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Effect of Different Vegetable Oils on Growth and Fatty Acid Profile of Rohu (*Labeo rohita*, Hamilton); Evaluation of a Return Fish Oil Diet to Restore Human Cardio-protective Fatty Acids

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ABSTRACT: Two experiments in the sequential order were conducted to determine the effects of different dietary lipid sources on the growth and fatty acid composition of rohu (*Labeo rohita*) and to examine the viability of a return fish oil finisher diet in restoring the human cardio-protective fatty acid profile. In the first experiment, fish were fed either with coconut oil (D1), olive oil (D2), sunflower oil (D3), linseed oil (D4) and fish oil (D5) as the main lipid source in the isonitrogenous diet for 90 days. No significant differences in growth were observed. Among the experimental diets moisture content of fish varied significantly ($p<0.05$) between the groups. Dietary lipid sources had a profound influence on the fatty acid profile of the muscle and liver as tissue fatty acid profile reflected the dietary fatty acid composition. Increased amounts of eicosapentaenoic acid and docosahexaenoic acid were observed in tissue of fish fed D4 and arachidonic acid was observed in the tissue of fish fed D3. We have also detected the metabolites of n-3 and n-6 pathway in D4 and D3 groups respectively, which prompted us to conclude that rohu, can desaturate and elongate C_{18} essential fatty acids to C_{20} and C_{22} HUFA. A second feeding trial was conducted using the animals from the five different treatment groups for the duration of 30 days with fish oil rich diet (D5). Feeding with fish-oil rich washout diet resulted in the near equalization of all the other treatment groups tissue fatty acid profiles to that of fish oil (D5) fed group. These results indicate that a finishing fish oil diet can be effectively used to restore the human cardioprotective fatty acid profile in rohu fed with vegetable oils as lipid source. (**Key Words** : Fish Muscle, Fish Liver, *Labeo rohita*, PUFA, Vegetable oils, n-3 and n-6 Fatty Acids, Wash-out Diet, Indian Major Carps)

INTRODUCTION

Fish provides 26.2% of total animal meat and has been considered as the fastest growing food source in Asia (Delgado et al., 2002). Several reports have exemplified the importance of fish in reducing the risk of cardiovascular diseases. Human epidemiological studies (Bang and Dyerberg, 1980; Mori et al., 1991) showed that risk of coronary heart disease was reduced in populations where fish is the major part of food. Fish oil is a major source of long chain polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3) which have been proven to have a role in reducing the of risk of cardiovascular diseases and other metabolic syndromes (Breslow, 2006).

As the wild capture fishery is reaching its stagnation, aquaculture is thought to be a reliable source for the fish oil. Ironically, 60% of the global fish oil production is being utilized by aqua-feeds, and if the same trends continue, by 2010, 85% of the global fish oil production will be utilized for aqua-feeds (Barlow, 2000). The aquaculture industry should explore alternate viable lipid sources to sustain its growth. The sustainable alternatives to fish oil are plant (vegetable) oils, which are rich in C_{18} PUFA, but devoid of highly unsaturated fatty acids (HUFA) (Sargent et al., 2002). However, most of the freshwater fishes are able to desaturate and elongate the C_{18} fatty acids into C_{20} and C_{22} fatty acids. The extent to which fish can convert C_{18} PUFA to $C_{20/22}$ HUFA varies with species, and is associated with their ability for fatty acyl desaturation and elongation (Tocher, 2003).

Freshwater aquaculture industry in India has attained a massive size in last two decades due to the "blue revolution". Presently three Indian major carps, catla, rohu, and mrigal account for more than 90% of freshwater aquaculture production (Veerina et al., 1999) and rohu is the

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Table 1. Composition of experimental diets (% of dry matter)

Ingredient	D1	D2	D3	D4	D5
Soybean meal	42.00	42.00	42.00	42.00	42.00
Casein[a]	20.00	20.00	20.00	20.00	20.00
Gelatin[b]	5.00	5.00	5.00	5.00	5.00
Dextrin[c]	10.00	10.00	10.00	10.00	10.00
Starch[c]	8.00	8.00	8.00	8.00	8.00
Coconut oil[d]	9.00	0	0	0	0
Olive oil[d]	0	9.00	0	0	0
Sunflower oil[d]	0.5	0.5	9.5	0.5	0.5
Linseed oil[d]	0.5	0.5	0.5	9.5	0.5
Fish oil[d]	0	0	0	0	9.00
Carboxymethyl cellulose[e]	2.0	2.0	2.0	2.0	2.0
Vitamin+mineral mix[e]	1.9	1.9	1.9	1.9	1.9
Vitamin C[f]	0.1	0.1	0.1	0.1	0.1
Betain hydrocloride[c]	1.0	1.0	1.0	1.0	1.0
Proximate composition (% of dry matter, except moisture)					
Moisture	8.42	8.85	8.51	8.65	8.68
Crude protein	33.93	35.12	35.67	34.75	34.26
Crude fat	10.34	9.84	9.78	9.45	10.27
Ash	12.54	13.68	13.54	12.23	11.98
Carbohydrate	45.61	46.84	45.35	46.4	47.27
Energy (kcal/100 g)	420.12	414.25	415.38	412.85	416.95

[a] Casein fat free, 79.5% crude protein (HiMedia Ltd, India). [b] Gelatin, 95.7% CP (HiMedia Ltd, India).
[c] SD Fine Chemicals Ltd., India. [d] Procured from a local market.
[e] Composition of vitamin mineral mix (EMIX PLUS) (quantity/2.5 kg): vitamin A 5,500,000 IU; vitamin D_3 1,100,000 IU; vitamin B_2 2,000 mg; vitamin E 750 mg; vitamin K 1,000 mg; vitamin B_6 1,000 mg; vitamin B_{12} 6 mg; calcium pantothenate 2,500 mg; nicotinamide 10 g; choline chloride 150 g; Mn 27,000 mg; I 1,000 mg; Fe 7,500 mg; Zn 5,000 mg; Cu 2,000 mg; Co 450 mg; Ca 500 g; P 300 g; l-lysine, 10 g; dl-methionine 10 g; selenium 50 ppm.
[f] Roche, India.

most preferred fish for culture. Farming practices for rohu are predominantly modified extensive or semi-intensive in nature. In these practices, locally available feed ingredients (dominantly of plant source) like rice bran and groundnut oil cake are used to formulate the fish feed. The ingredients of plant source are generally rich in saturated, monounsaturated and n-6 fatty acids (Veerina et al., 1999). In this context, the present study was carried out with two objectives. First, to determine how different dietary fatty acid groups (saturated, monounsaturated, n-6 PUFA, and n-3 PUFA) may influence the growth and the fatty acid profile of rohu; second, to determine the effectiveness of fish oil based finishing diet in restoring the profile of human health beneficial fatty acids in rohu fed with vegetable oils.

