized in rat liver during mitochondria and peroxisome proliferation. *J. Lipid Res.* 39, 583–593.
- Miller, M.R., Nichols, P.D., Carter, C.G., 2007. Replacement of dietary fish oil for Atlantic salmon parr (*Salmo salar* L.) with a stearidonic acid containing oil has no effect on omega-3 long-chain polyunsaturated fatty acid concentrations. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* 146 (2), 197–206.
- Montero, D., Robaina, L., Caballero, M.J., Gines, R., Izquierdo, M.S., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: a time course study on the effect of a re-feeding period with 100% fish oil diet. *Aquaculture* 248, 121–134.
- Mourente, G., Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 145, 389–399.
- National Research Council (NRC), 1993. Nutrient requirements of fish. National Academy Press, Washington, DC, USA.
- National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC.
- Regost, C., Arzel, J., Robin, J., Roselund, G., Kaushik, S.J., 2003. Total replacement of fish oil by soybean or oil with return to fish oil in turbot (*Psetta maxima*) I. Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture* 217, 465–482.
- Reis, B., Cabral, E.M., Fernandes, T.J.R., Castro-Cunha, M., Oliveira, M.B.P.P., Cunha, L.M., Valente, L.M.P., 2014. Long-term feeding of vegetable oils to Senegalese sole until market size: effects on growth and flesh quality. Recovery of fatty acid profiles by a fish oil finishing diet. *Aquaculture* 434, 425–433.
- Ricker, W.E., 1979. Growth rates and models. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), *Fish Physiology, III, Bioenergetics and Growth*. Academic Press, New York, pp.

- 677–743.
- Rinchard, J., Czesny, S., Dabrowski, K., 2007. Influence of lipid class and fatty acid deficiency on survival, growth, and fatty acid composition in rainbow trout juveniles. *Aquaculture* 264, 363–371.
- Rosenlund, G., Obach, A., Sandberg, M.G., Standal, H., Tveit, K., 2001. Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.). *Aquac. Res.* 32, 323–328.
- Rosenlund, G., Corraze, G., Izquierdo, M., Torstensen, B.E., 2010. The effects of fish oil replacement on nutritional and organoleptic qualities of farmed fish. In: Turchini, G.M., Ng, W.-K., Tocher, D.R. (Eds.), *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, pp. 487–522.
- Ruxton, C.H.S., Reed, S.C., Simpson, M.J.A., Millington, K.T., 2007. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J. Hum. Nutr. Diet.* 20, 275–285.
- Sargent, J.R., Bell, J.G., McGhee, J., McEvoy, J., Webster, J.L., 2001. The nutritional value of fish. In: Kestin, S.C., Warriss, P.D. (Eds.), *Farmed Fish Quality*. Fishing News Books Blackwell Science Ltd., Oxford, UK, pp. 3–12.
- Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, third ed. Elsevier, USA, pp. 181–257.
- Simopoulos, A.P., 2005. Fatty acids|omega – 3 polyunsaturated. In: Benjamin, C. (Ed.), *Encyclopedia of Human Nutrition*, second ed. Elsevier, Oxford, pp. 205–219.
- Stone, D.A.J., Oliveira, A.C.M., Plante, S., Smiley, S., Bechtel, P., Hardy, R.W., 2011a. Enhancing highly unsaturated omega-3 fatty acids in phase-fed rainbow trout (*Oncorhynchus mykiss*) using Alaskan fish oils. *Aquac. Nutr.* 17, E501–E510.
- Stone, D.A.J., Oliveira, A.C.M., Ross, C.F., Plante, S., Smiley, S., Bechtel, P., Hardy, R.W., 2011b. The effects of phase-feeding rainbow trout (*Oncorhynchus mykiss*) with canola oil and Alaskan pollock fish oil on fillet fatty acid composition and sensory attributes. *Aquac. Nutr.* 17, E521–E529.
- Thanuthong, T., Francis, D.S., Senadheera, S.P.S.D., Jones, P.L., Turchini, G.M., 2011a. Fish oil replacement in rainbow trout diets and total dietary PUFA content: I) effects on feed efficiency, fat deposition and the efficiency of a finishing strategy. *Aquaculture* 320 (1), 82–90.
- Thanuthong, T., Francis, D.S., Senadheera, S.P.S.D., Jones, P.L., Turchini, G.M., 2011b. LC-PUFA biosynthesis in rainbow trout is substrate limited: use of the whole body fatty acid balance method and different 18:3n – 3/18:2n – 6 ratios. *Lipids* 46, 1111–1127.
- Thanuthong, T., Francis, D.S., Senadheera, S.P.S.D., Jones, P.L., Turchini, G.M., 2012. Short-term food deprivation before a fish oil finishing strategy improves the deposition of n – 3 LC-PUFA, but not the washing-out of C₁₈ PUFA in rainbow trout. *Aquac. Nutr.* 18, 441–456.
- Tidwell, J.H., Allan, G.L., 2001. Fish as food: aquaculture's contribution: ecological and economic impacts and contributions of fish farming and capture fisheries. *EMBO Rep.* 2 (11), 958–963.
- Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* 11, 107–184.
- Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac. Res.* 41, 717–732.
- Tocher, D.R., Bell, J.G., Dick, J.R., Crampton, V.O., 2003. Effects of dietary vegetable oil in Atlantic salmon hepatocyte fatty acid desaturation and liver fatty acid composition. *Lipids* 38, 723–732.
- Torstensen, B.E., Li, Ø., Frøyland, L., 2000. Lipid metabolism and tissue composition in Atlantic salmon (*Salmo salar* L.)-effects of capelin-, palm- and oleic acid enriched sunflower oil as dietary lipid sources. *Lipids* 35, 653–664.
- Turchini, G.M., Francis, D.S., De Silva, S.S., 2006. Modification of tissue fatty acid composition in Murray cod (*Maccullochella peelii peelii*, Mitchell) resulting from a shift from vegetable oil diets to a fish oil diet. *Aquac. Res.* 37, 570–585.
- Turchini, G.M., Francis, D.S., De Silva, S.S., 2007. Finishing diets stimulate compensatory growth: results of a study on Murray cod, *Maccullochella peelii peelii*. *Aquac. Nutr.* 13, 351–360.
- Turchini, G.M., Torstensen, B.E., Ng, W.-K., 2009. Fish oil replacement in finfish nutrition. *Rev. Aquac.* 1, 10–57.
- Von Schacky, C., 2006. A review of omega – 3 ethyl esters for cardiovascular prevention and treatment of increased blood triglyceride levels. *Vasc. Health Risk Manag.* 2, 251–262.
- Yıldız, M., Eroldoğan, O.T., Engin, K., Gulçubuk, A., Baltacı, M.A., 2013. 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*. *Turk. J. Fish. Aquat. Sci.* 13, 453–464.
- Yıldız, M., Kose, I., Issa, G., Kahraman, T., 2015. Effect of different plant oils on growth performance, fatty acid composition and flesh quality of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Res.* 46, 2885–2896.