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The implications of substituting dietary fish oil with vegetable oils on the growth performance, fillet fatty acid profile and modulation of the fatty acid elongase, desaturase and oxidation activities of red hybrid tilapia, *Oreochromis* sp.

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ABSTRACT

A 75-day feeding trial was carried out to examine the effects of fish oil (FO) replacement with various alternative vegetable oil (VO) on the growth performance and feed utilization efficiency of red hybrid tilapia (*Oreochromis* sp.). The VO evaluated included canola oil (high in monoenes), perilla oil (high in n − 3 polyunsaturated fatty acids, PUFA), sunflower oil (high in n − 6 PUFA) and refined, bleached, deodorized palm olein (high in saturates) added as the sole lipid source in semi-purified experimental diets. The ability of hybrid tilapia to biosynthesize long chain-polyunsaturated fatty acids (LC-PUFA) from dietary 18:2n − 6 and 18:3n − 3 was also determined. With the exception of sunflower oil, results showed that total FO replacement by various VO did not compromise ($P > 0.05$) tilapia growth performance. However, dietary VO significantly ($P < 0.05$) influenced the fatty acid composition of tilapia fillet and whole-body lipids through changes in the overall fatty acid metabolism. The total Δ5 and Δ6 fatty acid desaturase activities were exclusively triggered in tilapia fed VO-based diets. Tilapia fed the VO diets, which contained no PUFA longer than C_{18}, recorded significant amounts of both n − 6 and n − 3 LC-PUFA in their tissue lipids. Dietary canola oil (CO) increased Δ-6 desaturation of 18:3n − 3 to the greatest extent despite the dietary 18:3n − 3 content being comparatively lower than dietary perilla oil (PeO). Beta-oxidation of 18:3n − 3 was observed to be highest in fish fed the PeO diet, indicating that the 18:3n − 3 content in the PeO diet exceeded the optimum substrate level for Δ-6 desaturase. Omega-3 LC-PUFA biosynthetic activities were highest in fish fed the CO diet and resulted in a comparatively higher eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) content than in fish fed other VO diets. However, endogenous LC-PUFA synthesis induced by the VO diets was insufficient to rival the n − 3 LC-PUFA content of fish fed the FO diet. It was also observed that β-oxidation of 18:3n − 3 and 18:2n − 6 was regulated by tissue n − 3/n − 6 PUFA ratio and proportional to their dietary level. This study provided the first comprehensive evidence of the significant impact of dietary oils with different fatty acid class profile on the *in vivo* fatty acid metabolism of tilapia.

Statement of relevance: Marine fish oil (FO) was traditionally used as the major lipid source in commercial tilapia feeds to provide energy and essential fatty acids as well as to impart palability to the feed. With the rising costs of FO and limited global supplies, the use of alternative lipid resources in aquafeeds, including tilapia feeds, has become more critical to enable the continued sustainability and scalability of the global aquaculture industry. Terrestrial vegetable oil (VO) are viable alternatives since their production is more sustainable and cost-effective. However, unlike FO, which is rich in n − 3 long chain-polyunsaturated fatty acids (LC-PUFA), VO are deficient in these essential fatty acids and but high in other fatty acid classes. The present study evaluated perilla oil (high levels of n − 3 polyunsaturated fatty acids), sunflower oil (rich in n − 6 PUFA), canola oil (high amounts of monounsaturated fatty acids) and palm oil (rich in saturated fatty acids) together with fish oil. By understanding how dietary lipid source with different major fatty acid classes modulates *in vivo* fatty acid metabolism, we can better formulate fish feeds that can optimize not only growth performance but also maintaining the health benefits of eating seafood for the human consumer in regards to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in farmed fish fillets. As far as we know, there is currently no information comparing the fatty acid metabolism of red hybrid tilapia fed diets with lipids containing different major fatty acid classes.

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1. Introduction

The farming of tilapia is the most popular type of aquaculture in the world (FAO, 2014) and its rapid global expansion is due in large part to the increasing use of factory-made formulated feeds (Ng and Romano, 2013). Marine fish oil (FO) was traditionally used as the major lipid source in commercial tilapia feeds to provide energy and essential fatty acids as well as to impart palability to the feed. With the rising costs of FO and limited global supplies, the use of alternative lipid resources in aquafeeds, including tilapia feeds, has become more critical to enable the continued sustainability and scalability of the global aquaculture industry (Turchini et al., 2009; Ng and Romano, 2013). Terrestrial vegetable oil (VO) are viable alternatives since their production is more sustainable and cost-effective. However, unlike FO, which is rich in n−3 long chain-polyunsaturated fatty acids (LC-PUFA), VO are deficient in these essential fatty acids and high in other fatty acid classes. For example, perilla oil (PeO) and linseed oil contain high levels of n−3 polyunsaturated fatty acids (PUFA); sunflower oil (SFO), corn oil, soybean oil are rich in n−6 PUFA; canola oil (CO)/rapeseed oil and olive oil have high amounts of monounsaturated fatty acids (MUFA); while palm oil and coconut oil are rich in saturated fatty acids (SFA). Much research had been conducted to evaluate the nutritional quality of these alternative plant-based lipid sources with different major fatty acid classes in fish feeds (Turchini et al., 2009, 2011).

In general, total or partial FO replacement by VO has no deleterious effect on the growth performance of a variety of warmwater fish species, including milkfish (Alava, 1998), sunshine bass (Wonnacott et al., 2004), barramundi (Raso and Anderson, 2003; Alhazzaa et al., 2011) and common carp (Steffens et al., 1995) as long as their essential fatty acid requirements are met and have been reported in some instances to enhance growth such as that observed in African catfish (Ng et al., 2003). Nevertheless, the use of dietary VO decreased the health beneficial n−3 LC-PUFA content of fish fillets and is considered a major challenge facing FO replacement in fish feeds (Turchini et al., 2009, 2011). Moreover, dietary fatty acids have been reported to affect fatty acid metabolism (Bell et al., 2001; Turchini et al., 2009; Torstensen and Tocher, 2010). Therefore, a better fundamental understanding on how dietary lipid source modulates *in vivo* fatty acid metabolism is needed to better formulate fish feeds that can optimize not only growth performance but also maintaining the health benefits of eating seafood for the human consumer in regards to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in farmed fish fillets.

Although we have previously reported on the effects of dietary VO in the replacement of FO (Ng et al., 2001, 2013; Bahurmiz and Ng, 2007), as far as we know, there is currently no information comparing the fatty acid metabolism of red hybrid tilapia fed diets with lipids containing different major fatty acid classes. Therefore this study aimed to examine the effects of individual VO with different fatty acid class profile as FO alternatives on growth performance, fish fatty acid composition and changes in the overall *in vivo* fatty acid metabolism in red hybrid tilapia. These fundamental findings will serve as the basis for current and future fish nutrionists to develop newer concepts and methods for improving the efficiency of conversion of fish feed to food and the healthiness of fish products for human consumers.

2. Materials and methods

2.1. Experimental diets

Five isonitrogenous and isolipidic semi-purified diets were formulated (Table 1). Different oils were used as the sole lipid source, *i.e.*, FO (control diet), CO (high in MUFA), PeO (high in n−3 PUFA), SFO (high in n−6 PUFA) and refined, bleached, deodorized palm olein (RBDPO) (high in SFA and MUFA). PeO was purchased in Japan while the other oil sources were purchased from various local suppliers or grocery stores. Casein and gelatin were used as the main sources of protein in the diets, whereas dextrin was included as the carbohydrate source. Chromic oxide was included in diet at an inclusion rate of 0.5% as an inert marker for the determination of apparent nutrient digestibility. All purified feed ingredients were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dry ingredients were mixed homogenously in a Hobart mixer and distilled water was added. The moist dough was screw-pressed through a 2 mm die and the feed pellets formed were fan-dried and stored frozen at −20 °C until use.

2.2. Fish and experimental conditions

Red hybrid tilapia fingerlings were purchased from a local fish farm. Upon arrival at the Fish Nutrition Laboratory, the fish were held in a 1000-L fiberglass tank and fed with pre-crushed commercial tilapia starter feed (Cargill, Malaysia). All fish were then acclimated to the experimental system for two weeks before the start of the feeding trial. During the acclimation period, all fish were fed with a conditioning diet with similar protein and energy content to the experimental diets but containing no added lipid prior to commencing the feeding trial. Groups of 15 fish with an average initial weight of 8.9 ± 0.0 g were randomly selected, weighed and stocked into a series of 15 aquaria. Each of the experimental diet was fed to randomly assigned triplicate groups of fish. Sand filtered water flowed into 95 L aquarium continuously at a flow-rate of 15 L h^{-1}. All aquaria were provided with respective air stones for continuous aeration. The flow-through aquaria system set-up and water quality parameters were as previously described by Ng et al. (2008). Fish were hand-fed twice a day at 0900 and 1700 h to apparent satiation for 75 days with feed consumption recorded daily. Fish were batch-weighed weekly.

2.3. Sample collection

At the beginning of the experiment, an initial sample of 20 fish was culled for whole-body analysis (proximate and fatty acid analysis), another 20 fish were killed, muscle and liver tissue excised and kept frozen for subsequent proximate composition and fatty acid analysis. These fish were starved for 48 h prior to sampling to ensure the gut was completely empty. After six weeks into the feeding trial, fecal material (intact strands) were siphoned and collected with a fine mesh net. Tilapia feces are rather unique in the sense that it is expelled in long strands encased within a clear gelatinous material. Fecal samples collected from

Table 1
Ingredient and proximate composition (g kg^{-1} diet) of experimental diets.