MATERIALS AND METHODS

Experimental design and diets

Rohu fingerlings were procured from the Vasai fish farm, Maharashtra, India. Fingerlings were then acclimatized for 15 days in 20 synthetic plastic tanks (763×521×410 mm^3) of 160 L capacity. Each tank was stocked with 14 fingerlings of uniform size (7.62-8.48 g). Complete randomized design with five different treatment diets, each having four replicates was used for the feeding trial. Five isoenergetic and isoproteic diets were formulated to supply 10% lipid in each diet. Coconut oil (D1), olive oil (D2), sunflower oil (D3), linseed oil (D4), and fish oil (D5) were used as the main lipid source in one of the five diets. The feed ingredients (Table 1) were procured from S. D. Fine chemicals, Agrimin or Hi Media labs (Mumbai, India). Vitamin B complex (Becosules) and different oils were procured from the local market. The proximate compositions and fatty acid profiles of the different diets (6-mm pellets) are shown in Table 1 and Table 2 respectively. All the diets were formulated to supply all the essential nutrients required by rohu (Murthy et al., 1996). The feeding trial was conducted for 90 days and was followed by a wash out study of 30 days using D5 as finisher diet. Fishes were fed twice a day till satiation. At the end of the feeding trial, fish were euthanized individually by immersing in 0.2% MS-222. Muscle and liver samples were collected immediately. The fish were starved for 24 h before sampling. Samples were stored at -80°C. All the husbandry and euthanizing protocols were approved by the institute's animal care committee.

Evaluation of growth parameters

The growth parameters of the rohu fingerlings were assessed by measuring their body weight after 90 and 120 days. The animals were starved overnight before the measurement of body weight. The growth performance was

Table 2. Fatty acid composition of the experimental diets*

Fatty acid	D1	D2	D3	D4	D5
8:0	9.37	ND	ND	0.12	0.26
10:0	5.73	0.03	ND	1.08	0.16
12:0	40.70	0.02	0.22	0.43	1.29
14:0	18.31	0.12	0.19	0.33	3.49
16:0	7.68	10.42	11.00	6.74	11.25
16:1	0.30	1.10	0.21	0.16	6.15
18:0	2.57	5.09	3.80	5.66	2.56
18:1 n-9	7.15	65.59	23.57	20.85	23.41
18:1 n-7	ND	7.17	4.90	ND	4.17
18:2 n-6	6.57	8.90	52.27	14.03	6.81
18:3 n-6	ND	ND	0.61	ND	0.12
18:3 n-3	1.26	1.47	1.94	48.49	2.08
18:4 n-3	ND	0.07	ND	0.35	0.27
20:1 n-9	0.36	0.02	0.38	0.12	10.10
20:1 n-7	ND	ND	ND	ND	1.97
20:2 n-6	ND	ND	0.17	ND	0.20
20:3 n-6	ND	ND	ND	ND	0.47
20:4 n-6	ND	ND	0.74	1.49	0.6
20:3 n-3	ND	ND	ND	ND	0.46
20:5 n-3	ND	ND	ND	0.04	6.60
22:1 n-9	ND	ND	ND	ND	7.70
22:5 n-3	ND	ND	ND	ND	1.07
22:6 n-3	ND	ND	ND	0.11	9.41
ΣSFA	84.36	15.68	15.21	14.36	19.01
ΣMUFA	7.81	73.88	29.06	21.13	53.50
ΣPUFA	6.83	10.44	55.73	64.51	26.49
Σn-6	6.57	8.90	53.62	15.52	7.60
Σn-3	1.26	1.54	1.94	48.99	19.89
n-3 to n-6 ratio	0.19	0.17	0.03	3.15	2.61

* Data expressed as area percentage of fatty acid methyl esters.
** ND: Not detected.

assessed using the following formulae:

Specific growth rate

$$= \left(\frac{(\log_e (\text{Final weight}) - \log_e (\text{Initial weight}))}{\text{Experimental periods in days}} \right) \times 100$$

Percentage weight gain

$$= \left(\frac{(\text{average final weight} - \text{average initial weight})}{\text{average initial weight}} \right) \div 100$$

Food conversion ratio

$$= \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

Analysis of proximate composition

Association of Official Analytical Chemists (1990) based methods were used to carry out the proximate analysis of diets and whole fish. Individual fish were homogenized. Moisture (drying at 105°C till constant weight), crude fat (ethyl-ether extraction using 1045 Soxtec Extraction Unit, Tecator, Sweden), crude protein (Kjeldahl method, 2200 Kjeltec Auto Distillation, Foss Tecator, Sweden), and total ash (incineration at 600°C for 6 h until constant weight) percentages were determined. Gross energy content was determined using Gallenkamp ballistic bomb calorimeter-CBB 330 010 L (Gallenkamp, Loughbrough, UK).

Extraction of lipids and preparation of fatty acid methyl esters (FAME)

Total lipid was extracted following the Folch et al. (1957) method. Evaporation of solvent was done under nitrogen stream and residues are weighed to quantify the amount of lipid extracted. The lipid residue was re-dissolved in chloroform/methanol (2:1, v/v) and then stored in a 25 ml conical flask with glass stopper under nitrogen at -20°C until needed. The FAME's were prepared from the isolated lipids by the AOAC (1990) method.

Gas chromatography

The FAMEs were analyzed by GC (Shimadzu 14B-GC) equipped with a flame ionization detector (FID) and a fused silica capillary Carbowax 250 column (25 m×25 mm ID, 0.25 mm film thickness) from Supelco (USA). Chromatographic data were recorded and integrated using GC-CLASSIC Software (Shimadzu, Japan). The oven temperature was started with 100°C, raised to 230°C at the rate of 4°C/min, and held at 230°C for 10 min, while the injector and detector temperature were both set at 260°C. The sample size was 1 µl. The carrier gas (nitrogen) was controlled at 103.4 kPa. Hydrogen and compressed air used for FID were maintained at 275.6 kPa. The relative percentage of individual fatty acids and fatty acid groups (SFA, MUFA, PUFA n-3 and PUFA n-6) were expressed as the area percentage of the sum of the identified fatty acids.

GC/MS measurements were performed on a Shimadzu QP2010 quadrupole mass spectrometer with ionization energy of 70 eV operating in positive electronic impact set to 100 µA, connected to a GC 8060 gas chromatograph (Shimadzu) equipped with a Carbowax (25 m×0.25 mm; 0.25-µm film thickness) column (Cromlab SA) with helium as the carrier gas. Injection was performed in split mode at 240°C. The FAMEs were separated at constant pressure (23.1 kPa) by following the same oven temperature program used for GC analysis.

Statistical analysis

Microsoft Excel and Unscrambler (version 9.6) were used to analyze the data. The differences of mean values were treated by Duncan's multiple range test (DMRT). Principal component analysis (PCA) (Wold et al., 1987) was performed on the data matrix of fatty acid composition of the muscle of rohu fingerlings fed different dietary lipids. The PCA was done to express the main information in the

Table 3. Growth performance of rohu fed with different experimental diets

Growth parameter	Days	Dietary treatment				
		D1	D2	D3	D4	D5
Percentage weight gain	0 to 90	159.54±11.05	163.56±12.47	161.14±11.76	162.73±12.02	158.97±10.88
	90 to 120	40.91±0.49	40.09±1.23	43.11±0.86	42.41±2.32	45.62±0.62
Specific growth rate	0 to 90	2.2±0.1	2.3±0.07	2.3±0.25	2.3±0.3	2.2±0.1
	90 to 120	0.49±0.05	0.48±0.01	0.51±0.0	0.41±0.02	0.54±0.0
Food conversion ratio	0 to 90	2.0±0.27	2.02±0.05	1.98±0.14	1.92±0.05	1.98±0.13
	90 to 120	1.22±0.01	1.24±0.03	1.16±0.02	1.51±0.1	1.09±0.0

variables by a lower number of variables, which are called principal components (PC1, PC2).

