Effect of dietary canola oil level on the growth performance and fatty acid composition of fingerlings of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study evaluated the suitability of canola oil as a source of supplemental dietary lipid for rainbow trout. Triplicate groups of the 30 fingerlings held under identical culture conditions were fed twice daily by iso-nitrogenous, iso-calorific and iso-lipidic diets for eight weeks. Experimental diets consisted of 30.3% protein, 18.7 kJg^{-1} energy and 16.7% lipid from fish oil (FO), canola oil (CO) and 1:1 blend of the two oils (FCO). Moisture, ash, protein, lipid, final body weight, condition factor, feed conversion ratio, survival and hepatosomatic indices were not affected by treatments. Specific growth rate and weight gain of fish reared on fish oil diet (FOD) and canola oil diet (COD) were significantly higher than those fed with the fish and canola oils diet (FCOD). Protein efficiency ratio was highest in fish fed with the COD. Whole body fatty acid compositions mirrored those of diet treatments. The highest amounts of HUFAs were detected in fish fed with FOD, which was significantly different from other treatments. In all treatments PUFAs/SFAs and n-6/n-3 ratios were higher than 0.45 and lower than 4, respectively. Our results indicate the fingerlings can be reared on diets in which FO has been replaced with CO, with no significant effects on fish growth performance.

Keywords: Rainbow Trout, *Oncorhynchus mykiss*, Fish oil replacement, Fish oil, Vegetable oil, Fatty acids, Body composition

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Introduction

In the course of just a few decades, fish farming has developed into a highly productive and efficient industry to produce animal protein for human consumption. In addition to good growing conditions, a prerequisite for productivity and economic sustainability in fish farming can be a reliable supply of effective feeds. For various reasons, fish meal and fish oil have historically been the dominant raw materials in the production of fish feeds. Due to the development of more energy dense feed types as well as general growth of the aquaculture industry, a significant proportion of the total global fish oil is used for its feed preparation. A lipid requirement equal to 100% of the world's total fish oil production is estimated by the year 2010 (New, 1999).

While marine oils are superior in their fatty acid composition, they also contain a variety of toxic compounds including polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and dioxin-like polychlorinated biphenyls (DL-PCB), particularly the non-ortho and mono-ortho substituted PCBs (Jacobs *et al*., 2002 a,b; Hites *et al*., 2004 a,b). These compounds are suspected to be carcinogenic and immunosuppressive in humans (Birnbaum and Tuomisto, 2000; Baccarelli *et al*., 2002; Van Den Heuvel *et al*., 2002).

It is also well-known that lipid oxidation is one of the major concerns in fish-derived food products. Polyunsaturated fatty acids (PUFAs) are more easily oxidized than saturated fatty acids (SFAs), and therefore, food products enhanced with the PUFAs n-3 are also more prone to lipid oxidation. There is potential human health risks associated with increased consumption of oxidized PUFAs n-3 products (Fritsche and Johnston, 1990; Kubow, 1993). Another important factor to limit a more common use of PUFAs n-3 enhanced food products is the development of off-flavors following lipid oxidation that may be offensive to consumers (Waagbø *et al*., 1993).

While it is obvious that a substitute must be found, replacing fish oil in aquaculture diets has its own difficulties as most of the vegetable oils are relatively poor sources of n-3 fatty acids. Exceptions to this are flaxseed and canola oils which are rich in alpha linolenic acid (18:3n-3) (53% and 12% respectively) (NRC, 1993). However, these oils lack longer chain n-3 highly unsaturated fatty acids (HUFAs n-3) and their inclusion in trout diets results in a significant decrease in the tissue levels of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) (Bell *et al*., 2002, 2003a). Moreover, enhancement of omega-3 fatty acid content in rainbow trout fillet was observed in farmed rainbow trout and brook trout as results of flaxseed oil inclusion in diet (Chen *et al*., 2006; Simmons *et al*., 2011).

Freshwater fish are capable of converting C18 PUFAs to the longer chain C20 and C22 PUFAs (Henderson and Tocher, 1987) which are the functionally essential fatty acids in vertebrates (Lauritzen *et al*., 2001).