	Experimental diets[a]				
Ingredient	FO	PeO	CO	SFO	RBDPO
Casein	306.0	306.0	306.0	306.0	306.0
Gelatin	60.0	60.0	60.0	60.0	60.0
Peruvian fish oil	100.0	–	–	–	–
Perilla oil	–	100.0	–	–	–
Canola oil	–	–	100.0	–	–
Sunflower oil	–	–	–	100.0	–
Palm oil	–	–	–	–	100.0
Dextrin	292.4	292.4	292.4	292.4	292.4
Vitamin mix	30.0	30.0	30.0	30.0	30.0
Mineral mix	40.0	40.0	40.0	40.0	40.0
Dicalcium phosphate	10.0	10.0	10.0	10.0	10.0
Chromic oxide	5.0	5.0	5.0	5.0	5.0
Carboxymethyl cellulose	15.0	15.0	15.0	15.0	15.0
Alpha-cellulose	141.6	141.6	141.6	141.6	141.6
Proximate composition					
Dry matter	893.9	905.2	911.3	900.8	905.8
Crude Protein	351.7	346.9	343.2	346.1	352.6
Crude Lipid	89.1	82.6	82.1	84.6	84.1
Ash	43.6	43.7	43.3	43.0	43.9

[a] Experimental diets nomenclature: fish oil (FO), perilla oil (PeO), canola oil (CO), sunflower oil (SFO), refined, bleached, deodorized palm olein (RBDPO).

each aquarium were pooled, freeze-dried and finely ground before analysis.

At the termination of the experiment, all fish were anaesthetized with tricane methane sulphonate (MS 222), individually weighed and their total length measured. A sample of five fish per tank was randomly sampled, killed and blood collected into heparinized tubes. The sampled fish were dissected to remove various tissues for analysis and then skinned and filleted. Liver, viscera, gonads and intraperitoneal fat were excised and weighed for the determination of hepatosomatic index (HSI), viscerosomatic index (VSI), gonadosomatic index (GSI) and intraperitoneal fat index (IPF). Liver and fillet samples were pooled and kept frozen for subsequent chemical analysis. The remaining fish from each aquarium were culled and stored frozen at −20 °C for subsequent whole-body proximate and fatty acid composition analysis.

2.4. Chemical analysis

Proximate analysis of diet ingredients, experimental diets and feces samples was conducted using standard AOAC methods (AOAC, 1997). Chromic oxide content of diets and feces was determined according to the wet-acid digestion method of Furukawa and Tsukahara (1966). All samples were freeze-dried to constant weight and finely ground before lipid extraction. Total lipid was extracted from samples using a modified Folch et al. (1957) protocol. Weighed samples were soaked overnight in chloroform/methanol/water containing potassium chloride in a separatory funnel. After separation, total lipid content of samples was determined gravimetrically after evaporation of solvents using a rotary evaporator. Fatty acids were esterified into methyl esters using the acid-catalyzed methylation method (Christie, 2003). An internal standard (23:0) was added for quantitative measurement of individual fatty acid present in the samples. Fatty acid methyl esters were resolved and analyzed by a gas-liquid chromatograph (Shimadzu GC-2010) equipped with a flame ionization detector and Shimadzu GC solution software as previously described (Teoh et al., 2011).

2.5. Calculations and statistical analysis

The apparent digestibility coefficients (ADC) of dietary nutrients were calculated from standard formula (Maynard and Loosli, 1972). The computation of the whole-body fatty acid balance was carried out as initially described by Turchini et al. (2007) and with further developments as described in Turchini et al. (2008) and Turchini and Francis (2009) to assess fish fatty acid metabolism. Briefly, this method consists of four steps whereby the first step is to calculate individual concentrations of fatty acid (mg per fish) in the diets, feces, and the initial and final fish whole-body. Subsequently, the individual fatty acid intake, excretion and accumulation were calculated. An overall fatty acid appearance or disappearance can be computed based on the difference between fatty acid accumulation, intake and excretion. This is followed by step 2, the computation of the SFA and MUFA, n − 3 and n − 6 PUFA balances. The amount of appeared/disappeared fatty acid needs to be converted from milligram (mg) per fish to micromoles (μmol) per fish. In step 3, the amount of each individual FA (μmol g^{-1} of fish/day) that has been used for β-oxidation or elongated and desaturated is quantified. The final step is to quantify the total net disappearance of fatty acids, the total accretion of longer chain fatty acids, the total accretion of Δ-9, Δ-6 and Δ-5 desaturated fatty acids and the net accretion of fatty acids produced *ex novo*. All the apparent *in vivo* enzyme activities were expressed as μmol g^{-1} of fish/day.

All experimental data were subjected to one-way analysis of variance (ANOVA) using SPSS 11.5 (SPSS Inc., Chicago, IL, USA) to determine if significant treatment effect was observed, a Duncan's multiple-range test was used to compare means. Treatment effects were considered at the $P < 0.05$ level of significance.

3. Results

3.1. Experimental diets

The five semi-purified experimental diets were isonitrogenous and isoenergetic, and the proximate composition was similar for all diets (Table 1). The main difference was the fatty acid composition of the diet as it reflected that of the added lipid source (Table 2). The FO diet was characterized by the highest content of LC-PUFA with 1.5% arachidonic acid (ARA), 5% EPA and 20% DHA. The highest amount of MUFA was present in the CO diet, which consisted of 60% 18:1n − 9. The PeO diet was rich in 18:3n − 3 (57%) and represented the highest n − 3 PUFA source while the highest n − 6 PUFA was represented by the SFO diet which was rich in 18:2n − 6 (63%). The RBDPO diet had the highest SFA (43%), comprised mainly of 16:0. All the VO diets contained no LC-PUFA and the ratio of n − 3/n − 6 PUFA ranged from 0.003 (SFO) to 5.35 (FO).

3.2. Growth performance and biometry

Tilapia gained weight by over 1000% after 75 days of feeding (Table 3). Fish fed the FO diet showed the highest final weight while the lowest final weight was reported in fish fed SFO diet, which was significantly lower ($P < 0.05$). However, no significant differences ($P > 0.05$) were observed in specific growth rate among all treatment groups. Fish fed the

Table 2
Fatty acid composition (% of total fatty acids) of experimental diets with different lipid sources.

	Experimental diets[a]				
Fatty acid	FO	PeO	CO	SFO	RBDPO
14:0	3.62	–[b]	–	0.19	1.02
15:0	1.02	–	–	–	–
16:0	22.57	6.56	3.98	5.88	37.51
16:1n − 7	4.58	0.13	0.17	0.12	0.20
16:2n − 4	0.12	–	–	–	–
17:0	1.19	–	–	–	0.07
16:3n − 4	0.64	–	–	–	–
17:1	0.51	–	–	–	–
18:0	6.14	1.46	1.68	3.43	4.04
18:1n − 9	14.10	14.47	59.65	23.33	44.65
18:1n − 7	2.37	1.09	3.56	0.77	0.26
18:2n − 6	3.13	16.28	19.55	63.46	10.65
18:3n − 6	0.14	1.02	0.50	–	–
18:3n − 4	0.23	–	1.29	–	–
18:3n − 3	0.94	57.11	8.59	0.20	0.22
18:4n − 3	0.78	–	–	–	–
20:0	0.40	–	0.42	0.24	0.36
20:1n − 9	0.91	0.92	–	0.18	0.13
20:2n − 6	0.30	–	–	–	–
20:3n − 6	0.11	–	–	–	–
21:0	0.17	–	–	–	–
20:4n − 6	1.49	–	–	–	–
20:3n − 3	0.17	–	–	–	–
20:4n − 3	0.35	–	–	–	–
20:5n − 3	4.99	–	–	–	–
22:0	0.29	–	0.17	0.62	–
22:1n − 9	0.15	–	–	–	–
22:4n − 6	0.22	–	–	–	–
22:5n − 3	0.93	–	–	–	–
24:0	0.24	–	–	0.22	–
22:6n − 3	20.62	–	–	–	–
24:1n − 9	0.46	–	–	–	–
Total SFA	35.65	8.02	6.24	10.57	43.00
Total MUFA	23.08	16.62	63.35	24.40	45.24
Total PUFA	35.17	74.25	29.97	63.67	10.87
Total LC-PUFA	28.89	–	–	–	–
Total n − 3 PUFA	28.79	57.11	8.63	0.20	0.22
Total n − 6 PUFA	5.39	17.14	20.04	63.46	10.65
n − 3: n − 6 PUFA	5.35	3.34	0.43	0.00	0.02

[a] See Table 1 footnote.
[b] Not detected.

RBDPO diet recorded the highest HSI which was significantly higher than fish fed the FO diet, while the lowest HSI was found for fish fed the PeO diet. The best feed conversion ratio (FCR) was observed in FO-fed fish which was significantly different from the FCR recorded in the RBDPO-fed fish. Fish fed the FO diet exhibited the lowest condition factor and significantly lower than that of PeO-fed or RBDPO-fed fish. The GSI of female fish was highest in fish fed the SFO diet and significantly higher compared to those fed the PeO diet. No significant differences were noted in fish survival among the different dietary treatments. Likewise, no significant differences were detected for the IPF and hematocrit values among the dietary treatments (Table 3).

3.3. Nutrient digestibility

ADC of dry matter ($ADC_{dry\ matter}$) ranged from 62.3 to 67.2% with no significant difference observed among the treatments (Table 4). Lipid digestibility was high and did not vary much numerically among treatments. Nevertheless, the FO diet had the lowest lipid digestibility (88.1%) which was significantly lower than the PeO, CO and SFO diets but not significantly different from the RBDPO diet.

