www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 18: 891-903 (2018) DOI: 10.4194/1303-2712-v18_7_07

RESEARCH PAPER

Effects of Alternative Oil Sources on Growth Performance, Lipid Metabolism and mRNA Level of Some Genes in Juvenile Black Sea Trout (*Salmo trutta labrax* Pallas,1811)

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Received 07 July 2017
Accepted 19 October 2017

Abstract

Black Sea trout (*Salmo trutta labrax* Pallas,1811) fed with diets based on 100% fish oil (FO), 67-33%, 50-50% and 33-67% soybean oil-linseed oil (SO-LO) during 90 days were affected in terms of weigth gain, specific growth rate, feed conversion ratio and hepatosomatix index significantly ($P<0.05$) while the survival rate was not affected at all. Tissue fatty acid profiles of the individuals were affected by the degree of dietary treatments ($P<0.05$). For instance, the highest linoleic acid (18:2n-6, LA) content of the muscle and liver tissues were exhibited in the group fed with 67-33 % SO-LO diet, where the highest linolenic acid (18:3n-3, LNA) was detected in the group fed with 33-67% SO-LO diet ($P<0.05$). In contrast to linoleic and linolenic acids, the highest values of palmitic (18:0), eicosepentaoneic (20:5n-3, EPA) and docohecsaenoic acids (22:6n-3, DHA) were indicated in group fed with fish oil based diet ($P<0.05$). Gene expression of desaturase in the muscle and liver tissues of individuals fed with 33-67% SO-LO included feeds were higher than those fed with FO. Similarly, gene expression of elongase both in the liver and muscle in individuals fed with (50-50% SO-LO, 33-67% SO-LO) and (50-50% SO-LO, 67-33% SO-LO) respectively were higher than those fed with FO. As a result, it was determined that use of 50-50% SO-LO in Black Sea trout diets is acceptable without an adverse effect on growth and feed conversion, and also this species have a capability of conversion 18:2n-6 to 20:4n-6 and 18:3n-3 to 22:6n-3.

Keywords: Black Sea trout, vegetable oil, fatty acids, desaturase, elongase.

Introduction

Fish production from aquaculture in the World constitutes 44.1 % of total production (capture fisheries and aquaculture) in 2014 in proportion to 2012, 2004, 2000 and 1990 which were expounded as 42.1 %, 31.1 % 25.7 %, 13.4 % respectively. World aquaculture production, achieve growth, even if exhibits slow growth rate (FAO, 2014; FAO, 2016) is seriously based on the availability of fish oil and fish meal (Petterson, 2010). In spite of increase in global fish oil consumption supplied by fish farming sector, fish oil amount added in fish feeds is decreasing consistently. The main reason of this decline is the decrease in fish oil amount supplied by catching and the increase in use of alternative cheaper oil sources such as animal and vegetable oils (Molnar *et al*. 2012). Due to the only lipid source in fish feeds is provided from wild fish, use of fish oil has became one of the most important problems of the aquaculture sector (Petterson, 2010). This is one of the major factors restricting development of fish farming (Almaida-Pagan *et al*. 2007). In recent years, intensive researches have been conducted to find sustainable alternatives to fish oil.

The best sustainable alternative to fish oil can be vegetable oils rich in C18 polyunsaturated fatty acids (PUFA) such as linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, but devoid of the n-3 HUFA (EPA, DPA and DHA) abundant in fish oil (Tocher *et al*. 2006; Toretensen *et al*. 2008; Gonzalez-Rovira, Mourente, Zheng, Tocher & Pendon, 2009; Aminikhoei, Choi, Lee & Kim, 2013). Because of easily accessible and high economic value of vegetable oils, production is increasing consistently (Singh *et al*. 2012). When compared with fish oil, vegetable oil is rich in nutrients, highly commercial also low in price as an advantage (Jonasson, 2008). Dietary fatty acid composition which may directly affects fish lipid metabolism and the tissue lipid composition can be altered by replacing fish oil with vegatable oil (Torstensen and Tocher, 2010). Several studies have shown the partial or complete replacement fish oil by vegetable oils can use in the different fish species (Guler and Yıldız, 2011; Kenari, 2011; Arslan, Sirkecioglu, Bayir, Arslan & Aras,

2012; Eroldogan *et al*. 2012; Ozsahinoglu *et al*. 2013; Yones, El-Saidy & Abdel-Hakim, 2013).

In present study, it is intented to determine effects of soybean oil and linseed oil used in place of fish oil on growth performance, fatty acid composition in the muscle and liver tissues, and expression levels of Δ6 desaturation ve elongation gene participated in the arachidonic acid (20:4n-6, AA), eicosapentaoneic acid (20:5n-3, EPA) and dokosahexaenoic asit (22:6n-3, DHA) synthesis from C18 PUFA such as linoleic and linolenic acids.

Materials and Methods

Fish and Maintenance

The study was carried out in freshwater Recirculating Aquaculture Systems (RAS). Juvenile Black Sea trouts (F4; 4th generation) (1.40±0.01g) were placed ramdomly in 50 L tanks and each tank was included 60 fish. Trial was carried out in triplicate. Fish were fed 3% of body weight/day at four times. Water temperature (15.53±0.85ºC) and mortality were recorded daily. Ammonia (0.07±0.07 mg/l), oxygen (8.83±0.22 mg/l) and pH (7.56±0.13) were measured weekly. Water in tanks was changed 20 times in a day. The cleaning of tanks were done by siphoning daily.

Experimental Diets

Diets were prepared using fish oil and vegetable oil, and oil content was adjusted as 15%. The control diet prepared with only cod liver oil. Soybean oil (SO) rich in linoneic acid (18:2n-6, LA) and linseed oil (LO) rich in linolenic acid (18:3n-3, LNA) were used in trial diets as vegetable oil sources at different rates: 67% soybean oil-33% linseed oil (67-33 SO-LO), 50% soybean oil-50% linseed oil (50-50 SO-LO), 33% soybean oil-67% linseed oil (33-67 SO-LO). Control diet was formulated by means of 100% fish oil (FO). (Table 1)

Chemical Analyses

Chemical analyses of diets were performed according to the standard methods of AOAC (1990): Crude protein was determined by the Kjeldhal procedure (N x 6.25), moisture by drying samples to constant weight at 105ºC, and ash content by incineration at 550ºC for 12 h in a muffle furnace.

Evaluation of Fish Performance

The growth performance of the fish was calculated by the following equations:

Table 1 Formulation and proximate composition of experimental diets (%)

	Experimental Diets			
Ingredients	FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO
Casein (vitamin-free)	43.19	43.19	43.19	43.19
Gelatin	8.64	8.64	8.64	8.64
Dextrin[1]	6.25	6.25	6.25	6.25
Wheat meal	14	14	14	14
CPSP90[2]	5	5	5	5
Cod liver oil	15	-	-	-
Soybean oil	-	10	7.5	5
Linseed oil	-	5	7.5	10
Vitamin mixture[3]	2	2	2	2
Mineral mixture[4]	3	3	3	3
Askorbic acid	0.06	0.06	0.06	0.06
CMC[5]	1	1	1	1
L-arginine	0.05	0.05	0.05	0.05
L-methionine	0.04	0.04	0.04	0.04
L-lysine	0.8	0.8	0.8	0.8
Coline chloride	0.16	0.16	0.16	0.16
Proximate composition				
Crude protein	55.25	55.68	54.29	54.52
Crude lipid	15.89	15.79	15.90	15.87
Ash	5.07	4.44	4.67	4.71
Moisture	5.10	5.06	6.44	6.92

[1]Water soluble 80%

[2]CPSP90:Concentrate of fish soluble protein (crude protein, 82-84% WW; crude lipid, 9-13% WW), Sopropéche S.A., Boulogne-sur-mer, France.

