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Effects of Different Vegetable Oils on the Fatty Acid Metabolism Based on Whole Body Fatty Acid Balance Method and Gene Expression of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

The objective of conducted research was to analyze the growth performance, fatty acid (FA) metabolism and gene expressions related to polyunsaturated fatty acid (PUFA) metabolism in *Oncorhynchus mykiss* fed diets containing fish oil (FO), sesame oil (SO), linseed oil (LO) and olive oil (OO). Based on the results of the whole body FA balance method of the study, it was concluded that rainbow trout used n-3 PUFAs as energy and this affected the oxidation of n-6 long-chain polyunsaturated FAs (LC-PUFAs). Gene expression level of the Δ-6 desaturase was the lowest for FO and the highest for SO fed fish ($P<0.05$). Fish fed with OO had the lowest and fish fed with FO had the highest expression levels of elongase gene ($P<0.05$). The elongase (ELOVL5) transcription rate was higher in fish fed with FO diet and lower in fish fed with OO diet ($P<0.05$). In conclusion, this research indicated that the final n-3 PUFA ratio in fish fed with vegetable oil diets can be increased by the addition of 18:3n-3 and the decreased level of total dietary 18:2n-6, the balancing of dietary FAs with a proper content of saturated FA (SFA) and monounsaturated FA (MUFA) is substantial for the best growth.

Introduction

Aquaculture is anticipated, to fill the rising gap between supply and demand of fish (Tacon et al., 2006). Dependence of fish meal and fish oil for the fish feed producing sector is a global debate (Tacon & Metian, 2015). The capacity of fish stocks has reached the sustainable limit and so the demand of fish oil for the fish feed industry cannot be met (Sargent & Tacon, 1999). Thus, vegetable oils are used as alternative sources of lipids for freshwater fish feeds (Montero et al., 2005; Wassef et al., 2009; Köse & Yildiz, 2013; Yildiz et al., 2018). Rainbow trout (*Oncorhynchus mykiss*) are one of the most commonly cultured freshwater fish species, and their farming is responsible for an important use of fish oil (Tacon & Metian, 2008). Thus, finding possible remedial strategies towards minimising the use of this product, while at the same time maintaining optimal nutritional quality of the final product, is a highly relevant objective (Turchini et al., 2011a).

Different vegetable oils are characterized by a wide range of fatty acid compositions such as palmitic acid (PA, 16:0), oleic acid (OA, 18:1n-9), linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3). Among all vegetable oils, the n-3 18C PUFA-rich vegetable oil sources are considered as favorable replacements to fish oil in aquafeed production. This is due to their ALA contents and potential for subsequent bioconversion to HUFAs (Tocher et al., 2011). Sesame oil contains about

80% unsaturated fatty acids with OA and LA (Akinosa et al., 2010). Linseed oil is rich in 18:3n-3 (Pickova & Mørkøre, 2007). Olive oil contains high (75%) level of 18C n-9 fatty acids (Yıldız & Şener, 2004). It is known that rainbow trouts need fatty acids in the linoleic series along with the fatty acids in the linolenic series in their diets (Sargent et al. 1997; Kiesling et al. 2001). Within the oils available for the use of aquafeed formulation, those rich in MUFA or n-6 PUFA are readily available, in growing supply, favourably priced and they provide easily digestible energy for growth the most abundant.

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are useful choice for human consumption (Ruxton et al., 2005; Siriwardhana et al., 2012; Tocher et al., 2019). Seafood is the only widely available and edible source of n-3 PUFA for human nutrition (Turchini & Francis 2009; Mahaffey et al., 2011). It is known that the dietary lipid source contains balanced levels of PUFAs and HUFAs. The consantration of n-3 HUFAs such as EPA and DHA and n-6 HUFA known as arachidonic acid (ARA, 20:4n-6) is high in fish oil, though vegetable oils are normally characterized by free of these FAs (Holub & Holub 2004; Turchini et al., 2011a). In general, freshwater fish species, such as rainbow trout, have the ability to convert 18C PUFA from n-3 and n-6 series FAs through to more HUFA via the desaturation and elongation pathways (Tocher & Sargent 1990; Glencross, 2009; Turchini et al., 2011a). In contrast, marine fish are unable to produce DHA from 18:3n-3 at a physiologically significant rate (Sargent et al., 2002) due to apparent deficiencies in one or more steps in the pathway (Tocher & Ghioni, 1999). The extent to which fish species can convert 18C PUFA to 20C-22C HUFA varies, associated with their complement of fatty acid desaturase and elongase enzymes. The production of EPA requires Δ-6 and Δ-5 desaturases. It is known that the dietary FA composition has a regulatory effect on enzyme activity that affects the biological transformation of FAs in cultured fish species. In particular, using more economical alternative oil sources instead of fish oil in diets increases the activity and transcription rate of desaturase and elongase enzymes, in charge of the production of LC-PUFAs from FAs in the n-3 and n-6 series of 18C (Torstensen & Tocher 2010; Thanuthong et al., 2011a; Garrido et al., 2019). The first step is the production of 18:3n-6 from 18:2n-6 and of 18:4n-3 from 18:3n-3 by the Δ-6 desaturase enzyme in the FA elongation and desaturation pathway. Then, these products are successfully elongated by elongase FA and desatured by Δ-5 desaturase enzyme to 20:4n-6 and 20:5n-3, respectively. For the production of 22:6n-3, the final product, more elongation, a Δ-6 desaturation and chain shortening steps are required (Sprecher et al., 1995; Turchini et al., 2007).

The FA elongation and desaturation metabolism abilities of cultured fish are an important research subject, and it is thought that a solution to the decreases of fish oil will be realised via a better understanding FA metabolism of fish. Additionally, there is limited research (Turchini et al., 2013; Eroldoğan et al., 2013; Ofori-Mensah et al., 2020) that include both the in vivo assessment of FA metabolism, deduced by using the whole body FA balance method, and genes involved in FA metabolism. In the earlier studies conducted by Turchini et al. (2013) reported that the apparent in vivo fatty acid b-oxidation was proportional to fatty acid dietary supply and limited apparent in vivo fatty acid bioconversion (elongation and desaturation) was recorded, primarily acting on n-6 PUFA. Additionally, In all treatments, dietary 20:5n-3 was actively bioconverted into 22:6n-3. Eroldoğan et al. (2013) reported very limited fatty acid elongation and desaturation when juvenile European sea bass were fed diets containing 510 g/kg fish meal. This shows that such high levels of fish meal may interfere with the in vivo bioconversion abilities thereby masking the elongase and desaturase activities in marine fish. Ofori-Mensah et al. (2020) from the results of the whole body fatty acid balance method, feeding diets containing the VOs led to slightly increased β-oxidation. Feeding 100% VO diets reduced whole body and liver content of EPA, DHA and ARA in juvenile gilthead seabream.

The primary aim of this study is to understand the molecular basis of HUFA biosynthesis in fish will enable us to manipulate and optimise the activity of the pathway to enable efficient and effective use of vegetable oils in aquaculture. Thus, the FA metabolism, with the monitoring of the fate of individual dietary FA, and gene expressions related to PUFA metabolism in *O. mykiss* fed with total replacement of fish oil with LO, SO or an OO based diet was evaluated in this research.