3.4. Fatty acid composition of fillet and fish whole-body

The fillet fatty acid composition of fish reflected the fatty acid profile of the diets (Table 5). Fish fed the FO or RBDPO diet recorded significantly higher SFA concentrations with 33.3% and 34.8% of total fatty acids, respectively, in comparison to fish fed the other diets ranging from 22.3 to 25.1%. The highest MUFA content was observed in fish fed the CO diet which was significantly higher than those fed the other four diets. The lowest concentration of PUFA (15.1%) was observed in fish fed the RBDPO diet and increased significantly to 47.8% for fish fed the PeO diet. Despite the four FO-deprived diet containing no PUFA longer than C_{18}, LC-PUFA was detected in the muscle lipids of fish fed the VO diets and ranged from 7.3%–14.3% of total fatty acids. The highest ratio of n − 3 to n − 6 was observed in fish fed the FO diet (4.7), followed by the fish fed the PeO diet (2.7), CO diet (0.47) and relatively lower value was recorded by the fish fed the SFO or RBDPO diet with the values of 0.02 and 0.07, respectively.

Table 4
Apparent nutrient digestibility (%) of diets with different lipid sources[a].

	Experimental diets[b]				
Nutrient	FO	PeO	CO	SFO	RBDPO
Dry matter	67.2 ± 0.2	62.3 ± 1.6	64.3 ± 1.0	65.9 ± 1.2	64.5 ± 2.7
Lipid	88.1 ± 1.7[a]	94.8 ± 0.8[b]	94.2 ± 0.6[b]	94.7 ± 0.6[b]	92.2 ± 1.8[ab]

[a] Values are the mean ± S.E. of triplicate groups of fish. Different superscripts in the same row indicate significant differences at $P < 0.05$.
[b] See Table 1 footnote.

The most abundant fatty acids in the initial and final whole-body fish lipids were 18:1n − 9 and 16:0 (Table 6). Similar to the fatty acid profile in fillet lipids, total SFA was the significantly highest in fish fed the RBDPO diet with the value of 2628.4 mg 100 g^{-1} fish. Fish fed the RBDPO or CO diets had significantly higher total MUFA, while the highest PUFA was shown for fish fed the SFO diet. Irrespective of the dietary lipid source, the n − 3/n − 6 ratios of the fish whole-body lipids were higher than the experimental diets. With the exception of the fish fed the SFO diet, the n − 3/n − 6 ratio increased from 0.19 in the initial fish to a range value of 0.3–20.2 in the final fish whole-body, depending on the dietary n − 3/n − 6 ratios. Total LC-PUFA concentration in the whole-body lipids of fish fed the VO-based diets ranged from 104.2 mg 100 g^{-1} fish in the RBDPO diet to 150.1 mg 100 g^{-1} fish in the CO diet, which were significantly lower than that of fish fed the FO diet, 314.9 mg 100 g^{-1} fish. Fish fed the FO diet had the significantly highest content of EPA + DHA. Among the four VO-based diets, the EPA + DHA content of fish fed the CO or PeO diets were relatively higher than fish fed the SFO or RBDPO diets. With respect to ARA, fish fed the SFO or PeO diet had significantly the highest and lowest amount in fish whole-body lipids, respectively. A significantly higher amount of 20:2n − 6 and 22:2n − 6 was noted in fish fed the SFO diet, while a significantly higher content of 20:3n − 3 was observed in fish fed the PeO diet.

3.5. In vivo fatty acid metabolism

Table 7 shows the total apparent *in vivo* fatty acid neogenesis, β-oxidation, desaturase and elongase activities in red hybrid tilapia. The FA

Table 3
Growth performance, feed utilization efficiency and biological indices of tilapia fed diets with different lipid sources for 75 days[a].

	Experimental diets[b]				
	FO	PeO	CO	SFO	RBDPO
Initial weight (g)	8.93 ± 0.01	8.93 ± 0.03	8.93 ± 0.03	8.94 ± 0.04	8.93 ± 0.02
Final weight (g)	125.21 ± 0.59[b]	111.26 ± 10.16[ab]	107.04 ± 7.10[ab]	100.88 ± 1.75[a]	109.91 ± 7.50[ab]
Percent weight gain[c]	1301.48 ± 7.87[b]	1146.25 ± 118.02[ab]	1098.54 ± 82.20[ab]	1029.21 ± 22.84[a]	1130.37 ± 85.56[b]
SGR (%/day)[d]	3.52 ± 0.01	3.35 ± 0.13	3.31 ± 0.09	3.23 ± 0.03	3.34 ± 0.09
HSI[e]	1.36 ± 0.07[ab]	1.20 ± 0.09[a]	1.54 ± 0.06[bc]	1.41 ± 0.08[ab]	1.70 ± 0.04[c]
VSI[f]	3.24 ± 0.17[a]	3.40 ± 0.22[ab]	3.57 ± 0.06[ab]	3.52 ± 0.11[ab]	3.76 ± 0.13[b]
IPF[g]	0.58 ± 0.04	0.54 ± 0.04	0.68 ± 0.03	0.62 ± 0.11	0.74 ± 0.19
GSI[h] female	2.28 ± 0.39[ab]	1.38 ± 0.49[a]	1.96 ± 0.80[ab]	3.60 ± 0.68[b]	3.00 ± 0.40[ab]
GSI male	0.93 ± 0.15[b]	0.23 ± 0.11[a]	0.30 ± 0.16[a]	0.66 ± 0.06[ab]	0.68 ± 0.18[ab]
FCR[i]	0.90 ± 0.00[a]	0.94 ± 0.05[ab]	0.98 ± 0.03[ab]	0.98 ± 0.00[ab]	1.02 ± 0.03[b]
Condition factor[j]	1.81 ± 0.01[a]	1.88 ± 0.02[bc]	1.83 ± 0.01[ab]	1.84 ± 0.02[ab]	1.91 ± 0.02[c]
Survival (%)[k]	100	97.78 ± 2.22	97.78 ± 2.22	97.78 ± 2.22	100
Hematocrit (%)[l]	31.42 ± 1.37	27.58 ± 1.53	28.08 ± 0.65	30.25 ± 2.92	33.33 ± 1.30

[a] Values are the mean ± S.E. of triplicate groups of fish. Different superscripts in the same row indicate significant differences at $P < 0.05$.
[b] See Table 1 footnote.
[c] Percent weight gain = (final weight − initial weight) / initial weight × 100.
[d] SGR, specific growth rate = [(ln final mean weight − ln initial mean weight) / days of feeding trial] × 100.
[e] HSI, hepatosomatic index = [liver weight (g) / body weight (g)] × 100.
[f] VSI, viserosomatic index = [visceral weight (g) /body weight (g)] × 100.
[g] IPF, intraperitoneal fat index = [intraperitoneal fat weight (g) / body weight (g)] × 100.
[h] GSI, gonadosomatic index = [gonad weight (g) / body weight (g)] × 100.
[i] FCR, feed conversion ratio = total dry feed fed (g) / wet weight gain (g).
[j] Condition factor = [final body weight / (total length)^3] × 100.
[k] Survival = (final fish number/initial fish number) × 100.
[l] Hematocrit = [height of packed red cells (mm) / height of packed red cells and plasma (mm)] × 100.

Table 5
Fillet fatty acid composition (% fatty acid) of tilapia fed diets with different lipid sources over 75 days[a].