Several studies conducted on freshwater fish indicated that vegetable oils can successfully replace fish oil in the feed without affecting their survival and growth (Wonnacott *et al*., 2004; Subhadra *et al*., 2006). Caballero *et al*. (2002) reported that

in rainbow trout (*O. mykiss*) up to 80–90% of vegetable oils (e.g. soybean, rapeseed, olive, and palm oils) can be used without compromising fish growth (Caballero *et al*., 2002). Evidence shows that partial replacement of fish oil by vegetable oils such as rapeseed, soybean, flaxseed or palm oils in fish feeds has no negative impacts on growth and survival of Atlantic salmon (*Salmo salar*) (Rosenlund *et al*., 2001), brook charr (*Salvelinus fontinalis*), (Guillou *et al*., 1995), gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Izquierdo *et al*., 2003) and rainbow trout (Greene and Selivonchick, 1990; Caballero *et al*., 2002).

The aim of the present study was to evaluate the effects of fish oil replacement with canola oil (relatively freely available and low-priced oil) on growth, feed conversion ratio and fillet fatty acid composition of rainbow trout fingerlings.

Materials and methods

A number of 270 rainbow trout fingerlings with a mean initial body weight of 16.5±0.5 g, were purchased from Cheshme Dimeh fish hatchery (Shahre kord, Chaharmahal and Bakhtiari, Iran) and used in this study. Initially, fish were acclimatized to the new environmental conditions on a commercial diet (SFT2 of Chineh feed production factory, Tehran, Iran) for a two week period within a semi recirculating system.

Experimental diets

Three iso-nitrogenous, iso-calorific and iso-lipidic semi purified experimental diets were formulated from 100% fish oil (FO), 100% canola oil (CO) and 1:1 blends of the two oils (FCO) (Table 1). The nutritional content of the diets are shown in Table 1. Diets were prepared and stored according to Abery *et al*. (2002) and De Silva *et al*. (2002).

Husbandry

This study was conducted indoors in a thermostatically controlled room. Fish were housed in nine 100 L fiberglass circular rearing tanks in a semi re-circulating system with an in-line oxygen generator and a physical and biological treatment plant (flow rate of 6 L min^{-1}). During the experiment, fish were kept under a 12-h light: 12-h dark cycle. The experiment was conducted at 13.6±1.3°C, water quality parameters were measured every second day using Aquamerck test kits (Merck, Darmstadt, Germany) with a mean pH of 7.3±0.2 and levels of ammonia and nitrate below 0.1 mg l^{-1}.

Table 1: Ingredient (%), proximate composition (% wet weight) and energy (kJ g^{-1}) of the experimental diets.

	FOD	COD	FCOD
Fish meal	58	58	58
Soybean meal	20	20	20
Wheat meal	8.6	8.6	8.6
Fish oil	8	0	4
Canola oil	0	8	4
Vitamin premix*	2	2	2
Mineral premix**	1.5	1.5	1.5
Lysine	0.07	0.07	0.07
Methionine	0.13	0.13	0.13
Choline chloride	0.2	0.2	0.2
Molasses	1	1	1
Salt	0.5	0.5	0.5
Proximate composition			
Moisture	9.80	10.20	9.40
Ash	14.59	14.19	15.29
Crude protein	30.66	30.26	29.97
Crude lipid	16.09	16.89	17.06
Crude fiber	2.16	2.31	1.95
NFE***	26.70	26.15	26.33
Energy****	18.56	18.71	18.68

Diet abbreviations, FOD: 100% fish oil diet; COD: 100% canola oil diet; FCOD: 50% fish oil diet and 50% canola oil diet.
*Contains (mg kg^{-1} food): E (30), K (3), niacin (40), thiamine (2), riboflavin (7), pyridoxine (3), folacin (1.5), pantothenic acid (18), biotin (0.7) and cyanocobalamin (0.18).
**Contains (mg kg^{-1} food): Mg (100), Zn (60), Fe (40), Cu (5), Co (0.1), I (1) and Antioxidant (100).
***NFE: nitrogen free extract, calculated by difference (100 – moisture – ash – crude protein – crude lipid – crude fibers).
****Calculated on the basis of 23.6, 39.5 and 17.2 kJ g^{-1} of protein, fat and carbohydrate, respectively

Experimental design

The 270 individual rainbow trout fingerlings were weighed and measured and were randomly distributed into nine 100 L fiberglass tanks (30 fish per tank) and randomly assigned to one of the 3 experimental treatments (3 replicate tanks for each treatment).