[3]Roche Performance Premix (Hoffman-La Roche, INC., Nutley, N.J., USA), composition per g of the vitamin mixture: vitamin A, 2645,50 IU; vitamin D3, 220,46 IU, vitamin E, 44,09 IU; vitamin B,12 13 mg; riboflavin,13,23 mg; niacin, 61,73 mg; d-pantothenic asit, 20,05 mg; menadione, 1,32 mg; folic asit, 1,76 mg; thiamin, 7,95 mg ve d-biotin, 0,31 mg.

[4]Bernhart Tomarelli salt mixture (ICN Pharmaceuticals, Costa Mesa, CA, USA), composition (g/100 g): calsium carbonate , 2.1; calsium phosphate dibasic, 73.5; citric acid, 0,.27; cupric citrate, 0.046; ferric citrate, 0.558; magnesium oxide, 2.5; manganese citrate, 0.835; potassium iodide, 0.001; potassium phosphate dibasic, 8.1; potassium oxide, 6.8; sodium chloride, 3.06; sodium phosphate, 2.14; and zinc citrate, 0.133.

[5]Carboximetilecellulose.

Weight gain (WG) (%) = [(final weight – initial weight) / initial weight (g)] x 100

Specific growth rate (SGR) (% body weight/day) = [(ln final weight − ln initial weight) /trial period (days)] × 100

Feed conversion ratio (FCR) = Total daily feed intake (g) / Weight gain (g)

Survival (%) = (final number of fish / initial number of fish) x 100, (Ebrahimi and Ouraji, 2012)

Hepatosomatic index (HSI) (%) = (liver weight / body weight) x 100, (Piedecausa, Mazon, Garcia & Hernandez 2007).

Tissue Samples

Tissue samples (muscle and liver) for the fatty acid analyses were collected randomly from the begining (representing the main stock) following the 30th, 60th and 90th days of (from each replicate) the experiment. All samples were stored at –80ºC. Tissue samples (muscle and liver) for determination of elongation and Δ6 desaturation gene expression were put in cryo tubes (1.5 ml) on 30th, 60th, 90th days of experiment, and added RNA later stabilizasyon reagent solution. All samples stored at –80ºC.

Fatty Acid Analysis

Total lipid was extracted from feed samples and tissues (liver and muscle) of fish by homogenization in chloroform/methanol (2/1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, in accordance with the procedure of Folch, Less and Stanley (1957). Fatty acid methyl esters (FAMEs) were prepared in accordance with methods found by Metcalfe and Schmitz (1961), and analyzed as described previously by Czesny and Dabrowski (1998). The obtained FAMEs were determined by gas chromatography (Agilent 6890 N) equipped with a flame ionization detector and fitted with a DB 23 capillary column (60 m, 0.25 mm i.d. and 0.25 μm). The carrier gas was hydrogen (2ml min^{-1}) and the split ratio was 30:1. The idendification of the individual fatty acids were determined by comparison of their retention times to the standard mix of fatty acids (Supelco 37 component FAME mix).

Gene Expression

Muscle and liver tissue samples were added in RNA later solution and stored at -80ºC prior to RNA extraction. The isolation of total mRNA from these tissues was carried out with RNeasy Lipid Tissue Mini Kit (Qiagen cat. no. 74804) via Qiacube robot (Qiagen, Hilden, Germany). Isolated RNA was quantified by measuring absorbance at 260-280 nm in a spectrophotometer (Nanadrop-Multıskan go-Thermo). Using 1/RNA concentration equality for cDNA synthesis from total RNA, RNA amount was determined. Tubes were added 1 μl of oligo $DT_{(20)}$(50 μm), 1 μl of 10mM dNTP and 11 μl RNA and ddH_2O. The reactions were incubated at 65ºC for 5 min. Then the tubes were added 4 μl of 5x First strand Buffer, 1 μl of 0.1 M DTT, 1 μl of RNAse out™ ve 1μl of sperscript III. The reactions were incubated at 50ºC for 45 min, and then 70ºC for 15 min. All cDNA (20 μL) were kept at -20ºC before quantitative PCR (qPCR). For real time PCR, tubes were added 15 μl of ddH_2O, 25 μl of FastStart TaqMan® Probe, 1 μl of hydrolis prob, 2 μl of forward primer and 2 μl of reverse primer. Tubes were added 45 μl from mix, and then 5 μl of cDNA. The expression of fatty acid desaturase and elongase genes in tissues were studied by quantitative (real time) PCR (Rotor-gene Q–Qıagen). The mRNA levels of the genes were normalized with β-actin for each sample and calculated according to Ct values (Pfaffl, 2001).

$$\text{Ratio} = (E_{\text{target}})^{\Delta CT\text{target (control-sample)}}_{\text{(control-sample)}} / (E_{\text{reference}})^{\Delta CT\text{reference}}$$

The PCR primers were designed from rainbow trout sequence of desaturase (Genbank accession no: AF301910.1), elongase (Genbank accession no: AY605100.1) (Sirkecioglu, 2011) and β-actin (Genbank accession no: AF254414) (Johansen and Overturf, 2005). For desaturation, forward and reverse primers were 5′-ACCTAAAGGGTGCCTCTGCT-3′ and 5′-TTGTCTCCCAGGACGAAGAC-3′. For elongation, forward and reverse primers were 5′-TCTTACTATGGGCTCTCTGCT-3′ and 5′-AGAAAAGGGCAATAAGTGTGA-3′. For β-Actin, forward and reverse primers were 5′-TGGCCGTACCACCGGTAT-3′ ve 5′-GCAGAGCGTAGTCCTCGTAGATG-3′. β-Actin was used as a housekeeping gene for normalising mRNA levels of the target genes.

Statistical Analysis

Four different diets were used, and measurements were made in triplicate in the dietary groups in present study. Data were analyzed by one-way analysis of variance. Differences between means were compared using Duncan's multiple range test. Differences were considered statistically significant at $P<0.05$. All statistical analyses were computed using SPSS 21.

Results

Fatty Acid Composition of the Diets

In present study, 18:0, ∑SFA, ∑MUFA, AA (20:4n-6), EPA (20:5n-3), DHA (22:6n-3), ∑HUFA,

EPA+DHA ve n-3/n-6 contents of fish oil diet (FO) were higher than those in vegetable oil (VO) diets, while LA (18:2n-6) ve LNA (18:3n-3) levels in the vegatable oil diets were higher than those in FO. 67-33 SO-LO diet has the highest level of LA (Table 2).

Growth Performance

At the end of trial, final weight, weight gain, SGR, FCR ve HSI were significantly influenced by dietary treatments ($P<0.05$), where the survival rate was not affected at all ($P>0.05$). Final weight, weight gain and SGR values were the highest in FO and 50-50 SO-LO diets, but the lowest in the 33-67SO-LO diet. 33-67 SO-LO diet had significantly higher FCR than those fed with FO, the 67-33 SO-LO and the 50-50 SO-LO diets ($P<0.05$). HSI was similar in fish fed the FO, the 67-33 SO-LO ve the 33-67 SO-LO (Table 3).