RESULTS AND DISCUSSION

Growth and biochemical composition

All the diets were well accepted by the experimental animals. The growth parameters of different experimental groups recorded at the 90 day are presented in Table 3. Percentage weight gain (PWG) varied from 158.97 to 163.56. Specific growth rates (SGR) in the current feeding trial varied between 2.2 in D1 to 2.3 in D4. No significant differences in PWG or SGR were observed between the fish fed different diets. Similar results were reported from the studies of *Catla catla*, catla (Priya et al., 2005), *Salmo salar*, Atlantic salmon (Thomassen and Rosjo, 1989), *Cyprinus carpio*, common carp (Voila and Amidan, 1978), and *Sparus aurata*, Gilthead seabream and *Dicentrarchus labrax*, seabass (Izquierdo et al., 2003), where fish did not exhibit any significant difference in growth, when fed with different lipid sources. Feed conversion ratios (FCR) were similar for all the treatments ranging from 1.92±0.05 in D4 to 2.02±0.05 in D2. These results suggest that different lipid sources can be used to formulate diet for rohu without significantly affecting the growth of fish.

At 90 days proximate composition of experimental animals was determined for all the treatments (Table 4). Moisture percentage of fish varied significantly different ($p<0.05$) among the treatments from 62.17±1.29% in D2 group to 76.93±0.04% in D5 group. In contrast to our observation, Frinsko et al. (1992) reported that in hybrid stripped bass (*Morone saxatilis*×*Morone chrysops*) the treatment group fed with fish oil showed lower moisture content than the treatment groups fed with either beef tallow, soybean oil or corn oil. No significant difference was observed with respect to the crude protein content at 90 days except that the D1 group had a greater protein content followed by D2 group (Table 4). D1 and 2 (Table 2) were rich in 16:0 and 18:1 n-9 respectively, mitochondrial β-oxidation of these fatty acids might have resulted in protein sparing action and subsequent protein accretion. Sargent et al. (2002) proposed that 16:0 and 18:1 n-9 are the most

Table 4. Proximate composition of the rohu fed different diets after 90 days

Parameters	Percentage composition					
	Initial	D1	D2	D3	D4	D5
Moisture (%)	77.8±0.3	76.93±0.04^{a}	62.72±0.49^{e}	67.17±1.29^{b}	75.75±0.03^{a}	76.23±0.09^{a}
Organic matter (%)	22.5±0.2	23.06±0.04^{c}	37.27±0.49^{a}	32.82±1.29^{b}	24.24±0.03b^{c}	23.76±0.09^{c}
Crude protein (%)	54.2±0.3	59.24±0.38^{a}	58.64±0.90^{a}	57.68±0.64^{a}	57.81±0.77^{a}	55.61±1.94^{a}
Crude fat (%)	13.5±0.8	12.25±0.35^{bc}	15.05±0.45^{a}	13.50±0.4^{ab}	11.05±0.85^{c}	11.5±0.1^{c}
Ash (%)	14.1±0.4	16.23±0.63^{a}	13.61±1.04^{a}	14.67±0.67^{a}	16.18±0.57^{a}	15.35±0.97^{a}
Total carbohydrate	18.7±0.4	12.27±0.10^{b}	12.09±0.31^{b}	14.14±1.71^{ab}	14.95±2.19^{ab}	17.53±0.87^{a}
Energy (kcal/g)	414±0.4	396.3±4.2^{bc}	420.8±6.4^{a}	408.2±0.6^{ab}	390.5±1.9^{c}	396.1±3.3^{bc}

Values within a row with different superscript letter are significantly different ($p<0.05$).

Table 5. Proximate composition of the rohu fed different diets on 120th day

Parameters	Percentage composition				
	D1	D2	D3	D4	D5
Moisture (%)	70.69±0.69^{b}	70.37±0.83^{b}	70.31±1.04^{b}	74.09±0.50^{a}	72.4±0.74^{ab}
Organic matter (%)	30.86±0.58^{a}	28.0±0.71^{bc}	29.84±0.88^{ab}	24.76±0.64^{d}	27.14±0.30^{c}
Crude protein (%)	64.28±0.53^{a}	57.83±0.80^{c}	60.69±0.55^{b}	62.85±0.14^{a}	59.6±0.45^{bc}
Crude fat (%)	12.72±0.33^{c}	13.08±0.11^{bc}	13.71±0.14^{b}	14.49±0.17^{a}	12.43±0.04^{c}
Ash (%)	11.96±0.29^{b}	14.86±0.5^{a}	14.02±0.33^{a}	11.23±0.63^{b}	14.38±0.51^{a}
Total carbohydrate	11.03±0.09^{b}	14.22±0.19^{a}	11.57±0.36^{b}	11.41±0.59^{b}	13.50±0.91^{a}
Energy (kcal/g)	415.74±0.48^{b}	405.9±1.43^{e}	412.49±2.05^{bc}	427.56±3.4^{a}	406±2.26^{e}

Values within a row with different superscript letter are significantly different ($p<0.05$).

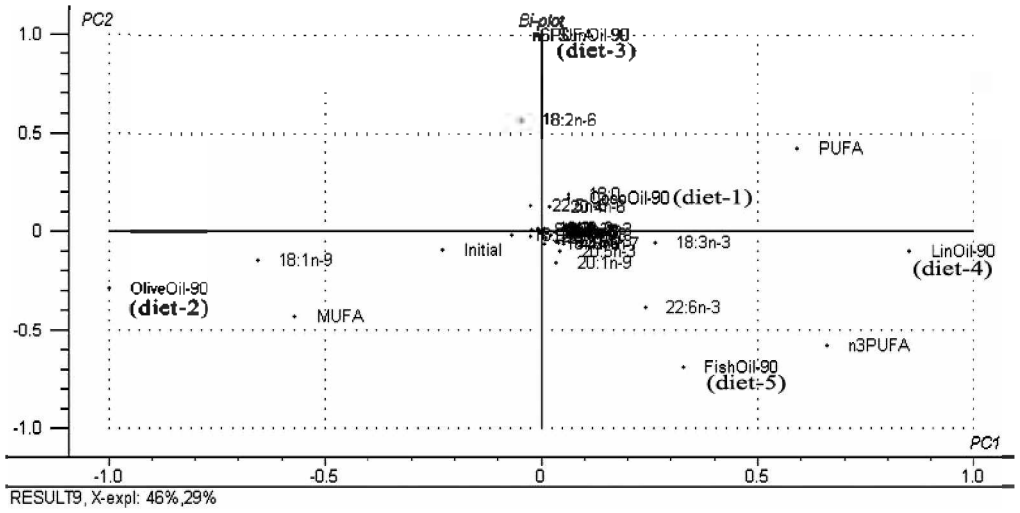

Figure 1. Principal component analysis (PCA) shows the differences in the muscle fatty acid profiles of fish fed different dietary groups. The fish fed different dietary lipids for 90 days (CocoOil-90=D1; OliveOil-90=D2; SunOil-90=D3; LinOil-90=D4 and FishOil-90=D5) were differentiated from the initial position based on fatty acid composition. PUFA, n-3 PUFA, MUFA, 18:2 n-6, 18:1 n-9 and 22:6 n-3 were found to be responsible for causing the differences among the samples.

preferred fatty acids for the mitochondrial β-oxidation and thus the source of metabolic energy in fish.