Fish were fed twice daily at approximately 08.30 and 17.00 h to apparent satiation for a period of 56 days. At the end of the experiment a sample of 54 fish (6 fish per replicate) was taken and euthanized in excess of anaesthetic application (Benzocaine 0.5 mg L^{-1}) for body composition (3 fish per replicate) and fatty acid profile analysis (3 fish per replicate).

Chemical analysis

The fish allocated for flesh analysis were filleted (denuded of skin and bone) and stored at −20°C until used for fillet proximate analysis. Fishes allocated for fatty acids analysis were stored at −80°C. Proximate analysis was conducted using

standard procedures (AOAC, 1990), percentage moisture (dried at 80°C to constant weight), protein (Kjeldahl nitrogen; N×6.25) in an automated Kjeltech (Model 2300, Tecator, Sweden), total lipid by chloroform/methanol extraction (2:1 v/v) (Folch *et al*., 1957) as modified by Ways and Hanahan (1964) and ash by incineration in a muffle furnace (Model WIT, C and LTetlow, Australia) at 550°C for 18h. Fatty acid analysis was carried out on each of the added dietary oils, experimental diets and fillet samples from each of the replicates. Fatty acid methyl esters (FAMEs) were prepared from aliquots of total lipids by acid catalyzed transmethylation with sulfuric acid in methanol overnight at 50°C (Christie, 1982). FAMEs were purified by TLC using hexane/diethyl ether/acetic acid (85:15:15 v/v/v) as solvent (Tocher and Harvie, 1988). Separation of FAMEs was carried out in Gas Chromatograph system (Agilent Technologies, 6890N, USA) equipped with a flame ionization detector (FID), and a cross-linked silica capillary column HP-88 (100 m, 250 µm ID, 0.2 µm film thickness), on-column injection and using helium as carrier gas with flow rate of 1.1 ml min^{-1}. The column was programmed for an initial temperature of 140°C held for 5 min, rising at a rate of 4°C min^{-1} to the final temperature of 240°C and held for 10 min. Injector and detector temperatures were 230°C and 260°C, respectively. The flow rates of compressed air and hydrogen were 300 ml min^{-1} and 30 ml min^{-1}, respectively. Identification and quantification of FAMEs were based on the comparison of the sample retention time with known standards (Sigma Chemicals, St. Louis, USA).

Statistical analysis

Mean values and standard deviation for each parameter measured for all treatments were calculated. The results were subjected to a one-way ANOVA to test the effect of vegetable oils blend replacement on fish performance. Data were analyzed using statistical packages SPSS v15 (SPSS Inc., Chicago, IL, USA). Differences between means were compared using Duncan's multiple range test at significance of differences (p<0.05) among dietary treatments. Linear regression analyses were performed between dietary and fillet fatty acid concentrations.

Results

Diet composition

The proximate compositions of the diets were similar across all treatments (Table 1). The fatty acid composition of the oils and experimental diets are presented in Table 2.

Table 2: Fatty acid composition (percentage of total fatty acids) of the oils and experimental diets.