Fatty Acid Composition of Fish Tissues

The fatty acid composition of fish tissues (muscle and liver) is demostrated in Table 4 and Table 5. In fish tissues (muscle and liver) and all experimental groups, palmitic acid (16:0) in ∑SFA, oleic acid (18:1n-9) in ∑MUFA and linoleic acid (18:2n-6, LA) in ∑n-6 PUFA were the predominant fatty acids. The highest percentage of ∑SFA was 16:0 (palmitic acid), followed by 18:0 (stearic acid) for all dietary oils ($P<0.05$). While the fish fed with 67-33SO-LO and 33-67SO-LO based diets had the highest oleic acid in the liver tissue, fish fed with FO and 67-33SO-LO based diets had the highest oleic acid content in the muscle tissue. Moreover, fish fed the FO diet had the highest ∑SFA, ∑MUFA and ∑HUFA contents, but ∑n-6 PUFA the lowest. While LNA level in the liver tissue increased with the increasing inclusion of linseed oil, AA level in the liver tissue increased with the increasing inclusion of

Table 2 Fatty acid composition (% of total fatty acids) of experimental diets

	Experimental Diets			
Fatty acids	FO	67-33SO-LO	50-50SO-LO	33-67SO-LO
C14:0	4.81±0.06	0.36±0.03	1.62±0.08	0.40±0.07
C15:0	0.40±0.01	0.12±0.05	0.15±0.02	0.05±0.02
C16:0	13.39±0.28	8.88±0.32	9.70±0.13	7.34±0.09
C17:0	0.99±0.15	0.14±0.01	0.22±0.14	0.22±0.11
C18:0	3.02±0.16	3.95±0.23	3.66±0.05	3.65±0.11
C20:0	0.32±0.07	0.26±0.02	0.25±0.03	0.26±0.07
∑ SFA	22.93±0.53	13.71±0.51	15.60±0.30	11.92±0.02
C14:1	0.36±0.04	0.14±0.11	0.12±0.02	0.08±0.07
C15:1	0.23±0.03	0.13±0.09	0.06±0.02	0.07±0.04
C16:1n-7	7.22±0.09	0.31±0.03	2.29±0.15	0.30±0.03
C17.1	0.74±0.24	0.10±0.03	0.25±0.05	0.13±0.06
C18:1n-7	4.96±0.19	1.27±0.02	2.22±0.11	0.74±0.64
C18:1n-9	20.55±0.87	19.96±0.29	19.67±0.07	17.92±0.17
C20:1n-9	2.33±0.03	0.10±0.01	0.75±0.05	0.13±0.02
C20:1n-11	0.63±0.10	0.04±0.01	0.20±0.01	0.08±0.09
C22:1n-9	0.05±0.01	0.06±0.03	0.09±0.03	0.25±0.21
C22:1n-11	0.98±0.01	0.05±0.00	0.34±0.03	0.09±0.01
C24:1n-9	0.69±0.18	0.08±0.07	0.19±0.01	0.05±0.01
∑ MUFA	38.74±0.48	22.24±0.36	26.18±0.45	19.84±0.73
C18:2n-6	3.58±0.11	39.13±0.27	25.23±0.70	27.95±0.29
C18:3n-6	0.26±0.01	0.27±0.00	0.21±0.02	0.28±0.07
C20:2n-6	0.52±0.19	0.06±0.01	0.18±0.07	0.06±0.01
C20:3n-6	0.29±0.17	0.05±0.03	0.17±0.19	0.05±0.00
C20:4n-6	0.64±0.09	0.04±0.00	0.26±0.12	0.05±0.01
C22:2n-6	0.56±0.03	0.09±0.03	0.16±0.02	0.02±0.02
C22:4n-6	0.33±0.11	0.16±0.11	0.07±0.01	0.16±0.14
∑ n-6 PUFA	6.18±0.26	39.80±0.14	26.28±0.45	28.57±0.49
C18:3n-3	1.51±0.07	22.93±0.32	29.27±0.71	38.24±0.33
C18:4n-3	2.71±0.12	0.07±0.01	0.91±0.05	0.33±0.20
C20:3n-3	1.22±0.20	0.03±0.01	0.28±0.03	0.04±0.01
C20:4n-3	0.39±0.16	0.05±0.03	0.08±0.01	0.05±0.00
C20:5n-3	10.40±0.13	0.31±0.01	0.28±0.16	0.34±0.06
C22:5n-3	1.76±0.21	0.38±0.39	0.55±0.03	0.17±0.02
C22:6n-3	14.24±0.32	0.47±0.06	0.49±0.26	0.50±0.09
∑ n-3 PUFA	32.23±0.08	24.24±0.67	31.86±0.27	39.67±0.23
∑ HUFA	30.35±0.43	1.64±0.46	2.52±0.88	1.44±0.09
n-3/n-6 PUFA	5.22±0.24	0.61±0.02	1.21±0.01	1.39±0.02
EPA+DHA	24.64±0.39	0.78±0.08	0.77±0.42	0.84±0.16

Table 3. Growth performance, survival and hepatosomatic index of Black Sea trout juveniles fed diets with different dietary lipid resources during 90 days

Days	Growth parameters	Experimental Diets			
		FO	67-33SO-LO	50-50SO-LO	33-67SO-LO
30th	Final weight (g)	2.80±0.16^{b}	2.71±0.09^{b}	3.07±0.07^{a}	2.73±0.11^{b}
	Weight gain (%)	199.42±10.58^{b}	192.84±9.27^{b}	218.30±7.34^{a}	193.97±7.52^{b}
	SGR (%)	2.22±0.17^{b}	2.12±0.16^{b}	2.52±0.11^{a}	2.14±0.13^{b}
	FCR	1.10±0.12^{ab}	1.14±0.12^{a}	0.93±0.04^{b}	1.14±0.13^{a}
	Survival rate (%)	98.89±1.92^{a}	99.33±1.15^{a}	100.00±0.00^{a}	98.33±1.53^{a}
	Hepatosomatic index	2.15±0.08^{a}	1.98±0.34^{a}	2.45±0.28^{a}	2.17±0.40^{a}
60th	Final weight (g)	4.21±0.26^{b}	4.09±0.24^{b}	4.74±0.32^{a}	3.84±0.19^{b}
	Weight gain (%)	318.60±43.65^{a}	295.30±15.03^{a}	324.32±49.48^{a}	283.08±26.18^{a}
	SGR (%)	1.92±0.23^{a}	1.80±0.09^{a}	1.95±0.27^{a}	1.73±0.15^{a}
	FCR	1.15±0.15^{a}	1.35±0.11^{a}	1.22±0.15^{a}	1.34±0.19^{a}
	Survival rate (%)	97.22±0.69^{a}	92.33±4.04^{a}	93.00±5.00^{a}	93.00±3.61^{a}
	Hepatosomatic index	1.50±0.60^{a}	1.44±0.44^{a}	1.49±0.08^{a}	1.75±0.06^{a}
90th	Final weight (g)	7.05±0.52^{a}	6.71±0.38^{ab}	7.08±0.76^{a}	5.82±0.28^{b}
	Weight gain (%)	502.22±37.14^{a}	478.29±34.35^{ab}	503.98±54.35^{a}	414.13±17.99^{b}
	SGR (%)	1.77±0.08^{a}	1.72±0.08^{ab}	1.77±0.12^{a}	1.56±0.05^{b}
	FCR	1.02±0.07^{c}	1.12±0.02^{b}	1.06±0.06^{bc}	1.30±0.04^{a}
	Survival rate (%)	97.22±0.69^{a}	92.33±4.04^{a}	93.00±5.00^{a}	93.00±3.61^{a}
	Hepatosomatic index	2.12±0.09^{a}	1.95±0.09^{ab}	1.81±0.06^{b}	1.99±0.14^{ab}

Means with different superscript letters in a row are significantly different (P<0.05)

soybean oil. Besides LA and LNA levels in both tissues of fish fed with VO diets had the higher than those fed with FO diet. Whereas EPA (20:5n-3), DHA (22:6n-3), EPA+DHA, n–3/n-6 and ∑HUFA levels in muscle and liver tissues of fish fed with FO diet were the higher than those fed with VO (P<0.05). In comparison to initial level, while LNA level in the muscle and liver tissues of fish fed with VO diets increased significantly, EPA and DHA decreased. But there was an opposite situation in fish fed with FO diet. Among the fish fed with diets with vegetable oil, the highest total HUFA level in both tissues was in 67-33SO-LO diet group at 30th day, but at 60th and 90th days in 50-50SO-LO diet group. In all sampling days, the n-3/n-6 rate of the muscle and liver tissues was lowest in fish fed with 67-33SO-LO diet when vegetable oil diets considered in itself.