Effect of different dietary lipid sources on muscle fatty acid profile

It is well known that changes in tissue fatty acid composition of fish are under various metabolic influences such as fatty acid desaturation, elongation (Henderson et al., 1995), and β-oxidation (Henderson and Sargent, 1985). Tissue fatty acid composition is affected by size or age of animals (Kiessling et al., 2001). The fatty acid compositions of tissue lipids are also readily influenced by the fatty acid composition of dietary lipid (Torstensen et al., 2000; Bell et al., 2001, 2002; Roselund et al., 2001). Muscle fatty acid composition of rohu fed different experimental diets for 90 days has also revealed the influence of dietary lipids (Table 6). At 90 days there was a clear trend between the dietary and muscle fatty acid profiles. Significant amounts of SFA (42.14%), MUFA (46.71%), n-6 PUFA (38.92%), and n-3 PUFA (30.89%) were found in fish fed diets 1, 2, 3 and 4 respectively. This observation was in coherence with previous studies (Reinitz and Yu, 1981; Mishra and Samantaray, 2004; Kim et al., 2008) where dietary fatty acids influenced the tissue fatty acid profile. The differences in the muscle fatty acid profiles could be examined further using the principal component analysis (PCA). The bi-plot of PC1 and PC2 (Figure 1) was used in this study since these two components summarized more variation (75%) in the data than any other pair of components. The fish fed different dietary lipids for 90 days (CocoOil-90 = D1; OliveOil-90 = D2; SunOil-90 = D3; LinOil-90 = D4 and FishOil-90 = D5) were differentiated from the initial position based on fatty acid composition. The bi-plot helps to determine which fatty acids are responsible for differences between samples. PUFA, n-3 PUFA, MUFA, 18:2 n-6, 18:1 n-9 and 22:6 n-3 were found to be responsible for causing the differences among the samples. Fish oil diet which was used as a control displayed a more uniform distribution of different fatty acid classes compared to the other experimental diets.

Effect of different dietary lipid sources on liver fatty acid profile

The dietary fatty acid profile was clearly reflected in the liver fatty acid composition of fish at 90 days (Table 7). Among the treatments studied, the highest levels of SFA (52.9%), MUFA (41.28%), n-6 PUFA (35.19%) and n-3 PUFA (36.15%) were found in the livers of fish fed with the D1 (84.36% SFA), D2 (73.88% MUFA), D3 (53.62% n-6 PUFA) and D4 (48.99% n-3 PUFA) respectively. Although SFA content was highest (52.9%) in the fish fed with D1, other treatment groups also had proportionately higher SFA content when compared to respective diets. In contrast to the observations of Mishra and Samantaray (2004) no significant amounts of arachidic acid (20:0) was detected in tissues of fish fed various experimental diets. However SFA content was greater in liver than that observed in the muscle. Izquierdo et al. (2003) proposed that high *de novo* fatty acid synthesis activity in fish liver may cause the high SFA content found in the fish livers. Feeding rohu with D1, D2, and D3 has resulted in significant reduction in the levels of 20:5 n-3 and 22:6 n-3 (Table 7). D5 contained substantial

amount of 22:6 n-3 (9.41%) which is reflected in the livers of fish fed with the same diet. Desaturation and elongation of ALA might be the reason behind the high amount (15.71%) of 22:6 n-3 found in the D5 fed fishes. Similarly desaturation and elongation of 18:3 n-3 might be the reason behind the high amount (7.93%) of 20:4 n-6 found in the D3 fed fishes. Feeding with high 18:3 n-3 diet (D4) has resulted in the deposition of high 18:3 n-3 and 22:6 n-3 but reduced EPA in liver of rohu (Table 8). However, a different trend was observed in Atlantic salmon (*Salmo salar* L.) when smolts were fed high 18:3 n-3 diet which showed increase in 18:3 n-3 and 20:5 n-3 but reduced 22:6 n-3 (Sargent et al., 2002). Madsen et al. (1998) concluded that preference for the 20:5 n-3 over the 22:6 n-3 in β-oxidation of fatty acids in the liver may the reason for the deposition of 20:5 n-3 compared to 22:6 n-3.

Evidence for Δ6 and Δ5 desaturase activity in rohu

A high amount of 22:6 n-3 (15.47%) was found (Table 7) in the liver of fish fed D4 containing 48.49% linolenic acid, the precursor of n-3 biosynthetic pathway. The concentration of 20:4 n-6 (7.93%) was relatively high in the liver of fish fed D3 containing 52% linoleic acid, the precursor of n-6 biosynthetic pathway. It is generally accepted that 18:3 n-3 is converted to 22:6 n-3 by a pathway combining the sequential action of Δ6, Δ5 and Δ4 desaturases with chain elongation reactions (Henderson and Tocher, 1987). However, Voss and co-workers (1991) proposed that Δ 4 desaturase may not be participating in the HUFA biosynthetic pathway. Instead, Voss et al. (1991) proposed that 22:6 n-3 may be biosynthesized by the sequential desaturation and chain elongation of 20:5 n-3 to 24:6 n-3, and 24:6 n-3 is finally chain shortened in peroxisomes to yield 22:6 n-3. In agreement with the suggestion of Voss et al. (1991), we detected using GC-MS, the other metabolites of n-3 pathway, 20:4 n-3, 22:5 n-3, 24:5 n-3 and 24:6 n-3, in the liver and muscle of fish fed D4 (Tables 6 and 7). These metabolites were not present in the other three vegetable oil based diets i.e., diets 1, 2 and 3 (Tables 6 and 7) where linolenic acid was not provided in

Table 6. Fatty acid composition (% of total fatty acids) of total lipid of muscle from rohu fed the experimental diets for 90 days