Materials and Methods

Experimental Diets

All animal experiments were conducted in accordance with the approval of Istanbul University Animal Experiments Ethics Committee (No: 2013/61). Four iso-lipidic (about 17% crude lipid) and iso-nitrogenous (about 47% crude protein) diets were prepared containing fish oil (FO), sesame oil (SO), linseed oil (LO) and olive oil (OO) only. Chromic oxide (Cr_2O_3) was added to diets as a marker for the calculations of digestibility. Diets (6 mm size) were prepared at the Sapanca Inland Waters Research Unit (Sakarya, Turkey) of Istanbul University as steam pressured pellets using a feed grinder (KAHL-L, 173). The ingredients of diet and proximate compositions are shown in Table 1. The FA compositions of the experimental diets are given in Table 2.

Experimental Conditions and Measurements

O. mykiss were supplied from the Sapanca Inland Waters Research Unit, Turkey. Fish with a mean initial body weight of 42.18±0.12 g g randomly stocked in

dublicate tanks (1000 L) of 30 fish each for each of the four treatments in this research center. Experimental fish were acclimated to experimental conditions two weeks prior to the study. During this time, fish was fed with commercial fish feed. The tanks were yielded with freshwater and the temperature was 12.4±0.3°C. Dissolved oxygen was preserved at 7.9±0.9 mg l^{-1}. During the experiment, 12 h light: 12 h dark photoperiod regime was utilized. The fish were fed twice a day (0830 and 1630 h) up to 2% of their body weight per day in the experiment, which lasted 60 days. Bulk weight increments of fish were measured every 2 weeks and feed intake was noted daily during the study. At the completion of the trial, fish were individually weighed to define the parameters of the growth performance. Additionally, 30 fish from each dietary group were collected for proximate and FA composition analysis. Fish samples placed in liquid nitrogen and were stored at -80°C for analysis of proximate composition and FAs. The fish growth performance was evaluated to Ricker (1979).

Weight gain (g)=(final weight-initial weight)

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

Feed conversiton ratio (FCR)=(feed intake/fish weight gain)

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

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

Fish faeces were collected daily throughout the feeding trial. A particular faeces collection columns were setted to the experimental tanks by using the Guelph system, which was identified by Cho et al. (1982). Tanks were siphoned after the last feeding (1630 hours) to take out the uneaten feed. Then faeces were collected overnight at regular intervals until the next morning (0830 hours). These collected faeces were stored in the freezer at -80°C until analysis. Nearly, 13-14 hour passed between the last feeding and the faeces collection.

Proximate and FA Analysis

The proximate composition of the diets and whole fish samples were analyzed by the methodology of AOAC (1995). With the methods of Folch et al. (1957), lipid was extracted from whole body of fish, liver, feed and faece samples. Methyl esters were prepared by transesterification method using 2M potassium hydroxide (Merck, Darmstadt, Germany) in methanol and n-hexane (Sigma-Aldrich, Steinhein, Germany) according to the Ichihara et al. (1996) with a modification; 10 mg of extracted oil were dissolved in 2 ml hexane followed by 4 ml of 2M methanolic KOH. Tube was vortexed for 2 min. The hexane layer was taken for GC analyses after the centrifugation at 4000 rpm for 10 min. FA composition of samples was conducted with gas chromatograph (Perkin Elmer Auto System XL) as described previously (Köse and Yildiz, 2013). All FA analyses were done in triplicate.

Table 1. Ingredients and proximate composition of the four experimental diets

	Diets			
	FO	SO	LO	OO
Diet formulation (g kg^{-1})				
Fish meal	350	350	350	350
Soybean meal	108	108	108	108
Whole-wheat meal	40	40	40	40
Wheat gluten	160	160	160	160
Corn gluten	80	80	80	80
Gelatine	50	50	50	50
Fish oil	150	0	0	0
Sesame oil	0	150	0	0
Linseed oil	0	0	150	0
Olive oil	0	0	0	150
Vitamin mixture[A]	30	30	30	30
Mineral mixture[A]	30	30	30	30
Cr_2O_3	2	2	2	2
Proximate composition (%)				
Dry matter	88.90	88.14	87.29	87.02
Crude protein	46.38	47.47	47.11	48.09
Crude fat	17.12	17.56	17.68	18.35
Crude ash	10.53	9.99	9.86	10.33
Crude cellulose	3.49	3.56	3.61	3.59
NFE[B]	11.38	9.56	9.03	6.56
Metabolisable energy (kj g^{-1})	19.69	19.81	19.68	19.78
Gross energy (kj g^{-1})	14.07	14.27	14.22	14.45

FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.

[A] Premix of vitamins and minerals according to NRC (1993) recommendations for fish.

[B] NFE: Nitrogen-free extract calculated by difference.

Digestibility Analysis and Whole Body FA Balance Calculations

Chromium oxide in the diets and faeces was calculated to Furukawa & Tsukahara (1966). FA digestibility was calculated with a standard formula: FA digestibility =100 - (100 (Cr_2O_3 in diet) / (Cr_2O_3 in faeces) x ((% FA in faeces) / (% FA in feed)).

The calculation of whole body FA balance was carried out the method of Turchini et al. (2007) and with more improvements by Turchini et al. (2008) and Turchini & Francis (2009) to evaluate the FA metabolism of fish. Datas of this method have been informed as apparent in vivo enzyme activity (nmol g^{-1} day^{-1}) and, for key FAs, their utilization was also stated and given as percentage of their net intake.

Real-Time PCR Analysis

The abundance of the elongase and Δ-6 desaturase genes were determined by Real-Time PCR analysis using 10 mg of fish liver tissues from each experimental group according to the method of Livak and Schmittgen (2001). The abundance of the elongase and Δ-6 desaturase genes were determined by Real-Time PCR analysis. Approximately 10 mg of fish liver tissues from each experimental group was used to extract the total RNA using the kit (Qiagen, USA). Nanodrop 1000 (ThermoFisherScientific, Inc USA, LightCycler® 480 System, Roche) was used to specify the purity of RNA. The A260 / A280 values were determined 2. cDNA was synthesized from 2 µg of RNA (20 µl reaction volume) using a high capacity RNA to cDNA reverse transcriptase kit (PE Applied Biosystems). Reverse transcription PCR amplification was completed using 1 µl cDNA diluted at 1:25 incubated at 95°C for 10 min to activate the heat-activated TaqDNA polymerase followed by 40 cycles at 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. Melting curve analysis was performed to test the specificity of the reaction at the end of these 40 cycles. The primer sequences used in the research are presented in Table 3. Each primer set was used at 200 nM concentration from a volume of 14 µl using Power SYBR Green PCR Master Mix (PE AppliedBiosystems). PCR primers were

Table 2 FA composition of four experimental diets

Fatty acid (g/kg)	Diets			
	FO	SO	LO	OO
14:0	62.9	24.1	17.9	13.2
16:0	200.2	192.5	124.4	150.8
18:0	53.5	65.6	49.4	37.0
20:0	7.4	ND	ND	4.9
Total saturates	324.1	282.3	191.8	206.0
16:1n-7	54.6	22.9	17.8	18.3
18:1n-9	169.2	222.0	182.6	530.8
20:1n-9	5.8	3.81	3.8	4.7
22:1n-11	8.4	2.0	1.8	1.4
Total monoenes	183.5	250.8	206.6	555.3
18:2n-6	80.5	282.2	172.1	109.1
20:4n-6	10.7	1.0	1.1	0.8
18:3n-3	19.9	12.5	329.6	12.3
20:3n-3	5.8	ND	2.2	ND
20:5n-3	97.8	2.98	3.08	3.19
22:6n-3	174.9	66.7	54.5	31.6
Total polyunsaturates	389.7	395.2	562.6	157.2
Total n-6 PUFA	91.2	283.2	273.2	109.9
Total n-3 PUFA	298.5	112.0	389.4	47.2
Total n-3 HUFA	278.6	99.6	59.82	34.8

ND: Not detected.
Data are mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05).
FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.