Gene Expression

Δ6 desaturation and elongation gene expression of muscle and liver tissues were affected by dietary treatments (P<0.05). Fish fed 33-67SO-LO diet had significantly higher Δ6 desaturation and elongation gene expression of muscle and liver tissues than those fed FO (P<0.05). At the end of trial, Δ6 desaturation gene expression of muscle and liver tissues of fish fed 67-33SO-LO and 50-50SO-LO diets were similar those fed FO. The highest elongation gene expression of liver tissue at 60th day was determined fish fed 50-50 SO-LO diet, whereas The highest elongation gene expression of muscle tissue at 60th day was determined fish fed 33-67 SO-LO diet (P<0.05). At the end of trial, Fish fed 67-33SO-LO and 50-50SO-LO diets had significantly higher elongation gene expression of muscle tissue than those fed FO. Moreover, Fish fed 50-50SO-LO and 33-67SO-LO diets had significantly higher elongation gene expression of liver tissue than those fed FO (P<0.05) (Figure 1).

Discussion

Similar to our study, several studies have shown that the replacement of fish oil by vegetable oils can be used in different fish species (Petterson, 2010; Guler and Yıldız, 2011; Kenari, Mozanzadeh & Pourgholam, 2011; Eroldogan *et al*. 2012; Yılmaz and Eroldogan, 2015). In our study, feeding diets containing 50-50 SO-LO had no any adverse effect on growth rate and feed conversion ratio, compared with fish fed completely fish oil diet. A similar result was obtained by Kutluyer *et al*. (2017). In a previous study, Arslan *et al*. (2012) found that Brown trout (*Salmo trutta)* fed with diets including a blend of soybean and linseed oils for 6 weeks had the greatest growth performance. The 30th day results of our study were similar to the results of Arslan *et al*. (2012), but after the 60th day of trial, the difference between the FO and 50-50SO-LO diet groups became insignificant. In an other study, Aminikhoei *et al*. (2013) found no significant differences in growth performance of juvenile rockfish (*Sebastes schlegeli*) fed with diets containing either fish oil, soybean oil, linseed oil, or a mixture of SO and LO for 8 weeks. Besides, similar to results in the 60th days of our study, Sirkecioglu (2011) revealed that growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed with 100% linseed oil for 8 weeks were similar to those fed with 100% fish oil. However, according to the results in the 90th day of our study, the lowest growth performance was seen in fish fed

Table 4. Fatty acid composition (% of total fatty acids) of muscle tissue Black Sea trout juveniles fed diets with different dietary lipid resources during 90 days