Fatty acid	Fish oil	Canola oil	FOD	COD	FCOD
14:0	0.06	-	0.07	0.04	0.04
15:0	0.32	-	0.23	0.08	0.14
16:0	20.73	5.77	22.71	14.33	17.41
17:0	0.72	0.07	0.72	0.41	0.56
18:0	4.16	1.27	5.85	4.46	5.08
19:0	2.89	3.78	2.94	3.13	3.04
21:0	0.18	0.21	0.25	0.16	0.19
22:0	0.24	0.14	0.20	0.13	0.20
23:0	0.21	0.57	0.22	0.37	0.33
24:0	0.18	-	0.14	0.07	0.13
SFAs	29.68	11.80	33.32	23.17	27.12
14:1	3.77	0.19	3.11	1.31	2.07
15:1	0.80	0.04	0.71	0.34	0.51
16:1n-7	5.24	0.34	4.92	2.54	3.61
17:1	0.71	-	0.65	0.26	0.41
18:1n-9	33.57	77.33	38.79	59.87	49.63
24:1n-9	0.44	-	0.41	0.24	0.39
MUFAs	44.52	77.91	48.59	64.57	56.61
18:2n-6	0.37	1.01	0.42	0.70	0.47
18:3n-6	0.05	0.38	0.04	0.21	0.14
20:2n-6	2.48	0.14	1.21	0.29	0.82
20:3n-6	0.18	-	0.20	0.22	0.28
20:4n-6	0.02	-	0.06	0.04	0.04
22:2n-6	0.72	-	0.65	0.40	0.62
22:5n-6	0.36	-	0.30	0.16	0.28
PUFAs n-6	4.18	1.53	2.88	2.03	2.65
18:3n-3	2.07	7.05	4.58	4.87	3.95
18:4n-3	0.32	1.71	0.60	1.25	0.87
20:3n-3	0.05	-	0.61	0.04	0.07
20:5n-3	5.90	-	2.95	0.96	2.29
22:5n-3	0.48	-	0.36	0.21	0.32
22:6n-3	12.82	-	6.65	2.91	5.82
PUFAs n-3	21.62	8.76	15.75	10.24	13.32
HUFAs n-3	18.72	-	9.60	3.87	8.11

-not detected
See Table 1 for diet abbreviations.

The fish oil diet (FOD) contained the highest level of SFAs (33.3%) predominantly in the form of palmitic (16:0) and stearic acids (18:0) which accounted for (22.7%) and (5.9%), respectively (Table 2). Monounsaturated fatty acids (MUFAs) concentrations were highest in the canola oil diet (COD) (64.6%), represented mainly as oleic acid (18:1n-9, 59.9%). The FOD was richest in PUFAs (n-6+n-3) (18.6%) with α-linolenic acid (18:3n-3, 4.6%) and linoleic acid (18:2n-6, 0.4%) as the principal fatty acids. The highest levels of EPA and DHA were in the FOD, with 3% and 6.7%, respectively. Fatty acids of the n-3 and n-6 series were observed with the highest concentration in the FOD, which accounted for (15.8%) and (2.9%), respectively. Levels of HUFAs n-3 were found in highest concentrations in the FOD with 9.6%.

Growth

The overall mortality was low and did not appear to be related to the dietary treatment (Table 3). The mean final body weight of fingerlings of rainbow trout ranged from 54.7±12.2 (fish and canola oils diet (FCOD)) to 58.5±8 (COD), and there were no significant differences between treatments. Similarly no significant differences were observed for survival and feed conversion ratio (FCR). Specific growth rate (SGR) and weight gain (WG) of the fish reared on FOD and COD were significantly (p<0.05) higher than the fish fed with the FCOD. No significant differences were observed for the biometric parameters such as condition factor (CF) and hepatosomatic index (HSI). Protein efficiency ratio (PER) was highest in the fish fed with the COD and only significantly (p<0.05) higher than those fed with FCOD.

Table 3: Mean (±SD) of growth, feed utilization and other body parameters of rainbow trout reared on the experimental diets.

	FOD	COD	FCOD
MIBW	16.12±0.27	16.44±0.30	16.82±0.11
MFBW	58.05±6.98	58.50±7.96	54.74±12.22
CF	1.22±0.10	1.18±0.11	1.19±0.11
SGR	2.29±0.02^{a}	2.27±0.02^{a}	2.10±0.10^{b}
WG	260.09±4.25^{a}	255.89±4.07^{a}	224.56±17.82^{b}
FCR	0.90±0.17	0.97±0.17	1.04±0.54
SR	100.00±0.00	96.67±5.77	90.00±17.32
HSI	1.21±0.13	1.16±0.08	1.11±0.10
PER	1.92±0.22^{ab}	2.24±0.17^{a}	1.49±0.49^{b}

Values in the same row with the same superscripts are not significantly different (p<0.05).
See Table 1 for diet abbreviations.
MIBW (g): Mean initial body weight, MFBW (g): Mean final body weight.
CF: Condition factor =100×(final weight (g))×(fork length (cm))$^{-3}$.
SGR (%day^{-1}): Specific growth rate =[Ln(final weight)−Ln(initial weight)]×(number of days)$^{-1}$×100.
WG (%): Weight Gain =(final weight−initial weight)×(initial weight)$^{-1}$×100.
FCR: Feed conversion ratio =(dry feed fed)×(wet weight gain)$^{-1}$.
SR (%): Survival rate =number of fish in each group remaining on day 56−(initial number of fish)$^{-1}$×100
HSI (%): Hepatosomatic index =(weight of liver)×(total fish weight)$^{-1}$×100.
PER: Protein efficiency ratio =(final weight−initial weight)×(mass of protein fed)$^{-1}$.