Table 3 Primer list for RT-qPCR analysis

Primer Name	Primer Sequences
D6DS-Forward	5′-ACCTAGTGGCTCCTCTGGTC-3′
D6DS-Reverse	5′- CAGATCCCCTGACTTCTTCA-3′
ELG-Forward	5′-GAACAGCTTCATCCATGTCC-3′
ELG-Reverse	5′-TGACTGCACATATCGTCTGG-3′
Act-Forward	5′-CAAGCAGGAGTACGACGAGT-3′
Act-Reverse	5′-CTGAAGTGGTAGTCGGGTGT-3′
GAPDH-Forward	5′-GTCTCAGTGGTGGACCTGAC-3′
GAPDH-Reverse	5′-GCCGTTGAAGTCTGAAGAGA-3′

prepared according to the previous paper of Δ6 desaturase (GenBank accession no. NM001124287.1) and elongase (GenBank accession no. NM001124636.1). The β-actin (GenBank accession no. NM001124235.1) of rainbow trout was used as a housekeeping control standardized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GenBank accession no. NM001124209.1). Gene expression primers were validated using the BLAST sequence alignment algorithm and specific PCR products were produced to determine the absence of primer-dimerization that could affect the analysis. The data were analyzed according to the method of Livak and Schmittgen (2001).

Statistical Analysis

Results were presented as means±SD (n = 3). For the proximate composition, chemical and gene expression analysis of diets, three samples per experimental diet were used whereas ten whole fish body for each dietary treatment were analyzed for the same measurements. The level of significance of difference (P<0.05) between dietary treatments was detected by one way analysis of variance (ANOVA) (Zar, 1984). Followed by the Tukey's test when significant difference was found. All statistical calculations were presented using SPSS 18.0.

Results

In the conducted research, no significant influence of dietary treatments on growth performance and feed efficiency parameters is observed (Table 4).

The lowest HSI and VSI values were observed in fish fed diets with FO and OO. However, fish fed with SO had the highest HSI value (Table 4; P < 0.05). In addition, fish fed with LO diet had the highest VSI value (Table 4; P < 0.05).

Dry matter was lower in fish fed the FO diet than it was in those fed the other dietary treatments. The crude lipid levels of fish fed OO diet were the lowest compared to the another dietary groups (Table 4; P<0.05).

The results of the FA composition of dietary groups showed that OO diet had the highest ratio of MUFA and LO diet had the highest ratio of n-3 PUFAs, especially 18:3n-3. Higher ratios of DHA were observed in the FO diet (P<0.05). Total saturates were higher in the FO diet (Table 2). Whole body FA composition of rainbow trout was affected by dietary treatments (Table 5). Higher levels of 20:4n-6, 20:5n-3 and 22:6n-3 were detected in FO group (P<0.05). Whole body 18:3n-3 and 20:3n-3 levels were the highest in fish fed with LO diet (P<0.05). Between the experimental groups, containing vegetable oil, levels of 20:5n-3 and 22:6n-3 were found the highest in fish fed with LO group. Higher amounts of SFA was observed in the FO group. Total monoenes were higher in the OO group and lower in the FO group.

The FA digestibilities of the experimental diets are given in Table 6. Digestibility of 20:4n-6, 20:5n-3 and 22:6n-3 in fish fed with FO diet was significantly higher compared to the other dietary groups (P<0.05). The highest digestibility in 22:5n-3 and 22:6n-3 were recorded in fish fed with FO diet. In the other experimental groups, this FA was not effectively digested (P<0.05). Generally, FA digestibility in fish was PUFA>MUFA>SFA in the present study.

Table 4 Growth parameters and proximate composition of fish fed four experimental diets

	Diets			
	FO	SO	LO	OO
Growth parameters				
Initial weight (g)[A]	42.10±0.14	42.35±0.10	42.10±0.14	42.20±0.14
Final weight (g)[B]	108.00±2.69	107.35±2.19	101.75±4.60	107.25±0.21
Weight gain (g)[C]	65.90±4.89[a]	65.00±5.05[a]	61.71±4.47[b]	65.06±4.10[a]
SGR (% day^{-1})[D]	1.59±0.49	1.57±0.46	1.59±0.69	1.57±0.34
Feed intake (g fish^{-1})	86.62±5.09[a]	85.31±4.67[a]	79.30±3.80[b]	86.59±4.88[a]
Feed conversion ratio	1.38±0.23	1.32±0.15	1.35±0.25	1.35±0.15
HSI (%)[E]	2.04±0.32[c]	2.54±0.48[a]	2.24±0.22[b]	2.06±0.33[c]
VSI (%)[F]	20.88±2.12[c]	21.53±1.13[b]	22.51±2.16[a]	20.13±1.47[c]
Survival (%)	100	100	100	100
Proximate composition (g/kg)[G]				
Dry matter	307.72±1.91	332.17±5.48	336.26±2.10	355.21±7.27
Crude protein	174.73±1.00	176.45±3.57	172.48±2.95	173.89±2.08
Crude lipid	167.34±4.59	154.48±3.51	157.78±3.92	131.84±0.65
Ash	19.32±4.10	18.20±1.43	20.00±3.94	17.76±4.23

Data = mean ± SD. Means with different superscript letter in a row are significantly different (P<0.05).
FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.
[A]n: 30 X 2.
[B]n: 30 X 2.
[C]n: 30 X 2.
[D]n: 30 X 2.
[E]n: 10 X 2.
[F]n: 10 X 2
[G]n: 3.

In this research, FA composition of the whole body and total net intake of FAs were calculated. Then these datas were utilized for the computation of the individual FAs appearance/disappearance, which were affected by the dietary treatments (Table 7). It was observed that OO group oxidized 18:1n-9 and the other experimental groups accumulated this FA in the body. Furthermore, 18:2n-9 was oxidized by SO group, 18:3n-3 was oxidized by LO group when compared to the other experimental groups. In addition, the highest oxidation amounts were found in 18C FA groups. Besides, 22:2n-6 FA was oxidized in all experimental groups of fish. The highest oxidation rate for this FA was found in the SO group and the lowest oxidation rate was found in the FO group (P<0.05).