Fatty acids	Initial	30th days				60th days				90th days			
		FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO	FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO	FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO
C14:0	2.88±0.02	3.28±0.14^{a}	1.25±0.02^{ab}	1.16±0.02^{b}	1.37±0.10^{b}	3.11±0.18^{a}	0.86±0.03^{b}	1.25±0.12^{b}	0.89±0.07^{b}	2.83±0.31^{a}	0.89±0.07^{ab}	1.20±0.19^{b}	0.76±0.12^{b}
C15:0	0.55±0.08	0.34±0.02^{a}	0.14±0.01^{b}	0.13±0.01^{b}	0.14±0.01^{b}	0.31±0.03^{a}	0.14±0.02^{b}	0.14±0.01^{b}	0.11±0.01^{c}	0.25±0.03^{a}	0.25±0.13^{a}	0.28±0.14^{a}	0.50±0.30^{a}
C16:0	18.75±0.61	15.63±0.29^{a}	12.96±0.12^{b}	11.77±0.24^{d}	12.41±0.32^{c}	15.13±0.24^{a}	10.98±0.35^{c}	12.02±0.42^{b}	10.22±0.07^{d}	15.85±0.85^{a}	11.92±0.92^{bc}	12.35±1.37^{b}	10.32±0.57^{c}
C17:0	0.45±0.04	0.56±0.01^{a}	0.24±0.02^{c}	0.23±0.01^{c}	0.26±0.01^{b}	0.48±0.03^{a}	0.21±0.01^{c}	0.35±0.09^{b}	0.22±0.02^{c}	0.39±0.04^{a}	0.17±0.01^{b}	0.39±0.25^{a}	0.49±0.33^{a}
C18:0	4.70±0.39	3.71±0.24^{b}	4.35±0.01^{a}	4.27±0.27^{a}	4.22±0.24^{a}	3.75±0.30^{b}	4.22±0.13^{a}	4.21±0.27^{a}	3.93±0.10^{ab}	3.87±0.03^{b}	4.55±0.14^{a}	4.33±0.17^{a}	4.54±0.25^{a}
C20:0	0.52±0.33	0.28±0.10^{b}	0.15±0.01^{b}	0.11±0.01^{b}	0.14±0.01^{b}	0.07±0.01^{b}	0.15±0.01^{a}	0.14±0.01^{a}	0.16±0.06^{a}	0.22±0.06^{a}	0.18±0.01^{a}	0.59±0.62^{a}	0.46±0.27^{a}
∑ SFA	27.85±0.65	23.80±0.49^{a}	19.09±0.14^{b}	17.67±0.48^{c}	18.54±0.67^{bc}	22.85±0.33^{a}	16.56±0.49^{c}	18.11±0.65^{b}	15.53±0.12^{d}	23.41±0.77^{a}	17.96±1.09^{bc}	19.14±1.43^{b}	17.07±0.48^{c}
C14:1	0.68±0.38	0.19±0.02^{a}	0.07±0.02^{b}	0.06±0.01^{b}	0.09±0.03^{b}	0.17±0.01^{a}	0.11±0.05^{a}	0.09±0.02^{ab}	0.06±0.01^{c}	0.31±0.21^{a}	0.24±0.07^{a}	0.74±0.81^{a}	0.81±0.69^{a}
C15:1	0.55±0.24	0.17±0.02^{a}	0.18±0.08^{a}	0.16±0.05^{a}	0.11±0.02^{a}	0.14±0.03^{a}	0.14±0.04^{a}	0.16±0.05^{a}	0.11±0.01^{a}	0.18±0.07^{a}	0.21±0.03^{a}	0.51±0.36^{ab}	1.08±0.74^{a}
C16:1n-7	3.46±0.05	5.39±0.24^{a}	0.94±0.61^{b}	0.94±0.58^{b}	1.71±0.11^{b}	5.41±0.30^{a}	1.09±0.06^{c}	1.60±0.16^{b}	1.11±0.07^{c}	5.16±0.36^{a}	0.83±0.11^{b}	1.74±0.61^{b}	1.12±0.83^{b}
C17.1	0.68±0.27	0.48±0.02^{a}	0.11±0.01^{c}	0.12±0.01^{bc}	0.14±0.02^{b}	0.43±0.01^{a}	0.16±0.03^{bc}	0.16±0.02^{b}	0.13±0.01^{c}	0.58±0.16^{a}	0.12±0.01^{b}	0.42±0.26^{ab}	0.41±0.24^{ab}
C18:1n-7	3.05±0.13	4.04±0.37^{a}	1.87±0.05^{b}	1.73±0.14^{b}	1.83±0.09^{b}	4.53±0.10^{a}	1.52±0.01^{c}	2.07±0.08^{b}	1.41±0.01^{c}	4.45±0.18^{a}	1.56±0.02^{c}	2.08±0.11^{b}	1.37±0.14^{c}
C18:1n-9	11.96±0.27	16.11±0.13^{c}	17.07±0.36^{b}	18.33±0.14^{a}	16.74±0.70^{bc}	19.98±1.09^{a}	18.32±0.17^{b}	17.28±0.04^{b}	17.47±0.01^{b}	18.89±0.58^{a}	18.01±0.34^{a}	14.99±1.56^{b}	13.75±1.76^{b}
C20:1n-9	0.61±0.24	1.28±0.02^{a}	0.16±0.02^{b}	0.17±0.06^{b}	0.17±0.01^{b}	1.32±0.07^{a}	0.16±0.01^{c}	0.30±0.01^{b}	0.11±0.02^{c}	1.25±0.05^{a}	0.11±0.01^{c}	0.49±0.19^{b}	0.72±0.23^{b}
C20:1n-11	0.33±0.09	0.39±0.02^{a}	0.11±0.01^{b}	0.08±0.01^{c}	0.10±0.01^{bc}	0.39±0.03^{a}	0.13±0.04^{b}	0.12±0.01^{b}	0.07±0.01^{c}	0.36±0.02^{a}	0.13±0.05^{c}	0.34±0.17^{bc}	0.59±0.14^{a}
C22:1n-9	0.84±0.49	0.47±0.02^{a}	0.07±0.02^{c}	0.07±0.01^{c}	0.13±0.01^{b}	0.32±0.10^{a}	0.21±0.09^{a}	0.15±0.01^{a}	0.21±0.12^{a}	0.42±0.13^{a}	0.18±0.06^{a}	0.51±0.32^{a}	1.03±0.33^{a}
C22:1n-11	0.55±0.02	0.63±0.01^{a}	0.21±0.02^{c}	0.19±0.02^{c}	0.25±0.02^{b}	0.63±0.03^{a}	0.20±0.02^{b}	0.25±0.02^{b}	0.31±0.16^{b}	0.62±0.02^{a}	0.26±0.07^{b}	0.35±0.11^{a}	0.69±0.42^{a}
C24:1n-9	0.57±0.12	0.52±0.06^{a}	0.24±0.01^{b}	0.22±0.06^{b}	0.26±0.04^{b}	0.46±0.04^{a}	0.18±0.02^{c}	0.24±0.01^{b}	0.24±0.04^{b}	0.48±0.01^{a}	0.25±0.02^{b}	0.58±0.52^{a}	0.62±0.57^{a}
∑ MUFA	23.28±1.93	29.67±0.70^{a}	21.03±0.20^{b}	22.07±0.96^{b}	21.53±0.99^{b}	33.78±1.42^{a}	22.22±0.17^{b}	22.42±0.09^{b}	21.23±0.20^{b}	32.70±0.69^{a}	21.90±0.35^{b}	22.75±3.35^{b}	22.19±2.17^{b}
C18:2n-6	8.09±0.14	4.84±0.03^{d}	24.34±0.57^{a}	21.92±0.87^{b}	18.49±0.37^{c}	6.49±0.05^{d}	27.12±0.06^{a}	19.82±0.73^{c}	21.05±0.18^{b}	5.54±0.17^{c}	26.96±0.99^{a}	17.22±2.30^{b}	16.87±1.88^{b}
C18:3n-6	0.41±0.12	0.16±0.02^{d}	1.32±0.01^{a}	1.10±0.11^{b}	0.81±0.01^{c}	0.22±0.03^{d}	1.55±0.13^{a}	1.14±0.05^{b}	0.98±0.02^{c}	0.22±0.09^{c}	1.28±0.18^{a}	0.56±0.16^{c}	0.75±0.13^{b}
C20:2n-6	0.59±0.07	0.55±0.04^{d}	1.05±0.10^{a}	0.90±0.02^{b}	0.77±0.03^{c}	0.60±0.02^{d}	1.22±0.05^{a}	0.99±0.07^{b}	0.77±0.03^{c}	0.56±0.04^{d}	1.37±0.06^{a}	1.08±0.04^{b}	0.82±0.06^{c}
C20:3n-6	0.37±0.06	0.28±0.02^{d}	1.44±0.03^{a}	1.13±0.02^{b}	0.73±0.01^{c}	0.32±0.02^{d}	1.66±0.06^{a}	1.21±0.02^{b}	0.99±0.03^{c}	0.31±0.02^{c}	1.81±0.08^{a}	1.04±0.16^{b}	0.93±0.07^{b}
C20:4n-6	0.95±0.03	0.87±0.01^{b}	0.97±0.01^{a}	0.63±0.05^{c}	0.55±0.09^{c}	0.68±0.05^{c}	1.11±0.14^{a}	0.86±0.11^{b}	0.51±0.02^{d}	0.73±0.07^{b}	1.07±0.04^{a}	1.02±0.24^{a}	0.75±0.09^{b}
C22:2n-6	0.89±0.62	0.20±0.09^{a}	0.09±0.01^{ab}	0.08±0.02^{a}	0.13±0.08^{ab}	0.11±0.04^{b}	0.80±0.69^{a}	0.14±0.07^{b}	0.06±0.02^{b}	0.36±0.04^{a}	0.18±0.09^{a}	0.79±0.82^{a}	0.70±0.41^{a}
C22:4n-6	1.08±0.16	0.12±0.03^{ab}	0.18±0.06^{ab}	0.09±0.03^{b}	0.26±0.14^{a}	0.14±0.02^{b}	0.24±0.05^{a}	0.24±0.06^{a}	0.12±0.04^{b}	0.32±0.03^{a}	0.28±0.06^{a}	0.64±0.37^{a}	0.95±0.72^{a}
∑ n-6 PUFA	12.38±0.41	7.02±0.04^{d}	29.39±0.58^{a}	25.85±0.95^{b}	21.74±0.52^{c}	8.56±0.09^{d}	33.70±0.79^{a}	24.40±0.57^{b}	24.48±0.07^{b}	8.04±0.27^{c}	32.95±1.18^{a}	22.35±1.74^{b}	21.77±0.80^{b}
C18:3n-3	0.98±0.02	1.16±0.19^{d}	8.76±0.15^{c}	13.52±0.74^{b}	15.68±0.05^{a}	3.46±0.17^{d}	10.26±0.24^{c}	11.87±0.19^{b}	18.81±0.08^{a}	3.27±0.09^{c}	10.74±0.47^{b}	11.56±1.20^{b}	16.45±1.44^{a}
C18:4n-3	0.88±0.06	1.52±0.08^{d}	3.02±0.03^{c}	4.06±0.45^{b}	4.68±0.15^{a}	1.52±0.10^{d}	3.28±0.22^{c}	3.88±0.31^{b}	5.29±0.10^{a}	1.22±0.17^{c}	2.65±0.30^{b}	2.18±0.49^{b}	3.45±0.41^{a}
C20:3n-3	0.72±0.03	0.20±0.01^{a}	0.11±0.01^{b}	0.09±0.02^{c}	0.10±0.01^{bc}	0.19±0.13^{a}	0.38±0.28^{a}	0.09±0.01^{a}	0.09±0.01^{a}	0.97±0.04^{c}	1.01±0.06^{c}	1.18±0.11^{b}	1.49±0.02^{a}
C20:4n-3	1.17±0.26	1.02±0.07^{b}	0.89±0.04^{c}	1.24±0.04^{a}	1.18±0.02^{a}	1.08±0.06^{c}	0.91±0.15^{d}	1.26±0.08^{b}	1.63±0.01^{a}	0.32±0.03^{c}	0.59±0.09^{b}	0.84±0.22^{a}	0.92±0.08^{a}
C20:5n-3	4.75±0.25	5.67±0.16^{a}	2.65±0.16^{c}	2.42±0.06^{c}	2.98±0.25^{b}	4.53±0.11^{a}	1.92±0.06^{d}	3.02±0.08^{b}	2.71±0.05^{c}	4.86±0.66^{a}	1.79±0.18^{c}	3.19±0.48^{b}	3.18±0.07^{b}
C22:5n-3	1.90±0.06	1.99±0.05^{a}	1.15±0.14^{b}	0.95±0.03^{c}	1.11±0.03^{b}	1.79±0.04^{a}	1.14±0.19^{b}	1.23±0.03^{b}	1.15±0.13^{b}	1.84±0.17^{a}	0.87±0.08^{c}	1.26±0.16^{b}	1.16±0.07^{b}
C22:6n-3	26.12±1.71	28.19±0.61^{a}	13.99±0.09^{c}	12.18±0.73^{d}	12.52±1.85^{d}	22.73±0.63^{a}	9.69±1.03^{c}	13.81±0.49^{b}	9.18±0.22^{c}	23.39±0.97^{a}	9.54±0.20^{c}	15.55±3.20^{b}	12.35±0.32^{c}
∑ n-3 PUFA	36.52±1.69	39.75±0.40^{a}	30.57±0.27^{c}	34.46±0.48^{b}	38.25±2.20^{a}	35.30±1.02^{b}	27.58±0.50^{c}	35.16±0.02^{b}	38.86±0.04^{a}	35.88±1.15^{a}	27.19±0.25^{b}	35.76±3.92^{a}	39.00±1.49^{a}
∑ HUFA	38.54±1.30	39.09±0.62^{a}	22.52±0.42^{b}	19.71±0.74^{c}	20.33±1.96^{c}	32.17±1.03^{a}	19.07±0.24^{c}	22.85±0.68^{b}	17.21±0.25^{d}	33.66±1.27^{a}	18.51±0.02^{c}	26.59±3.75^{b}	23.25±1.35^{b}
n-3/n-6PUFA	2.95±0.23	5.66±0.09^{a}	1.04±0.03^{d}	1.33±0.03^{c}	1.76±0.14^{b}	4.12±0.08^{a}	0.82±0.03^{d}	1.44±0.03^{c}	1.59±0.01^{b}	4.46±0.01^{a}	0.83±0.02^{c}	1.60±0.26^{b}	1.79±0.03^{b}
EPA+DHA	30.87±1.96	33.86±0.76^{a}	16.64±0.25^{b}	14.60±0.79^{c}	15.50±2.10^{c}	26.76±0.74^{a}	11.61±1.08^{c}	16.83±0.57^{b}	11.89±0.26^{c}	28.25±1.00^{a}	11.33±0.19^{c}	18.74±3.67^{b}	15.53±0.36^{b}