Fillet proximate composition

Results of the proximate analysis of fillet of fish receiving different dietary treatments are tabulated in Table 4. No significant differences between percent moisture, ash, protein and lipid content of fish fed with the experimental diets were observed (Table 4).

Table 4: Fillet proximate compositions (mean±SD) of rainbow trout reared on different diets, (% wet weight).

	Initial*	FOD	COD	FCOD
Moisture	78.44±0.61	76.96±0.20	76.97±0.60	76.43±0.17
Ash	1.34±0.03	1.28±0.05	1.26±0.04	1.29±0.08
Protein	15.29±0.81	17.77±0.16	18.70±1.02	18.95±0.24
Lipid	2.93±0.20	3.46±0.27	3.38±0.38	3.84±0.04

FOD: fish oil diet; COD: canola oil diet; FCOD: fish and canola oils diet.
See Table 1 for diet abbreviations.
*Statistics not performed on the initial sample.

Fillet fatty acid composition

The major fatty acid classes (SFAs, MUFAs and PUFAs) found in the highest concentration were palmitic, oleic, α-linolenic acids along with DHA, respectively (Table 5). The level of SFAs was observed in higher (p<0.05) concentrations for fish fed with the FOD compared to fish fed with COD and FCOD. Levels of MUFAs ranged from 53±0.4 (FOD) to 63.3±0.2 (COD) and were observed to be significantly higher in fish fed with the COD. The fillet of fish fed with the COD was particularly rich with oleic acid (58.8±0.9%) and α-linolenic acid (3.2±0.3%). DHA and arachidonic acid levels were found in higher concentrations in the fillet than in the diets. The highest level of EPA and DHA was observed in fish fed with the FOD (p<0.05). However, DHA was found in high concentrations within all of the dietary treatments, ranging from 5±0.2% (COD) to 10.7±0.4% (FOD). The level of n-3 fatty acids was higher in the fillet than those in the diet for each of the treatments, but level of n-6 fatty acids was higher in the fillet than that in the diet only for COD, with n-6/n-3 ratios ranging from 0.16±0.02 to 0.23±0.02 in the fillet. The highest HUFAs n-3 concentrations (p<0.05) were found in fish fed with the FOD (12.8±0.5%), while the lowest value was observed in fish fed with the COD (5.6±0.2%).

Regression analysis was used to identify dose response relationship between dietary and fillet fatty acids. As reported in Table 6, most of the fatty acid concentrations in the fillet were linearly correlated with the dietary fatty acid concentrations.

Table 5: Fillet fatty acid composition (percentage of total fatty acids) of rainbow trout reared on different diets (mean±SD).