	Experimental diets[b]				
Fatty acid	FO	PeO	CO	SFO	RBDPO
14:0	3.0 ± 0.0[c]	1.5 ± 0.1[a]	1.3 ± 0.1[a]	1.4 ± 0.1[a]	2.0 ± 0.0[b]
14:1n − 5	0.2 ± 0.1[b]	–	0.1 ± 0.0[ab]	0.0 ± 0.0[a]	0.1 ± 0.0[ab]
16:0	21.7 ± 0.5[c]	16.5 ± 0.0[b]	4.1 ± 1.1[a]	15.6 ± 0.3[ab]	23.2 ± 0.6[c]
16:1n − 7	5.5 ± 0.2[c]	2.2 ± 0.0[a]	1.9 ± 0.4[a]	1.6 ± 0.2[a]	3.9 ± 0.1[b]
17:0	0.9 ± 0.2[b]	0.1 ± 0.0[a]	0.2 ± 0.1[a]	0.2 ± 0.0[a]	0.2 ± 0.1[a]
16:3n − 4	0.3 ± 0.0	–	–	–	–
17:1	1.0 ± 0.1[b]	0.6 ± 0.0[a]	0.9 ± 0.2[b]	0.6 ± 0.0[ab]	0.6 ± 0.1[a]
18:0	6.7 ± 0.3[b]	5.6 ± 0.1[a]	5.5 ± 0.1[a]	7.1 ± 0.1[b]	5.9 ± 0.2[a]
18:1n − 9	20.0 ± 1.3[a]	18.7 ± 0.5[a]	37.6 ± 0.0[c]	20.0 ± 0.8[a]	32.8 ± 2.2[b]
18:1n − 7	3.5 ± 0.1[b]	2.2 ± 0.0[a]	4.2 ± 0.3[b]	2.2 ± 0.3[a]	3.7 ± 0.5[b]
18:2n − 6	2.2 ± 0.1[a]	10.2 ± 0.3[c]	11.4 ± 0.7[d]	27.0 ± 0.9[e]	6.4 ± 0.2[b]
18:3n − 6	0.1 ± 0.0[a]	0.4 ± 0.1[b]	1.0 ± 0.1[d]	1.4 ± 0.0[c]	0.9 ± 0.0[c]
18:3n − 4	0.1 ± 0.1	–	–	–	–
18:3n − 3	0.4 ± 0.0[a]	21.6 ± 0.5[c]	3.1 ± 0.4[b]	0.1 ± 0.0[a]	0.2 ± 0.1[a]
18:4n − 3	0.3 ± 0.0[a]	0.9 ± 0.1[b]	0.3 ± 0.0[a]	–	–
20:0	0.2 ± 0.0[ab]	0.1 ± 0.0[a]	0.2 ± 0.0[bc]	0.3 ± 0.0[c]	0.2 ± 0.0[ab]
20:1n − 9	1.0 ± 0.0[a]	1.0 ± 0.0[a]	1.8 ± 0.1[b]	1.1 ± 0.0[a]	1.6 ± 0.1[b]
20:2n − 6	0.3 ± 0.0[a]	0.5 ± 0.0[b]	0.5 ± 0.0[b]	2.1 ± 0.0[c]	0.3 ± 0.1[a]
20:3n − 6	0.1 ± 0.0[a]	0.6 ± 0.0[b]	1.2 ± 0.1[c]	1.9 ± 0.2[d]	1.3 ± 0.2[c]
21:0	0.1 ± 0.0[a]	0.2 ± 0.0[b]	–	–	–
20:4n − 6	2.0 ± 0.2[ab]	1.1 ± 0.1[a]	3.0 ± 0.1[bc]	5.4 ± 0.4[d]	4.1 ± 0.7[cd]
20:3n − 3	0.1 ± 0.0[a]	2.6 ± 0.4[b]	0.4 ± 0.0[a]	–	–
20:4n − 3	0.3 ± 0.0[b]	0.6 ± 0.0[c]	0.1 ± 0.0[a]	0.0 ± 0.0[a]	–
20:5n − 3	2.2 ± 0.2[c]	1.1 ± 0.1[b]	0.4 ± 0.1[a]	–	–
22:0	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.6 ± 0.1[a]	0.2 ± 0.1[a]	2.4 ± 0.6[b]
22:1n − 9	0.1 ± 0.0[a]	–	0.2 ± 0.0[b]	–	–
22:4n − 6	0.4 ± 0.0[a]	0.2 ± 0.0[a]	0.8 ± 0.1[b]	2.2 ± 0.2[d]	1.2 ± 0.0[c]
22:5n − 3	2.6 ± 0.2[c]	2.2 ± 0.1[c]	0.9 ± 0.0[b]	0.1 ± 0.1[a]	0.1 ± 0.0[a]
24:0	0.1 ± 0.0[a]	–	0.2 ± 0.1[a]	0.1 ± 0.1[a]	0.7 ± 0.0[b]
22:6n − 3	18.3 ± 1.5[c]	5.9 ± 0.5[b]	3.1 ± 0.3[a]	0.6 ± 0.3[a]	0.6 ± 0.2[a]
24:1n − 9	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
Total SFA	33.3 ± 1.0[c]	24.3 ± 0.1[ab]	22.3 ± 1.3[a]	25.1 ± 0.2[b]	34.8 ± 0.0[c]
Total MUFA	31.3 ± 1.1[b]	24.7 ± 0.5[a]	46.8 ± 0.5[d]	25.6 ± 0.2[a]	42.8 ± 1.8[c]
Total PUFA	29.8 ± 2.2[b]	47.8 ± 0.1[d]	26.4 ± 1.2[b]	40.8 ± 0.3[c]	15.1 ± 1.1[a]
Total LC-PUFA	26.1 ± 2.1[c]	14.3 ± 0.2[b]	10.0 ± 0.1[ab]	10.2 ± 1.1[ab]	7.3 ± 1.1[a]
Total n − 3 PUFA	24.3 ± 1.9[c]	34.9 ± 0.4[d]	8.4 ± 0.6[b]	0.8 ± 0.4[a]	1.0 ± 0.1[a]
Total n − 6 PUFA	5.2 ± 0.3[a]	13.0 ± 0.5[b]	17.9 ± 0.6[c]	40.0 ± 0.1[d]	14.1 ± 1.0[b]
n − 3: n − 6 PUFA	4.7 ± 0.1[d]	2.7 ± 0.1[c]	0.5 ± 0.0[b]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
EPA + DHA	20.5 ± 1.7[c]	7.0 ± 0.6[b]	3.5 ± 0.3[b]	0.6 ± 0.3[a]	0.6 ± 0.2[a]

[a] Values are the mean ± S.E. of triplicate groups of fish. Different superscripts in the same row indicate significant differences at $P < 0.05$.
[b] See Table 1 footnote.

neogenesis of fish was significantly different among the dietary treatments with the highest value observed in fish fed the PeO diet and the lowest value shown in fish fed the RBDPO diet. Elongation of SFA and MUFA was significantly higher in fish fed the PeO diet (1.915 μmol g^{-1} day^{-1}) compared to fish fed other diets. The highest oxidation of n − 3 PUFA (2.779 μmol g^{-1} day^{-1}) was observed in fish fed the PeO diet; while the elongation, Δ-5 and Δ-6 desaturation on n − 3 PUFA were significantly greatest in fish fed the CO diet. Fish fed the SFO diet had significantly higher elongation, oxidation and Δ-5 desaturation on n − 6 PUFA than the other fish. No Δ-5, Δ-6 desaturase and elongase activity were detected on either n − 3 or n − 6 PUFA for fish fed the FO diet. With respect to total oxidation, fish fed the SFO or PeO diets showed significantly higher activity with 3.698 μmol g^{-1} day^{-1} and 3.452 μmol g^{-1} day^{-1}, respectively, while the lowest activity was recorded in fish fed the RBDPO diet (0.749 μmol g^{-1} day^{-1}). Significantly higher total elongation activity was observed in the fish fed the PeO diet (2.053 μmol g^{-1} day^{-1}) compared to those fed the other diets, with the activities ranging from 0.439 to 1.180 μmol g^{-1} day^{-1}. However, fish fed the PeO diet had significantly lower total Δ-5 and Δ-6 desaturation than the other four VO-based diets, while fish fed the CO or SFO diets showed significantly lower total Δ-9 desaturase activities among the dietary treatments.

The apparent *in vivo* elongase activity on SFA and MUFA is shown in Fig. 1. Among all the fatty acids, 14:0 showed the highest elongase activity, which ranged from 0.542 μmol g^{-1} day^{-1} to 1.259 μmol g^{-1} day^{-1}, while no elongation on 14:0 was detected in fish fed the RBDPO diet, and fish fed the PeO diet showed the highest elongase activity on 14:0. Likewise, the highest elongation of 16:0 was observed in fish fed the PeO diet, followed by fish fed the FO diet and were significantly higher than the other three treatment groups. Fish fed the RBDPO diet had significantly higher elongation of 16:1n − 7, while significantly higher elongase activity on 18:1n − 9 was detected in fish fed the CO or RBDPO diets.

Total apparent *in vivo* desaturase activity is shown in Fig. 2. Fish showed relatively lower 14:0 desaturation to 14:1n − 5, while no Δ-9 desaturation of 14:0 was observed in fish fed the SFO diet. Fish fed the RBDPO had significantly higher Δ-9 desaturase activity of 16:0 and was significantly higher than fish fed the other diets. The Δ-9 desaturation of 18:0 was only observed in fish fed the FO or PeO diets. For 18:2n − 6, no Δ-6 desaturation was recorded in fish fed the FO or PeO diet, while fish fed the SFO diet showed the highest activity. The significantly highest Δ-6 desaturase activity of 18:3n − 3 was observed in fish fed the CO diet, while fish fed the SFO or RBDPO diet had the significantly lower activity compared to fish fed the PeO diet. A similar trend was also evident in the Δ-6 desaturation of 24:5n − 3 and Δ-5 desaturation of 20:4n − 3. With respect to the Δ-5 desaturation on 20:3 − 6, fish fed the SFO diet had the highest activity, followed by fish fed the RBDPO, CO and PeO diets.

Fig. 3 shows the apparent *in vivo* fatty acid β-oxidation. With regard to 18:1n − 9, no oxidation was observed in fish fed the FO or PeO diets, while fish fed the CO diet had significantly higher β-oxidation than fish fed the SFO or RBDPO diet. The highest β-oxidation of 18:3n − 3 and 18:2n − 6 was observed in fish fed the PeO and SFO diets, respectively. The β-oxidation on EPA and DHA was observed only in fish fed the FO diet.

Along the biosynthetic pathway of n − 3 PUFA, fish fed the FO diet showed no activity for all the involved enzymes (Fig. 4). Among the VO treatments, significant differences were detected on Δ-6 desaturation of 18:3n − 3 where the highest activities were found in fish fed the CO diet, followed by fish fed the PeO diet; while lower activities were detected in fish fed the RBDPO or SFO diets. For all the subsequent elongation, Δ-5 and Δ-6 desaturation, a similar trend among the dietary treatments was observed. Fish fed the SFO diet showed the highest enzyme activities along the biosynthetic pathway from 18:2n − 6 to 22:4n − 6 (Fig. 5), followed by the fish fed the RBDPO and CO diets. Fish fed the PeO diet showed minimal activities on the elongation of 18:3n − 6 and Δ-5 desaturation of 20:3n − 6. No Δ-6 desaturation on 18:3n − 3 and elongation on 20:4n − 6 was observed in fish fed the PeO or FO diet. On the dead-end pathway of n − 6 PUFA, fish fed the SFO-based diet has the highest elongation of 18:3n − 6 and was significantly higher than those fed the other VO diets, while no activity was shown by the fish fed the FO diet. The elongation on 20:2n − 6 was minimal for all the dietary treatments. Similar to the n − 3 PUFA pathway, the enzyme activities was decreasing gradually. Fig. 6 shows the apparent *in vivo* Δ-6 desaturation, elongation and β-oxidation on 18:2n − 6 and 18:3n − 3 as percentage of net intake. Regardless of the dietary treatment, the β-oxidation on 18:2n − 6 and

Table 6
Fatty acid content (mg/100 g fish) of initial and final fish whole-body[a].