The results were evaluated in 30th, 60th and 90th days individually, and significant differences between mean values of groups were shown with different superscripts in a row ($P<0.05$).

Table 5. Fatty acid composition (% of total fatty acids) of liver tissue Black Sea trout juveniles fed diets with different dietary lipid resources during 90 days

Fatty acids	Initial	30th days				60th days				90th days			
		FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO	FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO	FO	67-33 SO-LO	50-50 SO-LO	33-67 SO-LO
C14:0	2.16±0.21	2.48±0.12^{a}	1.09±0.02^{b}	1.10±0.15^{b}	1.11±0.04^{b}	2.75±0.08^{a}	0.80±0.03^{c}	1.29±0.29^{b}	0.72±0.05^{c}	2.85±0.24^{a}	1.16±0.17^{bc}	1.26±0.05^{b}	0.96±0.03^{c}
C15:0	0.37±0.06	0.22±0.01^{a}	0.11±0.01^{bc}	0.12±0.02^{b}	0.09±0.02^{c}	0.18±0.01^{a}	0.14±0.01^{b}	0.18±0.01^{a}	0.11±0.04^{b}	0.16±0.01^{b}	0.27±0.03^{a}	0.27±0.10^{a}	0.33±0.03^{a}
C16:0	19.24±0.53	15.96±0.04^{a}	11.39±0.12^{b}	10.59±0.16^{c}	8.56±0.18^{d}	15.00±0.27^{a}	8.23±0.08^{c}	11.53±1.12^{b}	7.41±0.13^{c}	18.06±0.02^{a}	13.00±0.62^{b}	13.08±1.01^{b}	11.64±0.66^{c}
C17:0	0.56±0.16	0.35±0.02^{a}	0.24±0.01^{b}	0.19±0.01^{c}	0.17±0.01^{d}	0.31±0.06^{a}	0.21±0.02^{b}	0.31±0.02^{a}	0.23±0.01^{b}	0.36±0.02^{a}	0.16±0.01^{c}	0.35±0.01^{a}	0.21±0.05^{b}
C18:0	4.61±0.11	3.33±0.01^{d}	5.83±0.01^{b}	6.01±0.03^{a}	4.39±0.03^{c}	4.00±0.02^{d}	5.97±0.03^{a}	5.02±0.11^{b}	4.74±0.05^{c}	4.12±0.01^{d}	5.23±0.07^{b}	4.36±0,13^{c}	5.57±0.10^{a}
C20:0	0.29±0.02	0.18±0.09^{a}	0.17±0.01^{a}	0.14±0.01^{a}	0.13±0.01^{a}	0.12±0.01^{b}	0.19±0.02^{a}	0.19±0.02^{a}	0.12±0.01^{b}	0.14±0.01^{b}	0.20±0.20^{b}	0.36±0.13^{a}	0.19±0.01^{b}
∑ SFA	27.23±0.64	22.52±0.45^{a}	18.83±0.09^{b}	18.15±0.29^{c}	14.45±0.26^{d}	22.36±0.29^{a}	15.54±0.15^{c}	18.52±1.56^{b}	13.33±0.18^{d}	25.69±0.20^{a}	20.02±0.89^{b}	19.68±1.32^{b}	18.90±0.62^{b}
C14:1	0.24±0.03	0.16±0.05^{a}	0.11±0.01^{b}	0.08±0.01^{b}	0.08±0.01^{b}	0.24±0.02^{a}	0.13±0.01^{b}	0.09±0.02^{c}	0.09±0.03^{bc}	0.18±0.01^{a}	0.37±0.17^{a}	0.30±0.19^{a}	0.19±0.07^{a}
C15:1	0.48±0.17	0.12±0.01^{a}	0.11±0.01^{ab}	0.08±0.01^{c}	0.10±0.02^{b}	0.07±0.02^{b}	0.16±0.02^{a}	0.08±0.02^{b}	0.10±0.01^{b}	0.10±0.01^{a}	0.12±0.02^{a}	0.25±0.16^{a}	0.17±0.01^{a}
C16:1n-7	2.56±0.18	4.79±0.11^{a}	1.33±0.01^{c}	1.58±0.08^{b}	1.65±0.01^{b}	6.12±0.13^{a}	0.88±0.02^{c}	1.86±0.20^{b}	0.88±0.03^{c}	7.13±0.80^{a}	1.67±0.45^{b}	1.84±0.18^{b}	1.92±0.19^{b}
C17.1	0.68±0.05	0.42±0.05^{a}	0.15±0.01^{b}	0.12±0.02^{b}	0.13±0.01^{b}	0.80±0.04^{a}	0.14±0.01^{c}	0.23±0.02^{b}	0.16±0.01^{c}	0.35±0.06^{a}	0.11±0.01^{b}	0.29±0.06^{a}	0.18±0.04^{b}
C18:1n-7	2.60±0.05	5.82±0.01^{a}	1.62±0.03^{b}	1.56±0.01^{c}	1.64±0.01^{b}	5.95±0.02^{a}	1.63±0.03^{c}	2.30±0.04^{b}	1.41±0.02^{d}	5.22±0.14^{a}	1.79±0.12^{c}	2.62±0.19^{b}	1.73±0.04^{c}
C18:1n-9	10.46±0.27	22.21±0.29^{d}	22.55±0.01^{c}	26.85±0.07^{a}	25.19±0.07^{b}	17.17±0.22^{c}	23.61±0.20^{a}	21.07±0.36^{b}	23.53±0.17^{a}	18.60±0.40^{b}	22.38±0.86^{a}	19.45±1.90^{b}	23.37±0.24^{a}
C20:1n-9	0.28±0.03	1.52±0.03^{a}	0.12±0.01^{b}	0.08±0.01^{c}	0.08±0.01^{c}	1.25±0.03^{a}	0.20±0.06^{c}	0.35±0.07^{b}	0.11±0.01^{c}	1.11±0.06^{a}	0.15±0.05^{d}	0.43±0.01^{b}	0.30±0.01^{c}
C20:1n-11	0.25±0.02	0.65±0.02^{a}	0.12±0.01^{b}	0.08±0.01^{c}	0.09±0.01^{c}	0.55±0.01^{a}	0.16±0.04^{c}	0.24±0.01^{b}	0.09±0.01^{d}	0.53±0.06^{a}	0.19±0.02^{c}	0.39±0.08^{b}	0.23±0.04^{c}
C22:1n-9	0.77±0.09	0.32±0.06^{a}	0.11±0.01^{b}	0.14±0.04^{b}	0.29±0.02^{a}	0.17±0.06	0.18±0.04	0.16±0.04	0.17±0.05	0.14±0.02^{b}	0.42±0.05^{ab}	0.72±0.34^{a}	0.25±0.01^{b}
C22:1n-11	1.13±0.72	0.71±0.02^{a}	0.25±0.02^{b}	0.26±0.02^{b}	0.26±0.01^{b}	0.78±0.02^{a}	0.34±0.09^{b}	0.27±0.03^{bc}	0.21±0.01^{c}	0.73±0.03^{a}	0.38±0.01^{c}	0.48±0.04^{b}	0.37±0.01^{c}
C24:1n-9	0.83±0.12	0.44±0.01^{a}	0.25±0.01^{b}	0.21±0.01^{c}	0.19±0.01^{d}	0.58±0.06^{a}	0.37±0.04^{b}	0.32±0.06^{b}	0.21±0.01^{c}	0.49±0.01^{a}	0.28±0.02^{b}	0.48±0.14^{a}	0.29±0.02^{b}