The apparent Δ-6 and Δ-9 desaturation activities were calculated for all FA groups (Figure 1). The β-oxidation value for MUFA and SFA was the highest in FO group (P<0.05). When the total β-oxidation value was examined for n-6 PUFAs, the highest value was seen in fish fed with the diet of SO, while the lowest β-oxidation value was observed in the fish fed with FO diet (Figure 2) (P<0.05). Total β-oxidation value for n-3 PUFAs was determined the highest in fish fed FO diet and the lowest in fish fed SO diet (P<0.05). Total Δ-6 desaturase was found the highest in fish fed with LO diet whilst was found the lowest in fish fed with FO diet (P<0.05). The total Δ-9 desaturation was determined the lowest in fish fed with the OO diet and the highest in fish fed with the SO diet (P<0.05).

The levels of apparent in vivo elongation, deposition and *β*-oxidation on some FAs, reported as percentage of the FA net intake, are shown in Table 8. In this study, the experimental fish were able to convert 18:2n-6 to 20:2n-6 and 18:3n-6. When the amount of the conversion of 18:2n-6 to 20:2n-6 by elongation was examined, the highest value was found in SO group, while the lowest value was found in the FO group (P<0.05). Similarly, based on the amount of conversion of 18:2n-6 to 18:3n-6 by desaturation, it was found that the highest value was in the SO group and the lowest value was in the FO group (P <0.05). In addition, when the amount of conversion of 18:3n-3 to 20:3n-3 by elongation was examined, the highest value was observed in the LO group and the lowest value was in the FO group (P<0.05). The amount of conversion of 18:3n-3 to 18:4n-3 by desaturation was found the highest in the LO group, whilst was found the lowest in the FO and OO groups (P<0.05). On the other hand, β-oxidation value of 18:2n-6 was determined to be the highest value in the SO group and the lowest in fish fed with the FO group. When the β-oxidation values of 18:3n-3 were examined, it was found that this value was determined the highest in LO group and the lowest in FO group (P<0.05). Furthermore, the β-oxidation values of 20:5n-3 were only observed in the FO group. In this study, the β-oxidation values of 22:2n-6 were determined, it was found the highest in SO group and the lowest in FO group (P<0.05). However, it was observed that the highest value of 18:2n-6 accumulation was found in the FO group and the lowest value was in the OO group (P<0.05). When the accumulation values of 18:3n-3 were examined, it was observed that the highest accumulation was in FO and SO groups, while the lowest accumulation was in the OO group. Based on the accumulation value of 22:6n-3, it was observed that the highest value was in FO group fish and the lowest value was in OO group fish (P<0.05).

Table 5 FA composition (% of total FAs detected) in the whole body of rainbow trout fed with FO, LO, SO and OO diets for 60 days

FAs	Fish groups				
	Initial	FO	SO	LO	OO
14:0	20.3±0.1^{b}	43.8±0.1^{a}	13.2±0.1^{d}	13.9±0.4^{c}	12.1±0.1^{e}
16:0	118.0±0.5^{d}	170.5±0.2^{a}	123.0±0.1^{c}	122.8±0.6^{c}	142.7±0.1^{b}
18:0	39.6±0.1^{ab}	39.8±0.2^{ab}	40.5±0.3^{ab}	45.8±0.8^{a}	32.0±0.9^{b}
20:0	ND	ND	ND	ND	ND
Total saturates	178.0±0.8^{c}	254.2±0.6^{a}	176.7±0.5^{c}	182.6±1.9^{bc}	186.9±1.1^{b}
16:1n-7	28.0±0.1^{b}	58.6±0.4^{a}	24.5±0.3^{b}	26.2±0.3^{b}	28.5±0.1^{b}
18:1n-9	398.1±1.1^{c}	236.9±1.5^{e}	403.6±0.2^{b}	259.3±1.0^{d}	534.9±0.5^{a}
20:1n-9	21.5±0.1^{a}	11.2±0.1^{d}	15.6±0.3^{c}	10.0±0.4^{d}	18.6±0.1^{b}
22:1n-11	9.8±0.1^{b}	10.4±0.1^{a}	7.0±0.3^{c}	5.3±0.1^{d}	3.4±0.0^{e}
Total monoenes	457.6±1.4^{b}	317.3±2.1^{c}	451.0±1.2^{b}	300.9±1.9^{c}	585.5±0.7^{a}
18:2n-6	193.6±0.3^{b}	100.4±0.2^{e}	236.7±0.4^{a}	152.0±1.0^{c}	115.6±0.4^{d}
20:4n-6	4.6±0.04^{b}	9.1±0.2^{a}	3.8±0.3^{bc}	2.5±0.0^{c}	2.2±0.1^{c}
18:3n-3	40.0±0.2^{b}	27.8±0.2^{c}	27.3±0.3^{c}	226.0±0.1^{a}	19.6±0.1^{d}
20:3n-3	2.9±0.1^{b}	2.5±0.1^{b}	1.3±0.3^{c}	7.15±0.4^{a}	1.27±0.1^{c}
20:5n-3	15.7±0.1^{c}	53.0±0.1^{a}	7.9±0.2^{d}	16.4±0.1^{b}	6.3±0.1^{e}
22:6n-3	60.0±0.1^{c}	199.2±0.1^{a}	47.17±0.3^{d}	63.5±0.9^{b}	40.7±0.4^{e}
Total polyunsaturates	317.0±0.7^{c}	392.2±0.9^{b}	324.4±1.8^{c}	467.7±2.5^{a}	185.8±1.2^{d}
Total n-6 PUFA	198.2±0.2^{b}	109.5±0.2^{e}	240.6±0.3^{a}	154.5±0.5^{c}	117.8±0.2^{d}
Total n-3 PUFA	118.7±0.1^{c}	282.7±0.1^{b}	83.8±0.3^{d}	313.2±0.4^{a}	68.0±0.2^{e}
Total n-3 HUFA	78.7±0.1^{c}	254.9±0.1^{a}	56.4±0.3^{d}	87.1±0.5^{b}	48.3±0.2^{e}

ND: Not detected.

Data = mean ± SD of three replicates (with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different (P<0.05).

FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.