Fatty acids	D1	D2	D3	D4	D5
14:0	4.45±0.10^{a}	0.37±0.07^{e}	0.36±0.03^{e}	0.22±0^{e}	0.91±0.05^{b}
16:0	21.65±1.11^{a}	14.74±0.83^{e}	13.30±0.91^{ab}	10.87±0.31^{e}	15.63±0.81^{ab}
16:1 n-9	1.90±0.08^{b}	1.22±0.07^{e}	0.62±0.04^{d}	0.57±0.01^{d}	2.20±0.07^{a}
18:0	11.16±0.60^{a}	8.78±0.59^{b}	8.81±0.60^{b}	12.0 ±0.15^{a}	7.79±0.4^{b}
18:1 n-9	14.93±0.56^{c}	39.55±1.39^{a}	22.26±0.29^{b}	17.74±1.15^{c}	17.14±0.88^{bc}
18:1 n-7	3.33±0.11^{b}	1.84±0.17^{e}	2.14±0.15^{e}	2.51±0.13^{bc}	4.87±0.20^{a}
18:2 n-6	9.19±0.37^{bc}	10.56±0.46^{bc}	22.76±1.08^{a}	11.03±0.72^{b}	6.79±0.49^{c}
18:3 n-6	ND	0.32±0.09^{ab}	0.56±0.1^{a}	0.1±0.01^{b}	0.19±0.05^{ab}
18:3 n-3	1.68±0.12^{b}	0.19±0.01^{b}	0.23±0.05^{b}	12.16±0.88^{a}	0.67±0.05^{b}
18:4 n-3	0.24±0.03^{a}	0.22±0.01^{a}	0.28±0.03^{a}	0.28± 0.05^{a}	0.22±0.01^{a}
20:1 n-9	1.08±0.1^{b}	1.48±0.13^{b}	1.08±0.03^{b}	0.88±0.01^{b}	6.52±0.65^{a}
20:2 n-6	0.5±0.05^{bc}	0.38±0.04^{c}	1.21±0.07^{a}	0.73±0.01^{b}	0.46±0.04^{bc}
20:3 n-6	2.55±0.08^{a}	1.63±0.04^{b}	2.97±0.15^{a}	1.59±0.07^{b}	0.79±0.05^{c}
20:4n-6, AA	5.60±0.26^{a}	3.92±0.29^{c}	6.00±0.37^{ab}	4.21±0.03^{bc}	3.25±0.03^{c}
20:3 n-3	0.12±0.02^{b}	ND	1.20±0.03^{a}	1.21±0.07^{a}	1.25±0.02^{a}
20:4 n-3	0.22±0.02^{b}	ND	0.83±0.06^{a}	0.82±0.06^{a}	0.48±0.04^{b}
20:5 n-3 (EPA)	0.88±0.04^{bc}	0.32±0.09^{e}	0.26±0.02^{e}	1.21±0.07^{b}	3.72±0.22^{a}
22:1 n-9	ND	ND	0.28±0.02^{a}	0.28±0^{a}	1.24±0.12^{a}
22:4 n-6	0.68±0.04^{a}	0.46±0.06^{ab}	0.58±0.04^{ab}	0.41±0.07^{ab}	0.30±0.02^{b}
22:5 n-6	4.45±0.28^{a}	2.78±0.09^{b}	4.84±0.14^{a}	2.18±0.23^{b}	1.03±0.01^{c}
22:5 n-3	1.16±0.19^{bc}	0.3±0.05^{d}	0.49±0.06^{cd}	1.48± 0.19^{bc}	2.61±0.14^{a}
22:6 n-3 (DHA),	6.53±0.33^{b}	5.02±0.05^{b}	5.13±0.23^{b}	12.86±0.94^{a}	16.99±0.93^{a}
24:5 n-3	ND	ND	ND	0.33±0.19^{a}	ND
24:6 n-3	ND	ND	ND	0.54± 0.02	ND
∑SFA	42.14±0.77^{a}	27.19±0.21^{b}	23.87±0.30^{b}	24.97±0.48^{b}	26.44±0.55^{b}
∑MUFA	24.06± 0.04^{cd}	46.71±1.02^{a}	28.79±0.12^{c}	23.89±0.82^{d}	34.81±1.59^{b}
∑PUFA	33.8±0.4^{c}	26.1±0.75^{d}	47.34±0.19^{b}	51.14±0.33^{a}	38.75±1. 41^{c}
∑n-6	22.97±0.21^{b}	20.05±0.74^{b}	38.92±0.58^{a}	20.25±0.49^{b}	12.81±0.67^{c}
∑n-3	10.83±0.14^{c}	6.05±0.1^{d}	8.42±0.38^{d}	30.89±0.12^{a}	25.94±0.74^{b}
n-3 to n-6 ratio	0.47±0.02^{e}	0.30±0.02^{d}	0.21±0.04^{d}	1.53± 0.02^{b}	2.02±0.01^{a}

Results are means±SE. AA = Arachidonic acid; EPA = Eicosapentanoic acid; DHA = Docosahexanoic acid.
SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids.
Values within a row with different superscript letter are significantly different ($p<0.05$), ND = Not detected.

Table 7. Fatty acid composition (% of total fatty acids by weight) of total lipid of liver from rohu fed the experimental diet for 90 days

Fatty acids	D1	D2	D3	D4	D5
14:0	9.42±0.33^{a}	0.39±0.06^{c}	0.28±0.04^{c}	0.32±0.03^{c}	1.47±0.07^{b}
16:0	20.09±1.09^{a}	16.71±0.49^{a}	15.38±0.82^{a}	14.78±0.66^{a}	17.82±0.21^{a}
16:1 n-7	2.00±0.04^{b}	1.14±0.13^{c}	0.85±0.06^{c}	0.82±0.03^{c}	3.7±0.07^{a}
18:0	10.15±0.3^{a}	8.71±0.43^{ab}	10.89±0.86^{a}	11.27±0.29^{a}	6.39±0.13^{b}
18:1 n-9	12.68±0.1^{c}	32.47±0.93^{a}	21.73±1.05^{b}	15.6±0.93^{c}	15.42±0.28^{c}
18:1 n-7	2.52±0.29^{b}	2.89±0.08^{b}	2.10±0.25^{b}	1.99±0.2^{b}	5.04±0.45^{a}
18:2 n-6	5.23±0.64^{bc}	8.13±0.93^{b}	18.12±0.86^{a}	6.82±0.58^{bc}	3.92±0.3^{c}
18:3 n-6	0.23±0.02	0.32±0.06	0.83±0.18	0.85±0.02	0.70±0.01
18:3 n-3	0.87±0.11^{b}	0.38±0.02^{b}	0.45±0.03^{b}	12.9±0.49^{a}	0.93±0.35^{b}
18:4 n-3	0.36±0.03	0.24±0.02	0.38±0.06	0.30±0.01	0.34±0.02^{c}
20:1 n-9	1.23±0.12^{b}	1.14±0.22^{b}	1.15±0.07^{b}	1.08±0.18^{b}	5.4±0.41^{a}
20:1 n-7	0.36±0.02^{c}	0.81±0.03^{b}	1.24±0.12^{a}	0.51±0.06^{c}	0.97±0.02^{a}
20:2 n-6	0.49±0.02	0.68±0.09	1.21±0.03	0.68±0.04	0.78±0.02
20:3 n-6	1.72±0.18^{abc}	1.92±0.34^{ab}	2.38±0.13^{a}	0.92±0.35^{bc}	0.52±0.04^{c}
20:4n-6, AA	4.61±0.19^{a}	5.96±0.83^{a}	7.93±0.67^{a}	3.68±0.26^{a}	3.21±0.55^{a}
20:3 n-3	0.32±0.02^{b}	0.33±0.02^{b}	0.28±0.02^{c}	1.67±0.17^{a}	1.87±0.02^{a}
20:4 n-3	0.28±0.02^{c}	0.29±0.02^{c}	0.25±0.02^{b}	0.89±0.12^{a}	0.47±0.04^{b}
20:5n-3 (EPA)	0.52±0.01^{c}	0.55±0.05^{c}	0.57±0.01^{c}	1.3±0.09^{b}	4.51±0.09^{a}
22:1 n-9	0.5±0.04^{b}	0.39±0.02^{b}	ND	1.12±0.02^{b}	1.78±0.03^{a}
22:4 n-6	0.52±0.04	1.16±0.19^{a}	0.57±0.03	ND	1.16±0.02^{a}
22:5 n-6	2.68±0.16^{bc}	5.34±0.68^{a}	4.15±0.26^{ab}	2.08±0.16^{bc}	0.51±0.03^{d}
22:5 n-3	0.35±0.03^{b}	0.39±0.02^{b}	ND	0.50±0.08	1.52±0.16^{a}
22:6n-3 (DHA),	6.87±0.14^{b}	5.02±0.21^{b}	4.58±0.17^{b}	15.47±1.4^{a}	15.71±1.26^{a}
24:5 n-3	ND	ND	ND	0.42±0.01	1.15±0.15
ΣSFA	52.9±0.46^{a}	28.01±0.8^{b}	29.46±0.17^{b}	29.51±0.09^{b}	28.91±0.55^{b}
ΣMUFA	22.05±0.24^{d}	41.28±0.90^{a}	28.84±0.73^{c}	19.31±0.75^{d}	33.79±0.11^{b}
ΣPUFA	25.05±0.53^{d}	30.71±2.04^{cd}	41.7±0.62^{b}	51.18±0.47^{a}	37.3±0.92^{c}
Σn-6	15.48±0.82^{c}	23.51± 2.46^{b}	35.19±0.19^{a}	15.03±1.30^{c}	10.8±0.07^{c}
Σn-3	9.57±0.06^{c}	7.2±0.22^{c}	6.51± 0.2^{c}	36.15±1.95^{a}	26.5±0.95^{b}
n-3 to n-6 ratio	0.61±0.11^{b}	0.30±0.59^{b}	0.18± 0.18^{b}	2.4±0.06^{a}	2.45± 0.01^{a}