∑ MUFA	20.28±0.35	37.16±0.27^{a}	26.72±0.05^{d}	31.04±0.16^{b}	29.70±0.07^{c}	33.26±0.12^{a}	27.80±0.23^{b}	26.97±0.43^{c}	26.96±0.04^{c}	34.58±0.97^{a}	27.86±1.67^{b}	27.25±1.38^{b}	29.00±0.53^{b}
C18:2n-6	6.32±0.09	3.58±0.02^{d}	18.90±0.09^{a}	16.55±0.01^{c}	16.78±0.02^{b}	3.27±0.06^{d}	22.36±0.26^{a}	14.37±0.32^{c}	18.74±0.07^{b}	2.87±0.06^{c}	18.73±0.89^{a}	13.63±1.10^{b}	14.18±0.57^{b}
C18:3n-6	0.21±0.01	0.38±0.15^{c}	1.42±0.01^{a}	1.53±0.01^{a}	1.06±0.01^{b}	0.19±0.03^{d}	1.59±0.02^{a}	0.68±0.03^{c}	1.14±0.01^{b}	0.10±0.01^{c}	0.94±0.06^{a}	0.46±0.05^{b}	0.51±0.01^{b}
C20:2n-6	0.96±0.02	0.81±0.03^{d}	2.59±0.01^{a}	1.80±0.02^{c}	2.37±0.02^{b}	1.05±0.04^{d}	2.94±0.02^{a}	1.71±0.07^{c}	2.13±0.04^{b}	0.72±0.08^{c}	3.54±0.29^{a}	3.05±0.01^{b}	2.99±0.28^{b}
C20:3n-6	0.71±0.12	0.49±0.02^{d}	3.19±0.01^{a}	2.71±0.05^{b}	2.32±0.02^{c}	0.56±0.02^{d}	3.18±0.01^{a}	1.53±0.05^{c}	2.32±0.03^{b}	0.38±0.03^{d}	3.54±0.09^{a}	1.63±0.05^{c}	1.98±0.13^{b}
C20:4n-6	2.86±0.26	1.51±0.02^{d}	3.11±0.02^{a}	1.99±0.02^{b}	1.59±0.01^{c}	0.74±0.15^{d}	3.53±0.01^{a}	2.61±0.09^{b}	1.78±0.02^{c}	0.88±0.04^{c}	1.74±0.10^{a}	1.45±0.05^{b}	0.92±0.05^{c}
C22:2n-6	0.51±0.32	0.60±0.39^{a}	0.16±0.02^{b}	0.10±0.01^{b}	0.11±0.02^{b}	0.12±0.03^{a}	0.18±0.01^{a}	0.25±0.12^{a}	0.27±0.18^{a}	0.25±0.02^{b}	0.39±0.15^{ab}	0.53±0.13^{a}	0.34±0.09^{ab}
C22:4n-6	1.07±0.07	0.23±0.04^{a}	0.14±0.02^{bc}	0.20±0.04^{ab}	0.11±0.03^{c}	0.22±0.03^{b}	0.33±0.04^{a}	0.11±0.03^{c}	0.12±0.05^{c}	0.24±0.04^{a}	0.28±0.01^{a}	0.28±0.06^{a}	0.21±0.05^{a}
∑ n-6 PUFA	12.64±0.68	7.60±0.16^{d}	29.51±0.06^{a}	24.88±0.13^{b}	24.34±0.11^{c}	6.15±0.10^{d}	34.11±0.29^{a}	21.26±0.07^{c}	26.50±0.09^{b}	5.44±0.14^{c}	29.16±1.61^{a}	21.03±0.97^{b}	21.13±0.90^{b}
C18:3n-3	0.69±0.08	1.21±0.01^{d}	5.45±0.02^{c}	7.04±0.01^{b}	10.30±0.01^{a}	1.49±0.06^{d}	6.48±0.05^{c}	7.21±0.14^{b}	11.69±0.08^{a}	1.21±0.04^{d}	6.09±0.31^{c}	7.67±0.29^{b}	11.35±0.01^{a}
C18:4n-3	0.44±0.04	0.89±0.02^{d}	2.22±0.03^{c}	3.10±0.03^{b}	3.76±0.02^{a}	0.71±0.03^{d}	2.01±0.02^{c}	2.22±0.07^{b}	4.19±0.01^{a}	0.55±0.05^{d}	1.60±0.06^{b}	1.14±0.19^{c}	2.29±0.02^{a}
C20:3n-3	0.46±0.01	0.34±0.01^{a}	0.11±0.01^{bc}	0.10±0.02^{c}	0.12±0.01^{b}	0.09±0.03^{b}	0.15±0.04^{a}	0.08±0.01^{b}	0.10±0.03^{ab}	0.62±0.02^{c}	1.38±0.04^{b}	1.32±0.12^{b}	2.27±0.35^{a}
C20:4n-3	0.36±0.12	0.82±0.01^{c}	0.81±0.01^{c}	0.93±0.01^{b}	1.74±0.02^{a}	0.61±0.04^{c}	0.66±0.03^{c}	0.96±0.05^{b}	2.25±0.03^{a}	0.25±0.03^{c}	1.02±0.13^{c}	1.83±0.17^{b}	2.36±0.33^{a}
C20:5n-3	4.77±0.06	3.94±0.06^{a}	2.74±0.02^{d}	2.84±0.04^{c}	3.81±0.03^{b}	3.70±0.02^{a}	1.82±0.01^{d}	2.78±0.19^{b}	2.38±0.06^{c}	2.84±0.05^{a}	1.56±0.03^{c}	2.18±0.03^{b}	2.14±0.02^{b}
C22:5n-3	1.96±0.17	1.07±0.02^{a}	0.87±0.05^{b}	1.06±0.06^{a}	0.93±0.01^{b}	0.83±0.10^{b}	1.14±0.33^{ab}	1.25±0.18^{a}	1.23±0.05^{a}	1.18±0.05^{a}	0.93±0.26^{a}	0.89±0.17^{a}	1.05±0.04^{a}
C22:6n-3	31.23±0.76	24.53±0.60^{a}	12.80±0.17^{b}	10.95±0.23^{c}	10.89±0.18^{c}	30.85±0.23^{a}	10.38±0.42^{c}	18.77±1.86^{b}	11.42±0.01^{c}	27.74±1.18^{a}	10.39±0.70^{c}	17.02±1.05^{b}	9.53±0.43^{c}
∑ n-3 PUFA	39.91±0.38	32.80±0.63^{a}	25.00±0.20^{d}	26.02±0.35^{c}	31.55±0.23^{b}	38.26±0.34^{a}	22.64±0.67^{c}	33.27±2.05^{b}	33.26±0.20^{b}	34.39±1.04^{a}	22.97±0.97^{b}	32.05±1.04^{a}	32.05±1.04^{a}
∑ HUFA	44.89±0.35	34.34±0.34^{a}	26.52±0.28^{b}	22.68±0.45^{d}	23.99±0.33^{c}	38.77±0.43^{a}	24.31±0.71^{c}	30.05±2.54^{b}	24.00±0.03^{c}	35.10±1.33^{a}	24.77±1.26^{c}	30.18±1.71^{b}	23.79±0.60^{c}
n-3/n-6PUFA	3.16±0.19	4.34±0.17^{a}	0.85±0.01^{d}	1.05±0.01^{c}	1.30±0.01^{b}	6.22±0.04^{a}	0.66±0.03^{d}	1.56±0.10^{b}	1.26±0.01^{c}	6.32±0.03^{a}	0.79±0.01^{c}	1.52±0.12^{b}	1.47±0.05^{b}
EPA+DHA	36.00±0.70	28.47±0.66^{a}	15.54±0.19^{b}	13.79±0.27^{d}	14.70±0.21^{c}	34.55±0.22^{a}	12.20±0.41^{c}	21.55±2.05^{b}	13.80±0.07^{c}	30.58±1.13^{a}	11.95±0.68^{c}	19.20±1.08^{b}	11.67±0.45^{c}