Fatty acid	Initial	FOD	COD	FCOD
14:0	0.03	0.04±0.01	0.04±0.01	0.04±0.01
15:0	0.09	0.16±0.01^{a}	0.06±0.00^{c}	0.10±0.02^{b}
16:0	10.11	17.45±0.29^{a}	12.85±0.28^{c}	14.13±0.39^{b}
17:0	0.14	0.50±0.01^{a}	0.32±0.04^{b}	0.36±0.01^{b}
18:0	2.90	4.46±0.10^{a}	3.78±0.09^{b}	3.90±0.16^{b}
19:0	3.07	3.72±0.45	3.76±0.09	3.41±0.11
21:0	0.21	0.19±0.06	0.18±0.04	0.18±0.02
22:0	0.77	0.55±0.06^{c}	0.93±0.55^{a}	0.71±0.05^{b}
23:0	0.72	0.28±0.02^{c}	0.49±0.03^{a}	0.42±0.02^{b}
24:0	0.41	0.41±0.03^{a}	0.25±0.03^{c}	0.34±0.04^{b}
SFAs	18.44	27.74±0.53^{a}	22.66±0.29^{c}	23.56±0.45^{b}
14:1	1.42	2.25±0.08^{a}	1.18±0.06^{c}	1.59±0.01^{b}
15:1	0.17	0.52±0.02^{a}	0.27±0.01^{c}	0.34±0.03^{b}
16:1n-7	1.77	4.49±0.09^{a}	2.46±0.20^{c}	3.20±0.13^{b}
17:1	0.18	0.48±0.03^{a}	0.22±0.05^{c}	0.34±0.02^{b}
18:1n-9	61.25	44.85±0.45^{c}	58.82±0.56^{a}	53.91±0.33^{b}
24:1n-9	0.10	0.40±0.03^{a}	0.33±0.04^{b}	0.35±0.02^{ab}
MUFAs	64.88	52.99±0.38^{c}	63.28±0.24^{a}	59.73±0.17^{b}
18:2n-6	0.71	0.41±0.05^{c}	0.78±0.06^{a}	0.54±0.03^{b}
18:3n-6	0.05	0.06±0.00^{c}	0.17±0.01^{a}	0.11±0.01^{b}
20:2n-6	0.88	0.89±0.06^{a}	0.46±0.05^{c}	0.60±0.06^{b}
20:3n-6	0.13	0.09±0.01^{b}	0.12±0.01^{a}	0.12±0.02^{a}
20:4n-6	0.86	0.13±0.03	0.17±0.05	0.17±0.01
22:2n-6	0.43	0.77±0.07	0.69±0.04	0.72±0.03

Table 5 continued:				
22:5n-6	0.33	0.24±0.03	0.24±0.02	0.26±0.02
PUFAs n-6	3.40	2.60±0.24	2.63±0.17	2.52±0.13
18:3n-3	4.27	1.94±0.07^{c}	3.23±0.25^{a}	2.76±0.03^{b}
18:4n-3	2.61	1.04±0.04^{b}	1.75±0.32^{a}	1.48±0.14^{a}
20:3n-3	0.53	0.30±0.03^{b}	0.62±0.07^{a}	0.40±0.05^{b}
20:5n-3	0.87	2.08±0.10^{a}	0.59±0.04^{c}	1.17±0.11^{b}
22:5n-3	0.25	0.59±0.05^{a}	0.21±0.02^{c}	0.38±0.02^{b}
22:6n-3	4.76	10.73±0.40^{a}	5.04±0.19^{c}	8.00±0.32^{b}
PUFAs n-3	13.28	16.67±0.52^{a}	11.43±0.04^{c}	14.19±0.45^{b}
HUFAs n-3	5.63	12.80±0.45^{a}	5.63±0.22^{c}	9.18±0.43^{b}
PUFAs	16.68	19.27±0.66^{a}	14.06±0.17^{c}	16.71±0.58^{b}
PUFAs/SFAs	0.90	0.69±0.04^{a}	0.62±0.02^{b}	0.71±0.04^{a}
n-6/n-3	0.26	0.16±0.02^{b}	0.23±0.02^{a}	0.18±0.01^{b}

See Table 1 for diet abbreviations.
Values in the same row with the same superscripts are not significantly different (p<0.05).

Table 6: Correlation between dietary fatty acid concentrations and fatty acid concentrations in fillet of rainbow trout fed with the experimental diets for 8 weeks.

Fatty acids	**Correlation coefficient (*r*)**	**Slope**
14:0	0.500	-0.167
16:0	0.995	0.556
18:0	0.953	0.492
SFAs	0.974	0.516
18:1n-9	0.988	0.664
MUFAs	0.985	0.644
18:2n-6	0.985	1.274
20:4n-6	0.971	-1.750
PUFAs n-6	0.502	-0.065
18:3n-3	0.157	0.218
20:5n-3	0.949	0.704
22:6n-3	0.959	1.390
PUFAs n-3	0.999	0.949
PUFAs	0.996	0.811

Discussion

The results of the present study suggest that canola oil can be used to replace fish oil without adverse effects on growth of rainbow trout, as reported in other studies (Glencross *et al*., 2003; Karayücel and Dernekbaşi, 2010). This was evidenced by the weight gain and feed conversion ratio of fish fed FOD and COD which ranged from 224.56±17.82% to 260.09±4.25% and 0.90±0.17 to 1.04±0.54, respectively, with no significant differences from fish fed with the control diet and COD. Considering the formulated diets were iso-nitrogenous, iso-calorific and iso-lipidic; varying only in lipid source, the differences in growth of fingerlings of rainbow trout signify that the fatty acid profile of the test diets may have influences on the growth of the species. This was also confirmed by the relative uniformity of the proximate composition of the fish amongst the different dietary treatments.