Table 6 FA digestibility (%) in the whole body of experimental fish fed with FO, LO, SO and OO diets for 60 days

Digestibility	Fish groups			
	FO	SO	LO	OO
14:0	87.31±0.32	91.82±0.38	90.72±0.27	91.29±0.18
16:0	85.53±0.14^{b}	94.73±0.41^{a}	93.75±0.36^{a}	89.82±0.49^{ab}
16:1n-7	95.81±0.36	93.72±0.29	93.70±0.46	93.79±0.28
18:1n-7	92.37±0.45	98.95±0.36	92.97±0.48	96.28±0.51
18:0	75.36±0.37^{b}	94.44±0.39^{a}	95.26±0.51^{a}	93.82±0.54^{a}
18:1n-9	93.48±0.33	97.10±0.41	94.39±0.42	95.10±0.28
20:1n-9	87.12±0.38	87.20±0.58	85.23±0.25	83.87±0.32
22:1n-9	83.80±0.25	90.21±0.36	91.70±0.45	89.90±0.53
24:1n-9	82.46±0.30^{b}	93.45±0.27^{a}	95.21±0.39^{a}	92.40±0.28^{a}
20:0	75.92±0.32^{b}	91.96±0.58^{a}	89.90±0.29^{a}	90.95±0.38^{a}
20:1n-11	85.18±0.29^{b}	97.62±0.36^{a}	98.50±0.48^{a}	96.63±0.33^{a}
22:1n-11	83.72±0.36	89.34±0.48	90.21±0.57	87.32±0.38
22:0	78.10±0.28	78.37±0.34	79.39±0.32	80.00±0.28
22:1n-13	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
24:0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
18:2n-6	95.34±0.53	96.17±0.43	95.12±0.54	97.96±0.50
20:2n-6	74.92±0.24^{b}	99.50±0.54^{a}	98.43±0.23^{a}	97.26±0.41^{a}
22:2n-6	96.37±0.35	100.00±0.00	100.00±0.00	100.00±0.00
18:3n-6	97.85±0.43	97.92±0.37	97.86±0.45	96.95±0.52
20:3n-6	93.60±0.18	91.57±0.28	92.65±0.43	93.70±0.52
20:4n-6	97.78±0.29^{a}	85.10±0.23^{b}	86.12±0.35^{b}	84.19±0.37^{b}
22:4n-6	95.45±0.56	96.38±0.27	97.33±0.58	95.34±0.56
24:4n-6	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
24:5n-6	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
22:5n-6	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
18:3n-3	96.28±0.32	98.29±0.30	98.00±0.15	98.04±0.41
20:3n-3	88.57±0.23	91.39±0.19	92.38±0.42	92.46±0.17
22:3n-3	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
18:4n-3	98.58±0.42	93.36±0.12	93.37±0.52	93.35±0.45
20:4n-3	97.27±0.28	96.47±0.27	97.40±0.38	92.76±0.48
20:5n-3	99.16±0.35^{a}	94.55±0.42^{b}	95.25±0.29^{b}	94.20±0.12^{b}
22:5n-3	97.08±0.52^{a}	74.30±0.31^{c}	78.29±0.35^{b}	76.30±0.19^{bc}
24:5n-3	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
24:6n-3	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
22:6n-3	97.76±0.52^{a}	88.24±0.35^{b}	87.10±0.43^{b}	87.92±0.35^{b}
SFA	80.44±0.29^{b}	90.26±0.42^{a}	89.80±0.35^{a}	89.18±0.37^{a}
MUFA	87.99±0.34^{ab}	93.45±0.39^{a}	92.74±0.44^{a}	91.91±0.36^{a}
PUFA	94.71±0.41	92.56±0.34	93.02±0.39	92.34±0.45

Data = mean ± SD of three replicates (with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different (P<0.05).
FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.

The magnitude of mRNA of Δ-6 desaturase and elongase abundance in fish liver is presented in Figure 3. The gene expression level of Δ-6 desaturase was the lowest in FO and the highest in SO fed fish (P<0.05). The expressions of Δ-6 desaturase gene were similar in fish fed with LO and OO (P>0.05). Fish fed with OO had the lowest and fish fed with FO had the highest expression levels of elongase gene (P<0.05). Elongase gene expression levels were found similar in fish fed SO and LO feeds (P>0.05).

Discussion

In the present research, growth performances of *O. mykiss* were not significantly influenced by the dietary FA compositions in comparison with the fish fed a diet containing 100% fish oil. Similar findings were observed with previous studies for *O. mykiss* fed vegetable oil supplemented diets (Rinchard et al., 2007; Turchini & Francis, 2009; Köse & Yildiz, 2013; Yildiz et al., 2018) indicating that replacing up to 80-100% of marine fish oil with vegetable oil sources had no significant effects on growth or feed conversion ratio. The difference in the higher level of substitution has been defined by the qualitative differences in EFA requirement in fish living in the salt water compared to freshwater (Qui et al., 2017).

Though, the proximate composition of whole fish body was affected by the total substitution of FO by SO, LO and OO in the dietary groups. Based on the proximate composition of the fish, the reason that the whole body lipid amount of the fish fed OO diet is lower than the other experimental groups can be explained by using the lipids stored in the bodies of the fish in order to produce energy from fatty acids. In the study, the protein (approximately 17%) and lipid levels (approximately 16%) in the whole body of the fish were similar to the levels stated in the earlier studies on *O. mykiss* (Turchini et al., 2013) and different species, such as *Salmo salar* (Torstensen et al., 2000) and

Table 7 Amounts of FA appearance/disappearance (mg/fish) in the experimental fish fed with FO, SO, LO and OO diets for 60 days

Appearance/disappearance (mg of FA per fish)	Fish groups			
	FO	SO	LO	OO
14:0	-276.19±8.83^{d}	68.98±4.92^{a}	12.52±0.63^{c}	20.87±0.96^{b}
14:1n-5	15.64±0.87^{c}	181.00±11.94^{b}	241.47±7.06^{a}	236.35±8.16^{a}
16:0	306.87±15.78^{c}	239.08±9.18^{d}	598.93±29.17^{a}	329.31±18.32^{b}
16:1n-7	245.65±6.43^{a}	246.57±10.06^{a}	210.72±8.48^{b}	145.96±14.87^{c}
18:1n-7	285.60±9.76^{a}	-16.33±0.91^{b}	-52.93±3.18^{c}	-90.67±5.12^{d}
18:0	113.96±12.09^{c}	-56.55±3.19^{d}	148.31±12.56^{b}	325.47±17.90^{a}
18:1n-9	1247.07±38.10^{b}	2380.81±101.06^{a}	1265.42±42.11^{b}	-1438.68±49.0^{c}
20:1n-9	85.44±6.12^{c}	184.68±14.10^{a}	115.11±9.04^{b}	185.13±10.12^{a}
22:1n-9	-33.51±4.56^{d}	89.81±6.13^{b}	98.27±8.02^{a}	33.86±1.98^{c}
24:1n-9	-73.91±3.98^{c}	20.95±2.01^{b}	28.24±3.10^{a}	-211.08±6.04^{d}
20:0	-30.10±3.67^{c}	-27.81±2.98^{c}	3.72±0.42^{a}	-18.05±0.86^{b}
20:1n-11	-26.03±3.08^{d}	-13.41±0.73^{b}	0.24±0.06^{a}	-22.82±1.01^{c}
22:1n-11	-1.85±0.09^{b}	10.48±1.56^{a}	10.34±1.28^{a}	-11.54±1.21^{c}
22:0	-3.01±0.85^{a}	-11.99±1.42^{b}	-12.98±0.97^{b}	-26.96±1.03^{c}
24:0	-18.46±0.78^{c}	-2.38±0.04^{b}	ND	11.42±0.98^{a}
18:2n-6	342.99±19.64^{a}	-464.69±16.40^{d}	236.58±17.43^{b}	6.34±0.62^{c}
20:2n-6	40.71±1.50^{d}	157.74±9.75^{a}	92.19±14.11^{b}	72.77±5.73^{c}
22:2n-6	-16.00±1.02^{a}	-137.97±11.92^{d}	-32.89±2.68^{b}	-77.38±6.14^{c}
18:3n-6	7.42±0.89^{d}	63.84±4.95^{a}	37.32±3.21^{b}	14.37±1.16^{c}
20:3n-6	24.22±2.64^{c}	102.66±15.30^{a}	38.72±2.79^{b}	47.35±3.52^{b}
20:4n-6	-0.07±0.00^{c}	22.94±1.03^{b}	53.07±3.12^{a}	24.40±2.10^{b}
18:3n-3	167.51±12.18^{a}	163.38±10.09^{b}	-1445.11±46.13^{d}	125.90±8.56^{c}
20:3n-3	13.49±1.02^{c}	31.34±3.15^{b}	224.43±12.84^{a}	18.95±1.04^{c}
18:4n-3	-68.33±5.07^{d}	25.77±1.12^{b}	236.35±14.98^{a}	17.03±1.14^{c}
20:4n-3	7.50±0.86^{c}	20.51±3.16^{b}	50.83±5.04^{a}	40.44±2.86^{a}
20:5n-3	-288.90±18.37^{d}	-6.64±0.79^{b}	44.27±4.08^{a}	-124.75±13.21^{c}
22:5n-3	139.52±12.84^{a}	85.91±4.99^{c}	117.68±13.97^{b}	57.36±8.02^{d}
22:6n-3	1368.73±31.10^{a}	267.54±26.05^{c}	562.66±39.16^{b}	92.04±8.04^{d}