Fatty acid	Initial	Experimental diet[b]				
		FO	PeO	CO	SFO	RBDPO
14:0	68.9	267.5 ± 23.0[b]	117.0 ± 4.6[a]	125.2 ± 36.7[a]	117.9 ± 11.4[a]	179.6 ± 2.4[a]
14:1n−5	2.6	9.1 ± 1.4[b]	4.8 ± 0.2[a]	6.4 ± 1.7[ab]	–	8.1 ± 0.1[ab]
15:0	13.2	47.2 ± 1.7[b]	7.1 ± 0.2[a]	6.2 ± 0.9[a]	6.9 ± 0.2[a]	8.7 ± 0.2[a]
16:0	660.8	1486.9 ± 98.9[b]	960.0 ± 36.9[a]	825.9 ± 35.5[a]	930.8 ± 49.4[a]	2090.4 ± 43.7[c]
16:1n−7	113.4	404.9 ± 40.0[c]	147.5 ± 5.7[a]	146.8 ± 8.5[a]	115.5 ± 6.1[a]	280.1 ± 4.4[b]
16:2n−4	3.6	5.1 ± 1.4	–	–	–	–
17:0	14.6	47.1 ± 1.8[b]	7.8 ± 0.3[a]	7.7 ± 0.1[a]	7.9 ± 0.5[a]	9.6 ± 0.4[a]
16:3n−4	1.1	14.2 ± 0.1[b]	4.0 ± 0.1[a]	–	–	–
17:1	8.4	37.9 ± 1.9[b]	7.4 ± 0.3[a]	10.7 ± 1.1[a]	7.6 ± 1.1[a]	10.3 ± 0.4[a]
18:0	157.5	340.9 ± 16.7[c]	260.3 ± 9.8[b]	196.2 ± 1.7[a]	282.0 ± 15.2[b]	295.0 ± 7.2[b]
18:1n−9	828.0	1378.6 ± 22.2[a]	1445.8 ± 55.7[ab]	2920.9 ± 42.5[c]	1580.3 ± 26.8[b]	3003.3 ± 67.7[c]
18:1n−7	91.9	218.3 ± 27.6[b]	128.7 ± 5.1[a]	234.8 ± 17.8[b]	101.4 ± 1.4[a]	183.5 ± 4.7[b]
18:2n−6	369.0	76.5 ± 7.1[a]	348.4 ± 13.9[b]	671.6 ± 4.7[c]	1553.1 ± 142.1[d]	374.1 ± 11.3[b]
18:3n−6	11.8	3.6 ± 0.4[a]	7.1 ± 0.3[a]	51.3 ± 1.5[b]	71.9 ± 15.5[b]	46.6 ± 1.5[b]
18:3n−4	3.1	10.4 ± 0.6[c]	4.3 ± 0.1[a]	–	–	4.4 ± 0.3[a]
18:3n−3	23.1	14.0 ± 0.7[a]	501.6 ± 20.3[c]	219.0 ± 2.5[b]	6.9 ± 0.0[a]	10.7 ± 0.0[a]
18:4n−3	2.7	5.8 ± 1.1[a]	10.0 ± 0.4[b]	15.9 ± 1.0[c]	–	–
20:0	12.4	14.3 ± 0.6[b]	8.0 ± 0.3[a]	18.2 ± 0.2[c]	12.8 ± 1.6[b]	12.4 ± 0.4[b]
20:1n−9	34.5	67.3 ± 1.5[b]	55.6 ± 2.0[a]	125.1 ± 0.1[c]	61.5 ± 3.3[ab]	142.9 ± 3.1[d]
20:2n−6	19.0	6.6 ± 0.4[a]	16.7 ± 0.7[b]	23.5 ± 1.3[b]	83.8 ± 4.6[c]	16.6 ± 0.8[b]
20:3n−6	14.1	3.2 ± 0.5[a]	6.6 ± 0.3[a]	25.5 ± 0.8[b]	48.3 ± 9.1[c]	27.2 ± 0.8[b]
21:0	2.2	6.0 ± 0.2	–	–	–	–
20:4n−6	18.3	21.0 ± 2.9[ab]	6.6 ± 0.2[a]	30.0 ± 1.0[bc]	52.2 ± 11.4[d]	47.1 ± 1.5[cd]
20:3n−3	5.1	3.1 ± 0.0[a]	63.3 ± 2.5[c]	20.1 ± 0.7[b]	–	–
20:4n−3	1.9	5.0 ± 0.5[a]	6.6 ± 0.3[b]	4.8 ± 0.3[a]	–	–
20:5n−3	5.4	19.8 ± 3.4[b]	–	3.2 ± 3.2[a]	–	–
22:0	6.5	8.6 ± 0.5[b]	3.3 ± 0.1[a]	8.0 ± 1.0[b]	13.8 ± 0.7[c]	3.8 ± 0.0[a]
22:1n−9	2.0	5.9 ± 0.1[a]	5.4 ± 0.2[a]	9.4 ± 0.7[b]	–	9.1 ± 0.2[b]
22:2n−6	1.1	–	–	–	1.8 ± 1.8	–
22:4n−6	10.4	7.8 ± 1.5[a]	–	10.1 ± 1.5[a]	34.7 ± 8.1[b]	21.2 ± 0.8[ab]
22:5n−3	14.7	45.9 ± 8.1[b]	13.0 ± 0.5[a]	17.6 ± 0.5[a]	–	–
24:0	5.4	4.0 ± 0.9[a]	–	3.7 ± 3.7[a]	3.0 ± 3.0[a]	28.9 ± 1.1[b]
22:6n−3	32.1	209.1 ± 49.9[b]	21.0 ± 1.0[a]	38.8 ± 2.2[a]	8.0 ± 2.4[a]	8.7 ± 0.6[a]
24:1n−9	7.8	9.9 ± 0.6[b]	–	6.8 ± 1.3[ab]	8.1 ± 1.6[b]	4.1 ± 0.3[a]
Total SFA	941.5	2222.4 ± 144.0[b]	1363.5 ± 52.3[a]	1191.0 ± 77.7[a]	1375.1 ± 76.0[a]	2628.4 ± 55.4[c]
Total MUFA	1086.0	2122.8 ± 93.9[b]	1790.5 ± 68.9[a]	3454.5 ± 67.7[c]	1874.4 ± 28.7[ab]	3633.2 ± 81.0[c]
Total PUFA	536.4	450.9 ± 75.0[a]	1009.2 ± 40.7[b]	1131.4 ± 10.5[b]	1860.7 ± 195.0[c]	556.5 ± 17.6[a]
Total LC-PUFA	101.9	314.9 ± 66.8[b]	117.0 ± 4.8[a]	150.1 ± 4.5[a]	143.2 ± 31.0[a]	104.2 ± 3.7[a]
Total n−3 PUFA	84.9	302.7 ± 63.7[b]	615.4 ± 25.0[c]	319.4 ± 0.3[b]	14.9 ± 2.4[a]	19.4 ± 0.6[a]
Total n−6 PUFA	443.7	118.6 ± 12.0[a]	385.4 ± 15.4[ab]	812.0 ± 10.8[c]	1845.9 ± 192.7[d]	532.7 ± 16.7[bc]
n−3: n−6 PUFA	0.2	20.2 ± 3.6[c]	12.1 ± 0.7[b]	2.8 ± 0.1[a]	0.1 ± 0.0[a]	0.3 ± 0.0[a]
EPA + DHA	37.5	228.9 ± 53.3[b]	21.0 ± 1.0[a]	42.0 ± 1.0[a]	8.0 ± 2.4[a]	8.7 ± 0.6[a]

[a] Values are the mean ± S.E. of triplicate groups of fish. Different superscripts in the same row indicate significant differences at $P < 0.05$.
[b] See Table 1 footnote.

18:3n−3 was high, ranging from 38.4 to 77.4% and from 27.5 to 83.8%, respectively. Fish fed the FO and PeO diets had the highest oxidation of 18:3n−3 and 18:2n−6, respectively; while fish fed the RBDPO diet had the lowest β-oxidation for both 18:3n−3 and 18:2n−6. With respect to 18:2n−6, fish fed the PeO or SFO diet showed β-oxidation of 68% of the net intake, which was significantly lower than fish fed the FO diet but significantly higher than the other two VO-based diets, while the fish fed the PeO diet had high β-oxidation on 18:3n−3, but was not significantly different from that of control treatment. Higher Δ-6 desaturase activities on these two fatty acids were observed in fish fed the RBDPO diet with a significant difference detected on 18:2n−6.

4. Discussion

4.1. Growth performance and biometry

It has been reported that total FO substitution by VO has no negative impact on growth or feed efficiency of red hybrid tilapia (Ng et al., 2001, 2013; Bahurmiz and Ng, 2007) as well as many other fish species (Turchini et al., 2009, 2011). However, it should be noted that in many of these feeding trials, experimental fish were fed practical diets which usually contains fish meal with its accompanying residual FO. In the present study, despite the small impact of dietary lipid source on the growth, feed utilization efficiency and biological indices of tilapia, the final body weight of fish fed the SFO diet was significanty lower compared to fish fed the FO diet. Tilapia fed the other VO-based diets also showed slightly lower growth response compared to the control fish fed the FO diet even though the differences were statistically not significant. Furthermore, fish fed the RBDPO diet had signficantly poorer FCR and higher HSI compared to fish fed the FO diet even though the quantitative differences were small. The use of semipurified diets with added VO as the sole source of dietary lipids and the two week conditioning of experimental fish with a lipid-free diet probably increased the potential for essential fatty acid deficiency in tilapia. Chou and Shiau (1999) reported that both n−3 and n−6 PUFA are important for maximal growth of hybrid tilapia. Nevertheless, the growth performance differences observed were small and the uniqueness of using these semipurified diets was that it allowed us to accurately determine the fatty acid metabolism in tilapia fed different lipid sources without the confounding effects of residual FO which contains LC-PUFA. This was the novelty of the present study.