In agreement with previous studies (Caballero *et al*., 2002; Martino *et al*., 2002; Glencross *et al*., 2003; Turchini *et al*., 2003b; Dernekbaşi *et al*., 2011), considerable differences were evident in the

fatty acid composition of rainbow trout fed with different lipid sources. There was a prominent increase in the levels of linoleic and α-linolenic acids in all treatments where fish were fed either with COD and/or FCOD.

As reported previously by other researchers, (Guillou *et al*., 1995; Bell *et al*., 2003; Turchini *et al*., 2003a, b; Torstensen *et al*., 2004; Chen *et al*., 2006, 2008; Simmons *et al*., 2011), high correlations for individual fatty acids as well as MUFAs and PUFAs were observed between the diets and fillets of fingerlings of rainbow trout (Table 6). There was, however, high correlation between the amount of SFAs in the diet and SFAs in the fillet, which was not in accordance with the findings of Turchini *et al*. (2003a, b) who postulated that SFAs were not used efficiently by Murray cod (*Maccullochella peelii peelii*) as an energy source and were subsequently deposited at an optimal level in preference to the other major fatty acid classes.

It is well known that freshwater fish have a dietary requirement for n-3 and n-6 fatty acids, predominantly in the form of α-linolenic and linoleic acids (Kanazawa *et al*., 1979, 1980; Guillou *et al*., 1995; Martino *et al*., 2002; Izquierdo *et al*., 2003; Tocher, 2003). In comparison to marine fish species, freshwater fish are also generally better adapted to de-saturation and elongation of these base fatty acids to higher homologs (Guillou *et al*., 1995; Tocher, 2003). This study observed α-linolenic acid in lower concentrations in the muscle than in the diets, it is therefore suspected that a high degree of metabolism of this fatty acid for β-oxidation and/or desaturation and elongation is taking place in fingerlings of rainbow trout. This is further bolstered by the presence of n-3 desaturation and elongation enzyme products in the form of 18:4n-3 and 20:3n-3 in fish fed COD and FCOD. These fatty acids were found in much lower concentrations in the diets. Likewise, fish fed with the COD and FCOD contained n-6 desaturation and elongation intermediates (18:3n-6 and 20:3n-6) and indicate an elongation and desaturation of linoleic acid via Δ6 desaturase. However, further desaturation of 20:3n-6 to 20:4n-6 and 20:3n-3 to EPA and ultimately DHA was shrouded by high concentrations of these fatty acids within the fillet of initial fish samples. The Department of Health of England recommends a minimum PUFAs/SFAs ratio of 0.45, and a maximum n-6/n-3 of 4.0. Table 5 shows that the fingerlings of all treatments met the PUFAs/SFAs and n-6/n-3 ratios. Despite the decrease in EPA and DHA in fillet from fish fed with FCOD, the trout fillets contained a relatively rich source of these fatty acids with a 200 g serving of the fillets from fish fed with FCOD supplying 704 mg of EPA plus DHA. This meets the intake of 500 mg day^{-1} of EPA plus DHA recommended by the International Society for the Study of Fatty Acids and Lipids (Simopoulos *et al*., 1999).

Conclusions

The results showed that, substitution of fish oil with canola oil in rainbow trout diet is possible without any negative effects on the growth and feed conversion ratio. However, the effect of the dietary oil source on the fillet fatty acid composition of the

fingerlings of rainbow trout could be a potential drawback for vegetable oil substitution from a human nutritional point of view, given the decrease in levels of EPA and DHA in fish fed with the vegetable oil diets. Further investigation into the benefits of other vegetable oils or indeed a blend of various vegetable oils is required in order to reduce usage of traditionally used fish oils, while simultaneously avoiding reduction in the human health protective properties found in fish flesh.

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