Results are means±SE. AA = Arachidonic acid; EPA = Eicosapentanoic acid; DHA = Docosahexanoic acid.
SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids.
Values within a row with different superscript letter are significantly different ($p<0.05$), ND = Not detected.

excess. Several metabolites of n-6 biosynthetic pathway (namely 18:2 n-6 and 20:3 n-6) were found in the liver and muscle (Tables 6 and 7) of fishes fed with D3. Linoleic acid is the precursor for the biosynthesis of arachidonic acid (Sprecher et al., 1999). We propose that the detection of n-3 biosynthetic pathway specific metabolites is a strong indication of HUFA biosynthetic capability of rohu to desaturate and elongate 18:3 n-3 into 20:5 n-3 and 22:6 n-3. Mishra and Samantaray (2004) also observed that desaturase and elongase enzyme systems are active in rohu. Although linoleic acid was present in significant quantity in D4 (Table 2), the concentrations of 20:4 n-6 and 22:5 n-6 (Tables 6 and 7) were significantly lower in D4 group compared to D3 fed fish. Tocher et al. (2001) concluded from their study that inclusion of high levels of 18:3 n-3 in the diet inhibits the production of 20:4 n-6 from 18:2 n-6. This fact can be explained with the observation of Sargent et al. (2002), that the affinity of desaturases to desaturate a C_{18} fatty acid is in the order of n-3>n-6>n-9 fatty acids.

Effect of finishing diet on the growth, biochemical composition and final tissue fatty acid profile

Incorporation of vegetable oils in experimental diets has clearly showed the effects of dietary fatty acid profile on the flesh quality, at least in the fatty acid profile of the muscle and liver (Tables 6 and 7). The fish fed diets 1, 2, and 3 possessed significantly low amounts of 20:5 n-3 (<1%) and 22:6 n-3 (4-6%). Similar changes have also been reported in Atlantic salmon (Thomassen and Røsjø, 1989), turbot, *Psetta maxima* (Serot et al., 2001), swordtail, *Xiphophorus helleri* (Ling et al., 2006), and Atlantic cod, *Gadus morhua* (Morkore et al., 2007) fed vegetable oils as the lipid source in the diet. Due to the importance of n-3 fatty acids for human health, fatty acid profile of fish fed plant oils is considered of inferior quality. After using feeds containing vegetable oils, switching to a fish oil diet is a way of increasing human cardio-protective n-3 fatty acids in fish flesh (Regost et al., 2003).

Table 8. Fatty acid composition (% of total fatty acids by weight) of total lipid of muscle from rohu the return fish oil diet for 30 (cumulative 120 days) days