Bell et al. (1991) suggested that feeding salmonid fish with a high level of n−6 PUFA-rich VO could be deleterious to their health. Similarly, tilapia fed the SFO diet which contains the highest levels of n−6 PUFA showed the lowest growth in the present study. Tilapia fed the

Table 7
Total apparent *in vivo* fatty acid neogenesis, β-oxidation, desaturation and elongation (μmol g^{-1} day^{-1}) in tilapia fed diets with different lipid sources for 75 days[a].

	Experimental diets[b]				
	FO	PeO	CO	SFO	RBDPO
SFA and MUFA					
FA neogenesis	0.711 ± 0.351[ab]	1.395 ± 0.121[c]	0.894 ± 0.061[bc]	0.650 ± 0.099[ab]	0.117 ± 0.005[a]
Total elongation	1.120 ± 0.430[a]	1.915 ± 0.194[b]	0.958 ± 0.012[a]	0.634 ± 0.099[a]	0.327 ± 0.012[a]
Total oxidation	0.033 ± 0.006[a]	0.002 ± 0.000[a]	1.210 ± 0.073[c]	0.410 ± 0.036[ab]	0.475 ± 0.237[b]
Total Δ-9 desaturase	0.536 ± 0.133[b]	0.612 ± 0.073[b]	0.143 ± 0.008[a]	0.126 ± 0.004[a]	0.402 ± 0.001[b]
n − 6 PUFA					
Total elongation	–	0.021 ± 0.001[a]	0.081 ± 0.003[b]	0.197 ± 0.034[c]	0.101 ± 0.002[b]
Total oxidation	0.261 ± 0.014[a]	0.671 ± 0.036[b]	0.643 ± 0.032[b]	3.280 ± 0.179[c]	0.269 ± 0.020[a]
Total Δ-6 desaturase	–	–	0.069 ± 0.001[a]	0.167 ± 0.036[b]	0.114 ± 0.002[ab]
Total Δ-5 desaturase	–	0.004 ± 0.000[a]	0.033 ± 0.001[b]	0.066 ± 0.015[c]	0.052 ± 0.001[ab]
n − 3 PUFA					
Total elongation	–	0.117 ± 0.005[b]	0.141 ± 0.006[c]	0.009 ± 0.005[a]	0.011 ± 0.001[a]
Total oxidation	1.323 ± 0.053[c]	2.779 ± 0.098[d]	0.303 ± 0.017[b]	0.008 ± 0.002[a]	0.004 ± 0.000[a]
Total Δ-6 desaturase	–	0.051 ± 0.002[b]	0.095 ± 0.002[c]	0.006 ± 0.004[a]	0.008 ± 0.001[a]
TotaL Δ-5 desaturase	–	0.023 ± 0.001[b]	0.046 ± 0.000[c]	0.003 ± 0.002[a]	0.003 ± 0.000[a]
Total					
Neogenesis	0.711 ± 0.351[ab]	1.391 ± 0.120[c]	0.894 ± 0.061[bc]	0.650 ± 0.099[ab]	0.117 ± 0.005[a]
Oxidation	1.617 ± 0.072[b]	3.452 ± 0.135[c]	2.155 ± 0.122[b]	3.698 ± 0.145[c]	0.749 ± 0.257[a]
Elongation	1.120 ± 0.430[a]	2.053 ± 0.199[b]	1.180 ± 0.010[a]	0.841 ± 0.059[a]	0.439 ± 0.009[a]
Δ-9 Desaturase	0.536 ± 0.133[b]	0.612 ± 0.073[b]	0.143 ± 0.008[a]	0.126 ± 0.004[a]	0.402 ± 0.001[b]
Δ-6 Desaturase	–	0.051 ± 0.002[a]	0.164 ± 0.000[b]	0.173 ± 0.040[b]	0.122 ± 0.002[b]
Δ-5 Desaturase	–	0.027 ± 0.001[a]	0.079 ± 0.001[b]	0.069 ± 0.017[b]	0.056 ± 0.001[b]

[a] Values are the mean ± S.E. of triplicate groups of fish. Different superscripts in the same row indicate significant differences at $P < 0.05$.
[b] See Table 1 footnote.

RBDPO diet led to a significantly higher HSI compared to control fish. The slightly larger livers might indicate an abnormal deposition of lipid which might be due to the beginnings of dietary EFA deficiencies (Sargent et al., 1989).

4.2. Nutrient digestibility

In the present study, the RBDPO diet contained relatively higher amounts of SFA, but mainly medium chain SFA while the FO diet had more long chain SFA which is less digestible compared to medium chain SFA. Therefore, fish fed the FO diet showed lower overall lipid digestibility but not significantly different from fish fed the RBDPO diet. Meanwhile, fish fed the PeO, CO or SFO diet showed higher overall lipid digestibility as the dietary SFA content is much lower than the FO diet. Furthermore, it has been reported that the apparent digestibility of fatty acids, particularly SFA, is directly related to the ratio of dietary PUFA/SFA and the reduced lipid emulsion induced by the long chain SFA can then decrease the efficiency of lipolysis (Caballero et al., 2002).

4.3. Fatty acid composition of fillet and fish whole-body

In terms of the relative amount in fish whole-body lipids, MUFA levels in fish fed the RBDPO diet was comparable to that of fish fed the CO diet. The results from the present study also indicated that the resultant fatty acid composition of fillet and fish whole-body lipids was not affected to the same magnitude as the changes varied depending on the tissue type (Bell et al., 2001; Turchini et al., 2009). Furthermore, metabolic factors such as preferential incorporation, fatty acid metabolism and β-oxidation regulate the incorporation fatty acids into fish tissue (Sargent et al., 2002; Turchini et al., 2009).

In the present study, tilapia fed various VO diets still showed an accumulation of n − 3 and/or n − 6 LC-PUFA despite the VO diets lacking

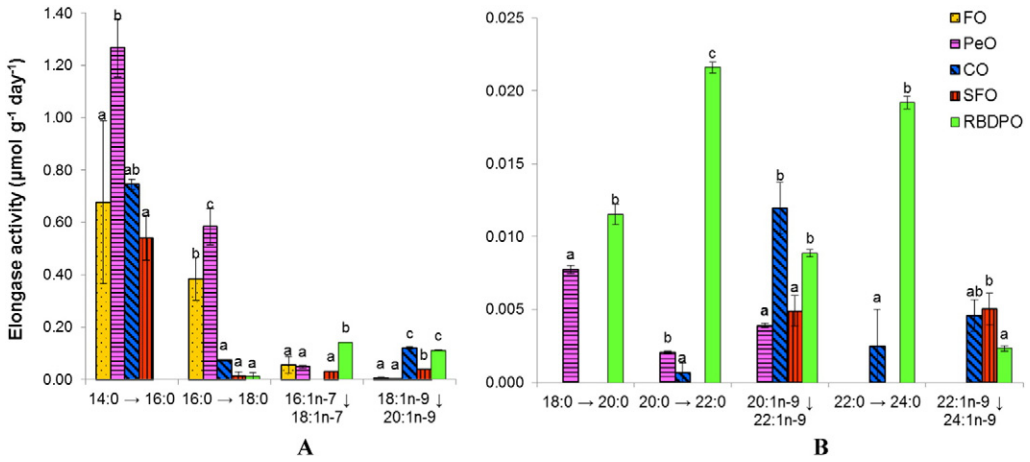

Fig. 1. The apparent *in vivo* elongase activity on SFA and MUFA in tilapia fed diets with different lipid sources over 75 days. Data were expressed as mean ± S.E. of triplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). The data are reported in A and B with different y-axis scales for added clarity.

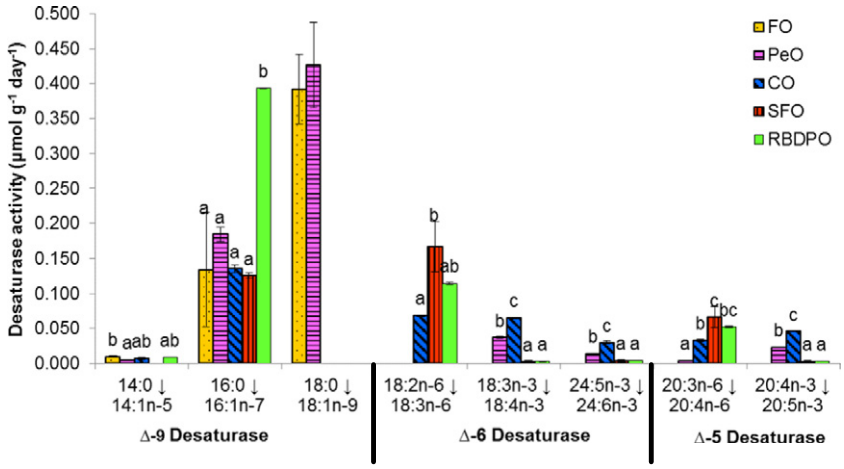

Fig. 2. The apparent *in vivo* desaturase activity in tilapia fed diets with different lipid sources over 75 days. Data were expressed as mean ± S.E. of triplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$).

in PUFA longer than C_{18}. Among the four VO treatments, the fillet LC-PUFA content was higher (not significantly) in fish fed the PeO diet, although for the whole-body, dietary CO resulted in a slightly higher deposition of LC-PUFA, which was 50% higher than that of the initial fish. With the exception of fish fed the PeO diet, the ARA content increased for fish fed the VO diets, especially a 1.5-fold increase was shown in fish fed the SFO diet. High dietary availability of 18:2n − 6 and 18:3n − 3 led to a significant deposition of their $C_{20/22}$ derivatives in fish lipids. The EPA + DHA content in fish fed the CO was superior compared to those fed the other four VO diets but the accumulation of the endogenous EPA + DHA was not enough to compensate for the reduced dietary LC-PUFA levels compared to fish fed the FO diet as similarly reported in other studies (Francis et al., 2007; Miller et al., 2008).