ND: Not detected.
Data = mean ± SD of three replicates (with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different (P<0.05).
FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.

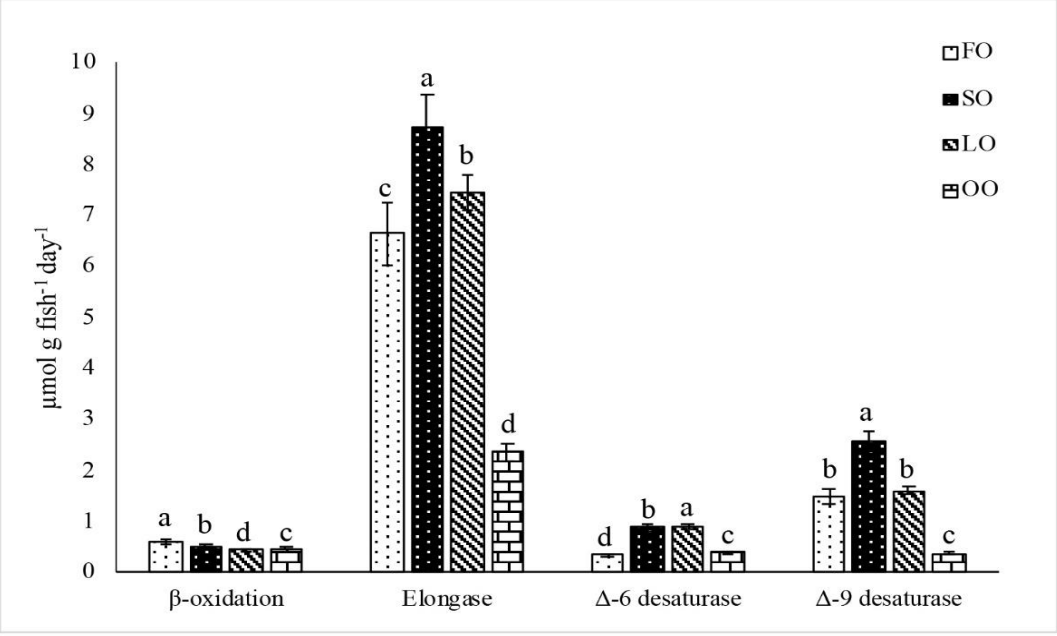

Figure 1. Total apparent in vivo FA β-oxidation, desaturation and elongation µmol g^{-1} day^{-1} detected by whole body FA balance method) in *O. mykiss* fed with FO, SO, LO and OO diets for 60 days[a]. [[a]Data = mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05)]

Diplodus puntazzo (Piedecausa et al., 2007).

As is well-documented, the fatty acid composition of fish tissues is reflective of that present in their diet (Turchini et al., 2009). In this research, substitution of marine fish oil with vegetable oils resulted in a decreased level of total n-3 PUFAs except for LO group because of consisting of 18:3n-3, and an increased level of n-6 PUFA in the whole fish body. Similarly, Drew et al., 2007 reported a higher level of LA and LNA and a lower level of EPA and DHA by the replacement of fish oil with vegetable oils on *O. mykiss*. Ofori-Mensah et al., 2020 stated that replacement of fish oil with vegetable oils resulted in a lower levels of EPA and DHA and a higher levels of LA and LNA on *Sparus aurata*. Generally, using vegetable oils instead of fish oil has resulted in a lower level of n-3 HUFAs, especially EPA and DHA and higher levels of 18C FAs, oleic, linoleic (LA) and linolenic (LNA) acids in tissues of some fish species such as rainbow trout (Köse & Yildiz, 2013; Yildiz et al., 2018), sea bass (Yildiz & Sener, 2004; Mourente & Bell, 2006; Eroldoğan et al., 2013; Yılmaz & Eroldoğan, 2015) and gilthead sea bream (Mourente & Bell, 2006; Wassef et al., 2009).

Many researches conducted on the desaturation and elongation activities, especially on salmonids, using ex vivo methods were published (Brown, 2005; Stubhaug et al., 2005; Turchini et al., 2007). Desaturase activity ranges, assessed by ex vivo methods, varry between different species and even within the same species (Turchini et al., 2006). Freshwater fish such as *O. mykiss* are capable of converting dietary LA and LNA to HUFA, such as ARA, EPA, DHA and might not require them in the diet (Sargent et al., 2002; Turchini et al., 2011a; Hixson et al., 2014). In vivo methods use whole body approach and give an estimate of the capacity of an organism to metabolise EFAs. Furthermore, in the estimation of the abilities of fish to synthesize 20C and 22C HUFA from dietary 18C PUFA existing in vegetable oils, the whole body approach can be thought suitable (Turchini et al., 2011b).

In this study, *O. mykiss* was able to elongate and desaturate dietary 18:2n-6 and 18:3n-3, and demonstrates elongase, Δ6 and Δ5 desaturase activities. These results are similar with previous studies on rainbow trout (Turchini & Francis, 2009) and murray cod (Turchini et al., 2011b). In contrast, *Esox lucius* destitutes this ability (Henderson et al., 1995). The datas of the study showed that total SFAs were high in fish feed although they were found low in the whole body of