Fatty acids	D1	D2	D3	D4	D5
14:0	1.11±0.11^{a}	1.22±0.06^{a}	1.07±0.18^{a}	1.18±0.09^{a}	0.81±0.03^{a}
16:0	17.25±0.98^{a}	18.76±0.90^{a}	16.68±0.71^{a}	15.09±0.69^{a}	17.42±1.22^{a}
16:1 n-7	1.73±0.12^{a}	2.35±0.11^{a}	2.5±0.33^{a}	2.31±0.1^{a}	2.66±0.34^{a}
18:0	11.20±0.61^{a}	10.15±0.81^{a}	10.69±0.99^{a}	10.29±1.15^{a}	9.25±0.81^{a}
18:1 n-9	13.23±0.97^{b}	19.76±1.07^{a}	17.37±1.02^{ab}	16.91±0.31^{ab}	14.96±0.41^{ab}
18:1 n-7	4.4±0.02^{a}	4.00±0.13^{a}	4.35±1.00^{a}	2.25±0.58^{a}	4.71±0.60^{a}
18:2 n-6	6.06±0.80^{a}	5.84±0. 38^{a}	7.50±0.72^{a}	4.74±0.62^{a}	4.15±0.44^{a}
18:3 n-6	0.24±0.01^{b}	0.25±0.01^{b}	1.15±0.01^{a}	0.27±0.03^{b}	1.25±0.01^{a}
18:3 n-3	0.48±0.10^{b}	0.48±0.05^{b}	0.35±0.07^{b}	2.56±0.52^{a}	0.39±0.10^{b}
18:4 n-3	0.28±0.01	0.30±0.01	0.31±0.05	0.32±0.05	0.28±0.04
20:1 n-9	4.85±0.75^{a}	5.00±0.20^{a}	4.11±0.70^{a}	5.01±0.34^{a}	4.88±0.57^{a}
20:1 n-7	0.69±0.12^{ab}	0.59±0.09^{ab}	0.28±0.04^{b}	0.36±0.05^{ab}	0.77±0.08^{a}
20:2 n-6	0.53±0.1^{a}	0.52±0.04^{a}	0.64±0.05^{a}	0.58±0.09^{a}	0.42±0.03^{a}
20:3 n-6	1.27±0.11^{a}	1.13±0.23^{a}	1.26±0.09^{a}	0.58±0.10^{a}	0.55±0.06^{a}
20:4 n-6, AA	5.26±0.23^{a}	4.85±0.36^{a}	4.73±0.57^{a}	3.53±0.29^{a}	3.97±0.16^{a}
20:3 n-3	0.48±0.01	0.49±0.01	0.51±0.01	0.5±0.14	0.52±0.01
20:4 n-3	0.5±0.07^{a}	0.41±0.06^{a}	0.7±0.08^{a}	0.75±0.13^{a}	0.86±0.16^{a}
20:5 n-3 (EPA)	4.55±0.35^{a}	5.03±0.53^{a}	4.92±0.26^{a}	5.47±0.65^{a}	4.26±0.28^{a}
22:1 n-9	0.74±0.07^{b}	1.15±0.11^{ab}	1.0±0.06^{ab}	1.45±0.17^{a}	1.10±0.08^{ab}
22:4 n-6	0.57±0.01	0.54±0.01	0.58±0.01	0.46±0.01	0.6±0.0.01
22:5 n-6	2.06±0.24^{a}	1.79±0.08^{a}	2.09±0.22^{a}	1.24±0.09^{a}	1.36±0.17^{a}
22:5 n-3	1.95±0.09^{a}	1.62±0.26^{a}	1.48±0.13^{a}	2.34±0.21^{a}	1.21±0.25^{a}
22:6 n-3 (DHA),	13.92±0.67^{a}	8.8±0.22^{b}	11.46±0.63^{b}	16.44±0.89^{a}	18.59±0.23^{a}
24:6 n-3	ND	ND	ND	1.57±0.10	ND
∑SFA	31.52±1.78^{a}	32.34±0.08^{a}	30.73±2.19^{a}	28.05±1.86^{a}	29.47±0.31^{a}
∑MUFA	30.33±0.39^{b}	35.61±1.40^{a}	31.59±0.18^{ab}	30.6±0.94^{b}	32.12±0.26^{ab}
∑PUFA	38.15±0.37^{ab}	32.05±0.59^{c}	37.68±2.11^{bc}	41.35±1.01^{a}	38.41±0.39^{ab}
∑n-6	15.99±0.55^{a}	14.92±0.33^{a}	17.95±1.66^{a}	11.4±1.05^{a}	12.3±0.52^{a}
∑n-3	22.16±0.24^{bc}	17.13±0.37^{d}	19.73±0.55^{cd}	29.95±2.24^{a}	26.11±0.21^{ab}
n-3 to n-6 ratio	1.38±0.03^{bc}	1.14±0.01^{c}	1.09±0.06^{b}	2.62±0.06^{a}	2.12±0.02^{a}

Results are means±SE. AA= Arachidonic acid; EPA = Eicosapentanoic acid; DHA = Docosahexanoic acid.
SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids.
Values within a row with different superscript letter are significantly different ($p<0.05$), ND = Not detected.

During the wash out study rohu were fed with D5 (rich in fish oil). No significant difference was observed in the growth of fish during the final wash out study (Table 3). Mourente and Bell (2006) did not observe any significant difference in the growth parameters of Atlantic salmon fed with a wash-out diet for 14 weeks after feeding with different vegetable oil diets. Analysis of proximate composition of the experimental groups has showed that D1 group had higher crude protein content till the 120th day. Moisture, organic matter and crude fat contents were not affected in all the groups at the end of 120 days compared to the 90 days. Regost et al. (2003) also reported similar results where turbot fed with a return fish oil diet after feeding with different vegetable oils.

In the muscle and liver tissues of rohu previously fed vegetable oils, the percentage of several fatty acids changed significantly after 30 days of wash-out period (Tables 8 and 9). The quantities of tissue fatty acids originated from the dietary vegetable oils (14:0, 18:1 n-9, 18:2 n-6 and 18:3 n-3) decreased, while 20:5 n-3 and 22:6 n-3, characteristic of fish oil increased and reached the same levels of fish that fed D5 (rich in fish oil) continuously throughout the experimental period (120 days). Similar observations were also recorded by Torstensen et al. (2005) where the fatty acid profile of Atlantic salmon fed with vegetable oil blends was restored with the final fish oil diet. The amounts of SFA (42.14%) in the muscle of fish fed D1, which was significantly high after 90 days from rest of the treatments (Table 6), has reduced after the washout period (Table 8). In D2 group, MUFA also has diluted from 46.71% after the 90 days to 35.61% after 120 days. Though 20:5 n-3 deposition was same in all the groups, in D2 group after wash-out period, 22:6 n-3 was significantly lower compared to other diet groups. In contrast, 20:5 n-3 levels increased but 22:6 n-3 levels were not restored by a fish oil return diet in post-smolt Atlantic salmon fed with different proportions of

Table 9. Fatty acid composition (% of total fatty acids by weight) of total lipid of liver from rohu fed the return fish oil diet for 30 (cumulative 120 days) days