4.4. In vivo fatty acid metabolism

The results of the present study showed that tilapia has an endogenous capacity to biosynthesisize LC-PUFA from C_{18} PUFA precursors *via* fatty acid desaturase and elongase enzyme as fish fed the VO diets showed an active fatty acid neogenesis, β-oxidation, desaturation and elongation. In contrast, dietary FO did not lead to any detectable Δ-5 and Δ-6 desaturase activity, or elongation of n − 3 and n − 6 PUFA. FO replacement with different VO and/or VO blends has been reported to increase the Δ-5 and Δ-6 desaturase activity as well as elongase activity in a variety of fish species. In Atlantic salmon, the hepatic desaturation and elongation activity increased with the inclusion of dietary VO or VO blends (Bell et al., 2001). Tocher et al. (2003a) reported that LC-PUFA biosynthesis in salmon livers significantly increased with the inclusion of dietary linseed oil and rapeseed oil. Salmon fed diets with 100% crude palm oil had a 10-fold greater hepatic desaturation and elongation compared to those fed diets containing 100% FO (Bell et al., 2002). Likewise, a study on gilthead seabream showed that Δ-5, Δ-6 desaturase and elongation increased in the larvae fed rapeseed oil or soybean oil-based diets (Izquierdo et al., 2008).

In the present study, different dietary VO manifested distinct effects on the *in vivo* fatty acid metabolism. With respect to n − 3 PUFA, fish fed the CO or PeO diets had higher enzymatic activities than those fed the RBDPO or SFO diets. Generally, for freshwater fish, dietary VO rich in 18:3n − 3 leads to increased Δ-6 desaturase activity (Tocher et al., 2002) and a greater desaturation of [1-^{14}C]18:3n − 3 than fish fed FO diets (Tocher et al., 2001). However, in the present study, fish fed the PeO diet showed no Δ-6 desaturation on 18:2n − 6, and the Δ-6 desaturation of 18:3n − 3 was lower than that of 18:2n − 6 in fish fed the SFO diet (Fig. 2). In the LC-PUFA biosynthetic pathway, Δ-6 desaturase bioconverts 18:3n − 3 to 18:4n − 3, 18:2n − 6 to 18:3n − 6 and then 24:5n − 3 to 24:6n − 3. Nevertheless, it has been reported that excessive dietary 18:2n − 6 in VO will compete with 18:3n − 3 for the Δ-6 desaturase enzyme thereby inhibiting the bioconversion which will subsequently result in minimal activities along the n − 3 PUFA biosynthetic pathway (Miller et al., 2007). Furthermore, the

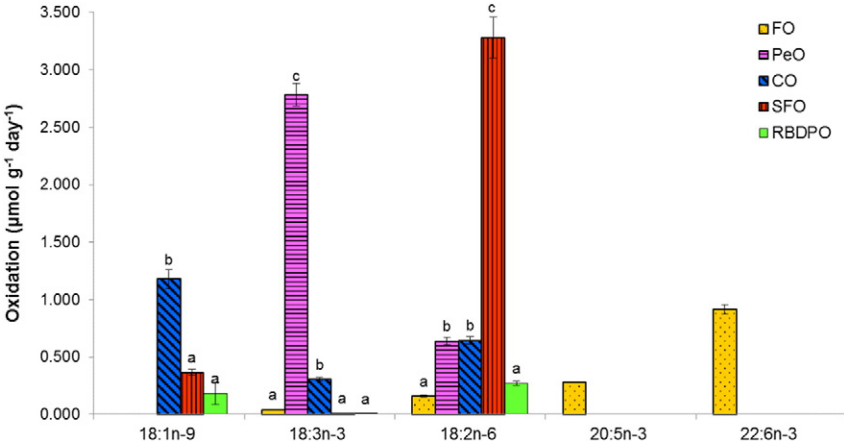

Fig. 3. The apparent *in vivo* fatty acid β-oxidation in tilapia fed diets with different lipid sources over 75 days. Data were expressed as mean ± S.E. of triplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). Only fatty acids that were oxidized >100 μmol g^{-1} day^{-1} are shown.

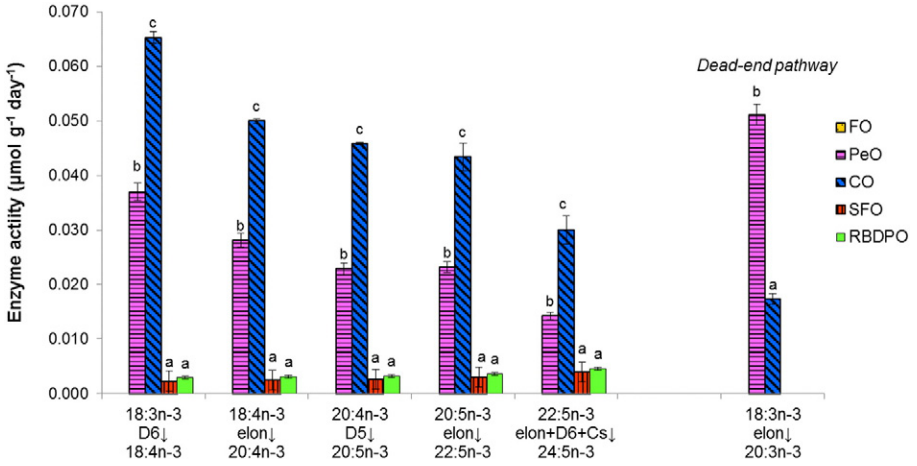

Fig. 4. The apparent *in vivo* elongase and desaturase activity of 18:3n − 3 pathway in tilapia fed diets with different lipid sources for 75 days. Data were expressed as mean ± S.E. of triplicates groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). D6: Δ-6 desaturase; D5: Δ-5 desaturase; elon: Elongase; Cs: chain shortening.

opposite is true where excessive dietary 18:3n − 3 inhibits the bioconversion of 18:2n − 6 to ARA (Miller et al., 2007). This likely explains the relatively lower amounts of ARA for tilapia fed the 18:3n − 3 rich PeO diet in the current study. Similary, a study on rats observed that the dietary garden cress seed oil (rich in 18:3n − 3) significantly reduced the level of ARA in both the serum and liver (Diwakar et al., 2008).

On the other hand, dietary CO increased the Δ-6 desaturation of 18:3n − 3 to the greatest extent despite the dietary content of 18:3n − 3 being substantially lower than dietary PeO (Fig. 2). This indicates that the 18:3n − 3 content in the PeO diet exceeded the maximal substrate level and adversely affect the Δ-6 desaturation on the C_{18} precusors in tilapia. Tocher et al. (2002) reported that feeding seabass with linseed oil (rich in 18:3n − 3) resulted in decreased hepatocyte desaturation. It was also shown that the expression of Δ-6 desaturase gene was completely inhibited by the high content of linseed oil. In constrast, a 6-fold increased of Δ-6 desaturase expression was shown in gilthead seabream fed with diets containing soybean oil and rapeseed oil (Izquierdo et al., 2008). Moreover, dietary rapeseed oil with a 18:3n − 3/18:2n − 6 ratio of 0.5 led to higher desaturase activities in Atlantic salmon than linseed oil (18:3n − 3/18:2n − 6 ratio of 2) (Tocher et al., 2000). In light of the above, it appears possible that the more optimal 18:3n − 3/18:2n − 6 ratio of the CO diet was the reason for the increased Δ-6 desaturase activity in the present study. Further research are warranted to confirm this as it can have dietary formulation implications to potentially maximize LC-PUFA synthesis in farmed tilapia to make a heathier seafood product for human consumers.

In fish fed the FO diet, no Δ-5, 6 desaturation was shown as dietary 18:2n − 6 and 18:3n − 3 were not being desaturated and elongated (Figs. 4 & 5). Similary, a study on Atlantic salmon showed that the three-fold higher levels of EPA and DHA contained in FO diet than VO diets was a potent feedback inhibition on Δ-6 and Δ-5 desaturase enzymes (Tocher et al., 2000). Fish fed the VO diets had increased fatty acid enzyme activities, although the EPA + DHA content was relatively lower compared to those fed the FO diet. This is in agreement with a previous study that showed that increased hepatocyte LC-PUFA biosynthesis did not improve the n − 3 LC-PUFA content in fish tissue to the level as those fed the FO diet (Tocher et al., 2002, 2006). With respect to n − 6 PUFA, fish fed SFO diet had the highest enzyme activities along the n − 6 PUFA biosynthetic pathway of elongase activities on 18:2n − 6 and 20:2n − 6 which likely explains the higher content of 20:2n − 6 and 22:2n − 6 in the fish fed this diet (Fig. 5).

It has been reported that increasing levels of dietary SFA increases stearoyl-CoA desaturase activity in fish liver (Polley et al., 2003). Thus, Δ-9 desaturation of 16:0 was higher in fish fed the RBDPO diet due to the high abundance of this fatty acid in the diet (Fig. 2). The products of Δ-9 desaturation, 16:1n − 7 and 18:1n − 9 were reported to be fatty acids that were being readily oxidized by trout mitochondria (Tocher

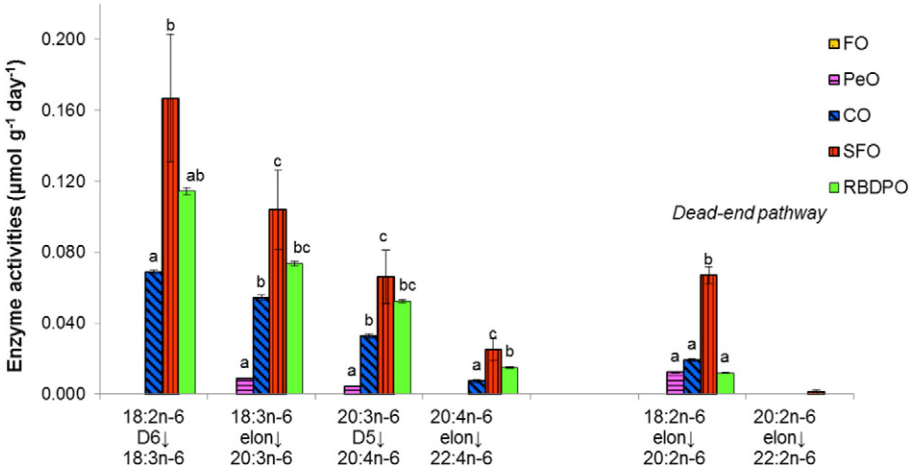

Fig. 5. The apparent *in vivo* elongase and desaturase activity of 18:2n − 6 pathway in tilapia fed diets with different lipid sources for 75 days. Data were expressed as mean ± S.E. of triplicates groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$). D6: Δ-6 desaturase; D5: Δ-5 desaturase; elon: Elongase.