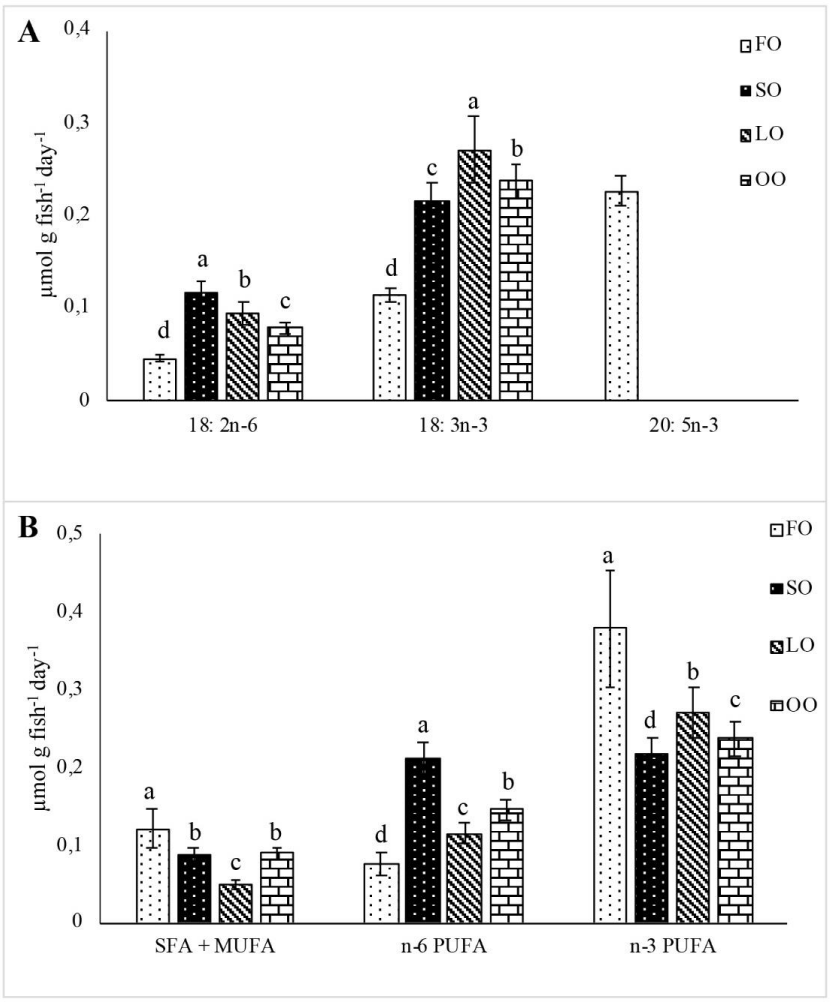

Figure 2. Total apparent in vivo β-oxidation (µmol/g of fish/day) in *O. mykiss* fed with FO, SO, LO and OO diets for 60 days[a]. A) The three main β-oxidised FAs. B) FA classes. [[a]Data = mean ± SD of three replicates (n = 3). Means with different superscript letter in a row are significantly different (P<0.05)]

fish. Especially the ratio of 18:0 is high in diet and is low in the whole body of fish. This result showed that the fish use 18:0 as an energy via the β-oxidation. On the other hand 18:1n-9 is higher in the whole body of fish compared to diets. The results indicated that fish produce 18:1n-9 from 18:0 via desaturation. Though, the highest conversion was observed in the SO group. The ratio of 18:2n-6 in the whole fish body decreased in SO and LO groups. On the other hand, 20:4n-6 levels in the fish body were significantly increased compared to FA composition of diets. These results indicate that the fish synthesize 20:4n-6 from 18:2n-6. This result has also been reported by Cabellero et al. (2002) and Turchini & Francis (2009).

The results of the study indicate that 18:3n-3 was converted to 20:3n-3 in dietary treatments, especially containing vegetable oils. As previously stated, the major level of dietary 18:3n-3 was converted to 20:3n-3 in trout via the elongation (Turchini & Francis, 2009; Tocher, 2010; Thanuthong et al., 2011b). Also, these findings clearly show that the dietary fatty acid composition is critical in determining the fatty acid composition of fish tissues, and that in vivo fatty acid metabolism can have a measurable effect.

The results of the lipid digestibility obtained in the present study ranged between 74–99%. Bakke et al. (2010) reported that the lipid digestibility generally lies in the range of 85–99% in cultivated fish species. Due to the increasing level of unsaturated FA in the dietary groups of this study, the digestibility of the individual FA increased in agreement with earlier results on *Atlantic salmon* (Røsjø et al., 2000; Menoyo et al., 2007), *Salmo trutta* (Turchini et al., 2005), Rainbow trout (Ng et al., 2010).

Based on the results of the whole body FA balance method of the study, it was concluded that rainbow trout used n-3 PUFAs as energy and this effected the oxidation of n-6 LC-PUFAs. However, it was found that 18:2n-6 was β-oxidized the highest in fish fed SO diet. When β-oxidation of 18:3n-3 was examined, it was found that the highest value was in LO group. This is because 18:3n-3 and 18:2n-6 are easily β-oxidized if found in high amounts in the feeds. Similarly, in studies on rainbow trout and Atlantic salmon, 18:3n-3 and 18:2n-6, as well as MUFA, are readily β-oxidized if found in high amounts in the feeds (Stubhaug et al., 2007; Turchini & Francis, 2009; Senadheera, 2012). Also, the β-oxidation of 20:5n-3 is observed for fish fed with FO diet in conducted research. Stubhaug et al. (2007) stated that the 20:5n-3 is actively β-oxidised if exist in high amounts in the feed. These datas have shown that the FAs in feeds are β-oxidized proportionally to their usability. The level of 18:3n-3 β-oxidation was every time higher than 18:2n-6. This indicate that 18:3n-3 is a favored substrate for β-oxidation. This result is similar with earlier publishing data on different species fed diets with a high content of 18C PUFA (Francis et al., 2009; Senadheera et al., 2011).

The elongase, Δ-6 and Δ-5 desaturase enzymes are necessary for the biosynthesis of 18C PUFA up to long chain PUFA. Δ-6 desaturase is known to be a single enzyme required twice within the long chain PUFA biosynthetic pathway, and acting on both n-6 and n-3 PUFA (Sprecher et al., 1995). It was probable to measure Δ-6 desaturase transcription rate is effective in increasing the number of double bonds of FAs in the LA series in this study. Unfortunately, the reason why the Δ-5 desaturase enzyme activity, which increases the

Table 8 Apparent in vivo elongation, β-oxidation and deposition (detected by the whole body FA balance method) (nmol/g of fish/day) of specific FAs in *O.mykiss* fed with FO, SO, LO and OO diets for 60 days

	Fish groups			
FAs	FO	SO	LO	OO
18:2n-6				
Elongase (18:2n-6→20:2n-6)	29.31±0.39^{d}	118.48±9.56^{a}	66.54±1.28^{b}	52.61±0.89^{c}
Desaturation (18:2n-6→18:3n-6)	23.47±0.12^{d}	148.19±12.54^{a}	96.77±9.10^{b}	63.84±3.27^{c}
β-oxidation	45.12±3.01^{d}	117.22±10.93^{a}	93.68±12.10^{b}	78.14±6.09^{c}
Deposited	193.18±28.42^{a}	ND	187.8±29.65^{b}	5.04±0.18^{c}
18:3n-3				
Elongase (18:3n-3→20:3n-3)	9.78±0.21^{c}	23.69±1.12^{b}	163.05±36.20^{a}	13.79±0.26^{c}
Desaturation (18:3n-3→18:4n-3)	75.75±6.91^{c}	281.07±22.06^{b}	720.76±56.18^{a}	52.58±1.99^{c}
β-oxidation	113.76±14.07^{d}	216.42±25.24^{c}	271.59±36.43^{a}	237.97±19.21^{b}
Deposited	133.57±19.54^{a}	135.95±26.15^{a}	ND	100.83±12.11^{b}
20:5n-3				
Elongase (20:5n-3→22:5n-3)	486.18±42.06^{a}	215.03±14.79^{c}	350.12±34.27^{b}	68.41±4.09^{d}
β-oxidation	226.17±14.07	ND	ND	ND
22:2n-6				
β-oxidation	10.56±0.76^{d}	95.01±16.12^{a}	21.77±0.98^{c}	51.29±1.29^{b}
22:6n-3				
Deposited	925.29±76.49^{a}	188.72±18.97^{c}	381.39±26.09^{b}	62.49±2.18^{d}

ND: Not detected.