Fatty acids	D1	D2	D3	D4	D5
14:0	3.15±0.08^{b}	8.06±0.38^{a}	7.76±0.38^{a}	2.58±0.16^{b}	6.77±0.38^{a}
16:0	24.42±0.83^{a}	22.62±0.74^{ab}	22.32±1.09^{a}	22.01±0.75^{ab}	22.95±0.67^{b}
16:1 n-7	2.09±0.15^{a}	2.05±0.21^{a}	2.88±0.20^{a}	2.47±0.09^{a}	2.96±0.25^{a}
18:0	6.11±0.40^{c}	6.80±0.60^{ab}	6.65±0.54^{ab}	8.89±0.34^{a}	5.04±0.81^{bc}
18:1 n-9	5.66±0.83^{b}	14.11±0.98^{a}	14.69±0.83^{a}	13.97±0.77^{a}	12.7±0.83^{a}
18:1 n-7	11.44±0.51^{a}	2.08±0.39^{c}	2.66±0.25^{bc}	4.20±0.36^{ab}	2.91±0.17^{bc}
18:2 n-6	2.90±0.57^{b}	2.60±0.18^{b}	6.73±0.60^{a}	3.70±0.64^{ab}	4.23±0.13^{a}
18:3 n-6	0.42±0.08	0.45±0.11	0.31±0.07	0.46±0.05	0.28±0.05
18:3 n-3	0.27±0.06^{b}	0.36±0.04^{b}	0.42±0.05^{b}	1.07±0.16^{a}	0.22±0.06^{b}
18:4 n-3	0.26±0.06^{a}	0.32±0.06^{a}	0.50±0.03^{a}	0.35±0.05^{a}	0.44±0.07^{a}
20:1 n-9	5.16±0.47^{a}	4.84±0.58^{a}	6.16±0.80^{a}	6.00±0.23^{a}	6.36±0.48^{a}
20:1 n-7	1.43±0.42^{a}	0.40±0.06^{a}	1.12±0.26^{a}	0.62±0.11^{a}	0.48±0.11^{a}
20:2 n-6	0.40±0.06^{a}	0.28±0.06^{a}	0.37±0.06^{a}	0.36±0.06^{a}	0.36±0.08^{a}
20:3 n-6	0.41±0.06^{a}	0.39±0.08^{a}	0.40±0.09^{a}	0.28±0.01^{a}	0.40±0.01^{a}
20:4 n-6, (AA)	3.71±0.49^{a}	2.54±0.40^{a}	3.93±0.12^{a}	2.37±0.59^{a}	3.03±0.44^{a}
20:3 n-3	0.30±0.05	0.35±0.02	0.40±0.11	0.27±0.06	0.45±0.06
20:4 n-3	0.61±0.06	0.45±0.08	0.60±0.10	0.50±0.09	0.45±0.06
20:5 n-3 (EPA)	5.08±0.66^{a}	4.86±0.26^{a}	3.77±0.29^{a}	4.39±0.45^{a}	4.37±0.47^{a}
22:1 n-9	1.18±0.19^{bc}	2.23±0.37^{a}	1.52±0.23^{b}	1.55±0.05^{b}	0.43±0.08^{c}
22:5 n-6	0.60±0.08^{ab}	1.09±0.25^{a}	0.38±0.08^{b}	0.53±0.04^{ab}	0.34±0.07^{b}
22:5 n-3	1.28±0.2^{ab}	0.60±0.11^{bc}	1.02±0.09^{ab}	1.42±0.12^{a}	0.29±0.05^{c}
22:6 n-3 (DHA),	14.74±1.16^{a}	15.53±0.93^{a}	10.14±0.84^{a}	15.75±1.18^{a}	16.17±1.17^{a}
∑SFA	36.36±0.59^{a}	40.33±0.22^{ab}	39.27±2.39^{a}	36.37±0.34^{a}	37.24±0.47^{a}
∑MUFA	32.66±0.12^{a}	29.85±0.24^{a}	31.76±2.28^{a}	32.18±1.20^{a}	31.73±0.19^{a}
∑PUFA	30.98±0.51^{a}	29.82±0.37^{a}	28.97±0.94^{a}	31.45±1.58^{a}	31.03±1.86^{a}
∑n-6	8.44±1.2^{a}	7.35±0.15^{a}	12.12±0.58^{a}	7.7±0.02^{a}	8.64±0.26^{a}
∑n-3	22.54±0.69^{a}	22.47±0.53^{ab}	16.85±0.36^{b}	23.75±1.60^{a}	22.39±1.60^{ab}
n-3 to n-6 ratio	2.67±0.06^{b}	3.05±0.01^{a}	1.39±0.02^{c}	3.08±0.02^{a}	2.59±0.01^{b}

Results are means±SE. AA = Arachidonic acid; EPA = Eicosapentanoic acid; DHA = Docosahexanoic acid.
SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids.
Values within a row with different superscript letter are significantly different ($p<0.05$), ND = Not detected.

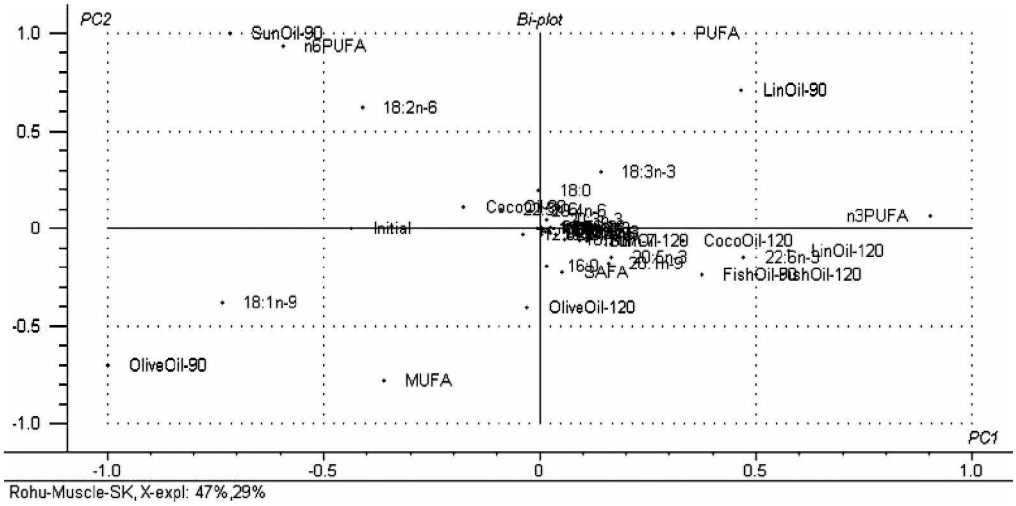

Figure 2. The bi plot of principal component analysis shows the impact of wash-out study. All the fish fed for 90 days with different lipid sources were fed for 30 days with fish oil based diet and different diet groups were named as CocoOil-120, OliveOil-120, ... to distinguish from that of fish fed for 90 days. Fish groups of wash-out study were found close to each other compared to fish groups for 90 days which are distributed in different quadrants indicating the influence of wash-out study.

rapeseed oil (Bell et al., 2003). The n-6 fatty acids have also undergone significant dilution in the D3 group, 38.92% after the 90 days to 17.95% after the 120 days. In two different studies where sea bass and gilthead sea bream were fed 60% rapeseed oil, linseed oil or soybean oil or 80% linseed oil, a fish oil finishing diet restored 22:6 n-3 values, but not 20:5 n-3 values, to those seen in fish fed fish oil diet until the completion of the study (Montero et al., 2003, 2005). These studies started with larger fish, compared to the smaller start weight (8 g) in the present study. It is difficult to compare the present study with the sea bass and sea bream studies (Montero et al., 2003, 2005) since many factors in addition to feeding period such as initial fish weight, weight increase, growth rate and carcass lipid percentage must be taken into account.

The bi plot of PCA (Figure 2) also revealed the impact of wash-out study on fatty acid profile of muscle. All the fish fed for 90 days with different lipid sources were fed for 30 days with fish oil based diet and different diet groups were named as CocoOil-120, OliveOil-120, ... to distinguish from that of fish fed for 90 days. The results for fish groups of wash-out study were found to be similar compared to fish fed different diets for 90 days, which were distributed in different quadrants indicating the influence of wash-out study (Figure 2). Some authors have argued that the depletion of the fatty acids originates from vegetable oil diets after feeding with return fish oil diet is the result of simple dilution (Robin et al., 2003; Jobling, 2004). Our data using rohu supports these findings.

It is difficult to predict the time required to reach a relatively stable fatty acid composition of tissue in fish fed a particular diet. It could be relatively short or continue to evolve over a longer period (Tidwell and Robinette, 1990). In case of turbot, though the initial differences were partly reduced, the fatty acid profiles of fish initially fed on different oil sources still differed among experimental groups, even after 2 months the same diet (Regost et al., 2003). However, in the present study, the washout duration of 30 days was sufficient to achieve desirable changes in the fatty acid composition. Using fast-growing juvenile fish, it was possible to obtain a desirable tissue fatty acid composition by changing the dietary fatty acid composition in a relatively short period of time. The final fish oil rich wash-out diet resulted in equalization of the fatty acid profile of the fish fed vegetable oil diets to that of fish fed fish oil diet demonstrating the practicality of a final wash-out diet in commercial aquaculture operations.

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