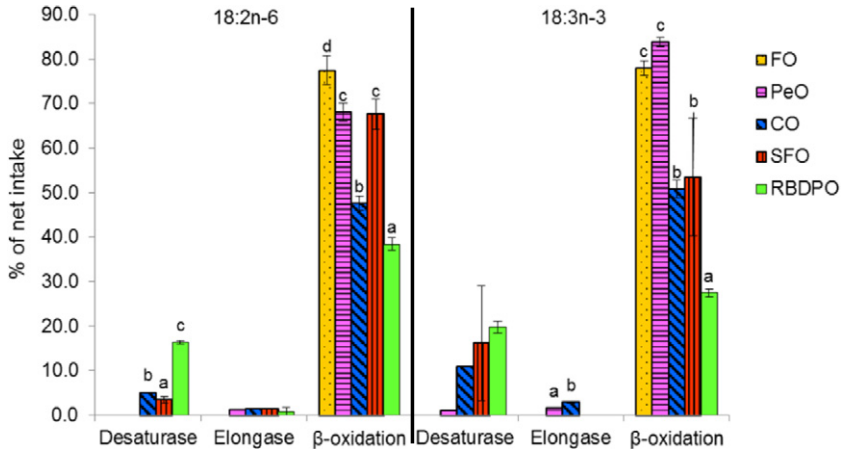

Fig. 6. Apparent *in vivo* desaturation, elongation and β-oxidation on 18:2n − 6 and 18:3n − 3 expressed as % of net intake in tilapia fed diets with different lipid sources for 75 days. Data were expressed as mean ± S.E. of triplicate groups of fish. Among each fatty acid group, different letters indicate statistically significant difference ($P < 0.05$).

et al., 1996). Despite of this, the availability of these two fatty acids were still high in fish fed the dietary RBDPO. This is in agreement with a study on trout whereby the biosynthesis surpassed catabolism (Tocher et al., 1996). Meanwhile, Δ-9 desaturation that acted on 18:0 was observed exclusively in fish fed the PeO or FO diets. This might be, in part, ascribed to the relatively lower or absence of the total apparent Δ-5 and Δ-6 desaturase activity in fish fed these two diets. Basically, dietary fatty acids could hinder fatty acid synthetase and stearoyl-CoA desaturase to a certain degree, and in particular, 18:2n − 6 appears to be a predominant inhibitor (Jeffcoat and James, 1984). This could explain the cause for the lower total Δ-9 desaturase activity for fish fed the CO or SFO diets, since these contained 19.6% and 63.5% 18:2n − 6 (of total fatty acids), respectively. Moreover, dietary 1% conjugated 18:2n − 6 inhibited Δ-9 desaturase activity and thus led to increased SFA but slightly decreased MUFA in the muscle of European seabass (Makol et al., 2012). In general, dietary SFA has an inhibitory effect on fatty acid synthesis, whereas dietary PUFA plays the determining role in the enzyme activities (Jeffcoat and James, 1984). As such, neogenesis was stimulated in fish fed the PeO and CO diets to compensate dietary 14:0 deficiencies, while the SFA content in the RBDPO diet was sufficient to satisfy the needs of the fish, thereby diminishing neogenesis.

The apparent *in vivo* elongase activity acting on SFA and MUFA was predominant among all the total fatty acids being elongated. Despite elongase activity being the highest on 14:0 in the present study, the excessive availability of dietary 16:0 in the RBDPO diet exerted a feedback inhibition on the elongase activity and thus no elongation of 14:0 occured for fish fed this diet. Subsequently, the elongase enzyme shifted towards 16:1n − 7, thereby leading to significantly higher 18:1n − 7 in fish fed the RBDPO diet compared to those fed the PeO or SFO diet, despite the dietary 18:1n − 7 content in the RBDPO diet being the lowest among all the treatment groups. Meanwhile, the higher content of 18:1n − 9 provided in diets had increased the elongation of 18:1n − 9 in fish and resulted in a discernible higher amount of 20:1n − 9 in fish fed the dietary CO and RBDPO (Fig. 1). The results of present study showed that elongase activity of n − 3 and n − 6 PUFA was related to their dietary availability and type. Elongase activity was favourably higher in the n − 3 PUFA pathway for fish fed the CO and PeO diets, while for the n − 6 PUFA pathway, fish fed the SFO and RBDPO diets showed relatively higher elongase activity than other three dietary treatments (Figs. 4 & 5). Collectively, the present study provided further confirmation that the increased levels of enzyme precursors coupled with the lower availability of enzyme products dictate the fatty acid desaturation (Tocher et al., 2003b) and elongation (Bell et al., 2002) in fish.

Irrespective of the dietary treatment, β-oxidation was the predominant fate for both 18:2n − 6 and 18:3n − 3. In fish fed the FO diet, β-oxidation on these two fatty acids were >77% of net intake while EPA and DHA were also oxidized for energy production. A higher dietary content of 18:2n − 6 and 18:3n − 3 in fish fed the SFO and PeO diets, respectively, led to higher oxidation of these respective fatty acids in tilapia (Fig. 6). This is in agreement with previous studies that reported that a surplus of dietary fatty acids upregulates fatty acid catabolism for energy production (Torstensen and Stubhaug, 2004; Stubhaug et al., 2007). This indicated that 18:2n − 6 and 18:3n − 3 are readily oxidized substrates, particularly the surplus of 18:3n − 3 in the PeO diets. Generally, β-oxidation occurs in mitochondria and peroxisome, whereby mitochondrial β-oxidation oxidizes short- ($<C_8$), medium- (C_{8-12}), and long- (C_{14-20}) chain fatty acids by adenosine triphosphate (ATP) generating oxidative phosphorylation to produce energy. However, due to the scarcity of very-long-chain acyl-CoA synthetase in mitochondria, very long chain fatty acids ($>C_{20}$) are only oxidized in peroxisomes (Reddy and Hashimoto, 2001). DHA is potently resistant to β-oxidation and more inclined to be transfered to the mitochondrial matrix, mostly stored in tissue membrane (Almaida-Pagan et al., 2007). Given that carnitine palmitoyltransferase (CPT)-I and CPT-II play an important regulatory step in catabolism as they activate and transport long-chain fatty acids into the mitochondrial matrix (Power and Newsholme, 1997), hepatic CPT-II in brown trout had significantly increased activities when fed a FO-based diet than those fed a CO-based diet (Turchini et al., 2003). Moreover, EPA and DHA were observed to improve the peroxisomal β-oxidation in liver (Reddy and Hashimoto, 2001). But, with respect to energy production, the efficiency of peroxisomal β-oxidation is only half of the mitochondrial β-oxidation (Stubhaug et al., 2007).

The abundant amount of dietary 18:1n − 9 is considered as a reason for the higher oxidation of 18:1n − 9 in fish fed the CO diet. 18:1n − 9 are known to be efficiently oxidized at high concentrations (Bell et al., 2001) to generate ATP (Sargent et al., 2002). It has been reported that high dietary MUFA drive the protein sparing effect and enhance growth in Atlantic salmon post-seawater transfer (Tocher et al., 2000). Sargent et al. (2002) addressed that metabolic energy produced *via* fatty acid β-oxidation is not only used for growth and but also contributes to reproduction. Menoyo et al. (2004) suggested that an imbalance in tissue n − 3/n − 6 PUFA ratio (low in n − 3 PUFA) might cause energy depletion and subsequently increase fatty acid β-oxidation. However, fish fed the RBDPO diet showed a lower total β-oxidation and the β-oxidation of 18:3n − 3 and 18:2n − 6 were relatively lower which is antagonistic to the high desaturase activity on these two C_{18} PUFA precursors. Thus, the fatty acid β-oxidation is not merely regulated by the tissue n − 3/n − 6 PUFA ratio, but β-oxidation of these fatty acids is proportional to their dietary level. Indeed, the use of dietary palm oil extends the shelf-life and freshness of seafood since a decreased PUFA content in the fish fillets can decrease their susceptibility to lipid peroxidation (Ng et al., 2001).

In conclusion, the results of the present study showed that dietary VO influenced the fatty acid composition of tilapia fillet and whole-body lipids as well as regulating the overall fatty acid metabolism in tilapia. The availability of dietary C_{18} PUFA precusors and enzyme products dominated the fatty acid desaturation, elongation and neogenesis in tilapia, while total Δ-5 and Δ-6 desaturase activities were exclusively triggered by the VO diets. Among all the VO, CO appears to be the most suitable alternative oil source as it led to higher desaturation (Δ-5 and Δ-6) and elongation on n − 3 PUFA, which consequently resulted in higher n − 3 LC-PUFA in fish tissues. Nevertheless, this could not rival the n − 3 LC-PUFA content of fish fed the FO diet. In addition, fatty acid β-oxidation of 18:3n − 3 and 18:2n − 6 is regulated by the tissue n − 3/n − 6 PUFA ratio and proportional to their dietary level.

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