Data = mean ± SD of three replicates (with each mean based on the analysis of 10 fish). Means with different superscript letter in a row are significantly different (P<0.05).

FO: Fish oil, SO: Sesame oil, LO: Linseed oil, OO: Olive oil.

number of double bonds in the LNA series, cannot be analyzed genetically is due to the sequence of rainbow trout Δ-5 desaturase is unknown. Similar approaches have been reported by Thanuthong et al. (2011a). The Δ-6 desaturase is the first enzyme related to the conversion of 18C PUFA to more unsaturated long chain PUFA. This enzyme converts 18:2n-6 and 18:3n-3 to 18:3n-6 and 18:4n-3, respectively. In fish liver of all groups devoid of fish oil, the magnitude of mRNA of Δ-6 desaturase abundance was higher compared to the fish livers fed with FO diet in the present investigation. In earlier studies (Tocher, 2003; Torstensen & Tocher, 2010; Pratoomyot, 2010) it was determined that Δ-6 desaturase enzyme transcription rate increased due to the addition of vegetable oil to diets. In addition, 18:2n-6 FA was found in the highest ratio in SO diet, lowest ratio in the FO diet and similar ratio in the LO and OO dietary groups. The Δ-6 desaturase gene expression level (Figure 3) was examined and it was found similar to the 18:2n-6 levels in the feed. This is due to the Δ-6 desaturase activity was influenced by substrate availability. Similar results with rainbow trout were reported by different researchers (Tocher, 2003; Torstensen & Tocher, 2010). Thanuthong et al., (2011b) stated that in the livers of fish under all treatments deprived of fish oil, the magnitude of mRNA of Δ-6 desaturase abundance was higher than in the livers of fish fed the FO diet. It was observed that the elongase (ELOVL5) transcription rate was statistically higher in fish fed with FO diet, rich in HUFA and lower in fish fed with OO diet, rich in 18:1n-9, in conducted study. Additionally, elongase transcription rate was found similar in fish fed with LO, containing 18:3n-3 and SO dietary groups, containing 18:2n-6. Thanuthong et al., (2011b) reported that the transcription rate of elongase was lower in fish fed P28 (mixture of beef tallow, linseed oil and sunflower oil) compared to fish fed P67 (mixture of linseed oil and sunflower oil), but in general no differences were noted between fish fed FO and fish oil-deprived diets. Different results have been reported for replacement of marine fish oil with alternative lipids on elongase enzyme activity in rainbow trout. The transcription rate of elongase is not clarified yet, because of the different results from the studies, occasionally suggesting that the replacement of marine fish oil increases the transcription rate of elongase, where in different cases no modification was noted (Tocher et al., 2006; Miller et al., 2008; Pratoomyot et al., 2008; Thanuthong et al., 2011a).

In conclusion, this paper indicates that the fully addition of SO, LO and OO to diets had no negative effect on growth and feed efficiency. Based on the data of the growth performance and chemical analysis of the research, sesame, linseed and olive oils can be fully added instead of fish oil for rainbow trout diets. The calculations of the whole body FA balance method shows that *O. mykiss* is able to elongate and desaturate EFAs with good efficiency. Furthermore, this investigation present that the final n-3 PUFA ratio in fish fed vegetable oil diets may be increased by the addition of 18:3n-3 and the increased level of total dietary 18:2n-6, the balancing of dietary FAs with an appropriate content of saturated and monounsaturated FAs is important for the best growth. When formulating aquafeed towards maximising the potential in vivo n-3 long chain PUFA biosynthesis in cultured fish, priority should be given to using alternative oil sources rich in 18:3n-3 irrespective of any concern as to their 18:2n-6 content. It is concluded that future studies should be conducted on genetic research aiming to increase the enzymes that function in biosynthesis of FAs.

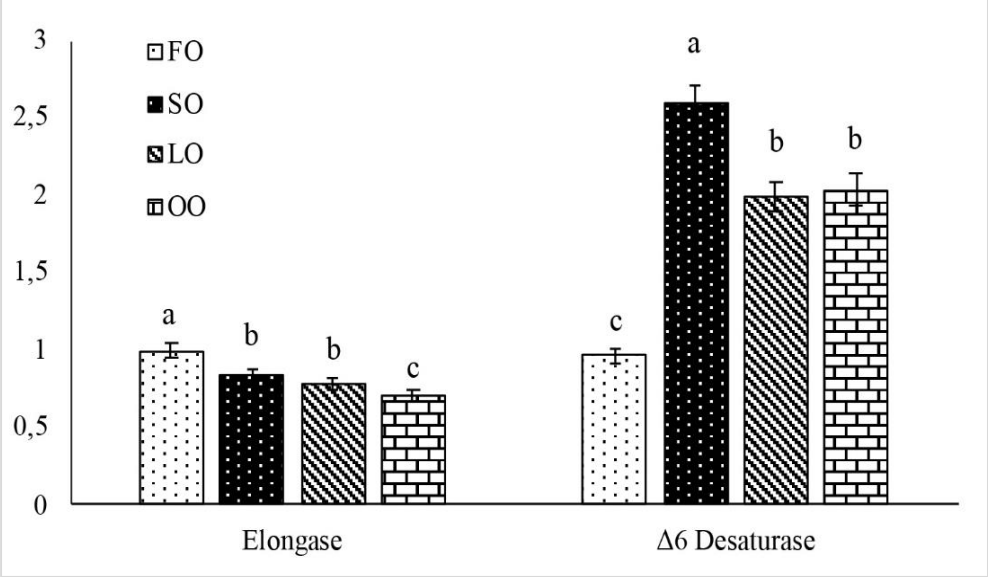

Figure 3. Differential gene expression of FA Δ-6 desaturase and elongase in the liver of rainbow trout fed with FO, SO, LO and OO experimental diets[a]. [[a]Data presented as mean±SD (n = 3). Means with different superscript letter in a row are significantly different (P<0.05)]

Ethical Statement

All animal experiments were conducted in accordance with the approval of Istanbul University Animal Experiments Ethics Committee (No: 2013/61).

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Author Contribution

Second author was consulted in the study. First and second authors planned the experiments. First author carried out the animal experiment. First author performed the analysis of fish and feed samples. First and Third authors performed the analysis of gene expressions. The first and second authors evaluated the data. First author wrote the manuscript in consultation with second author.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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