The results were evaluated in 30th, 60th and 90th days individually, and significant differences between mean values of groups were shown with different superscripts in a row (P<0.05).

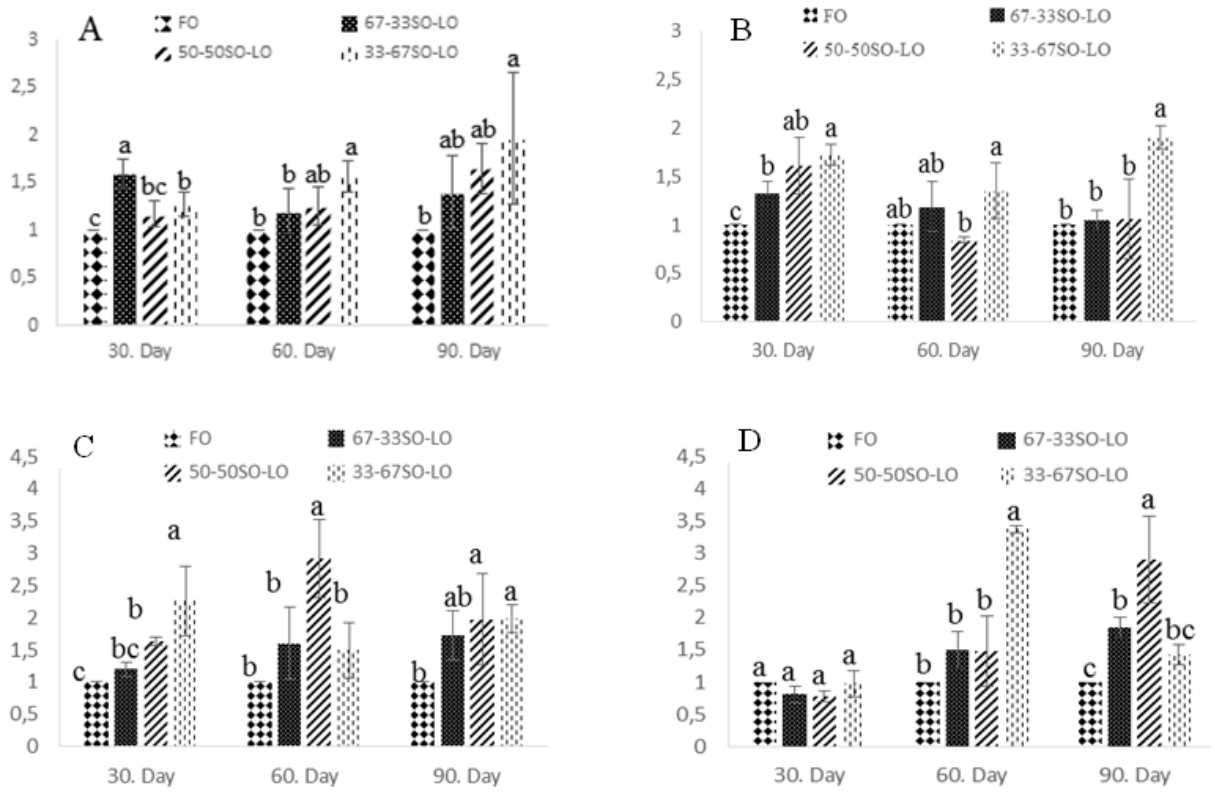

Figure 1. Δ6 desaturase (A) and elongase (D) gene expression of muscle tissue, and Δ6 desaturase (B) and elongase (C) gene expression of liver tissue of Black Sea trout juveniles fed diets with different dietary lipid resources during 90 days.

with diet containing the highest amount of linseed oil (33-67SO-LO). Montero, Robaina, Caballero, Gine's and Izquierdo (2005) revealed that, after 142 days of feeding, European sea bass (*Dicentrarchus labrax*) fed with diets containing 80% linseed oil exhibited significant lower growth. Our results suggest that the use of 50-50SO-LO in place of fish oil is advisable for juvenile Black Sea trout.

Similar to our study, Aminikhoei *et al*. (2013) determined that the highest percentage of saturated and monounsaturated fatty acids in liver and muscle of fish were palmitic acid (16:0) and oleic acid (18:1n-9) respectively. Also Cengiz, Unlü and Bashan (2010) reported that palmitic acid and oleic acid were the predominant saturated (SFA) monounsaturated fatty acids (MUFA) in the muscle of 9 freshwater fish species. In aquaculture diets, the replacement of fish oil by vegetable oil changes fatty acid composition of the diet (Torstensen and Tocher, 2010). The fatty acid composition in the fish diets influences the composition of fatty acids in their tissues (Trushenski, Boesenberg & Kohler, 2009; Babalola and Apata, 2011). Similar observations were made in our study. The replacement of fish oil by vegetable oils (soybean and linseed oils) decreased EPA and DHA ratios, but increased linoleic acid and linolenic acid in fish tissues. Because 18:2n-6 level of diets with vegetable oil (soyabean oil and linseed oil) were richer than those with fish oil, linoleic acid (18:2n-6) levels of muscle and liver tissues of fish fed with vegetable oil were higher than those fed with FO Previous studies announced in similar results (Dadras, 2013; Drew, 2007; Pratoomyot, Bendiksen, Bell & Tocher, 2008). It was reported that the amount of linoleic acid (18:2n-6) in the diets increased with fish oil substitution by vegetable oil (Karalazos, 2007). It was also reported that the use of vegetable oil rich in linoleic acid to substitute fish oil might cause increasing of arachidonic acid in fish tissue (Torstensen and Tocher, 2010). Similar to our study, Mourente *et al.* (2005) and Pratoomyot *et al.* (2008) found that arachidonic acid level of vegetable oils were lower than fish oil. In our study, arachidonic acid level in the tissues (muscle and liver) of the fish fed with vegetable oil diets except for 33-67 SO-LO were higher than those fed with fish oil diet. This may arise from high linolenic acid level in the diets. Because arachidonic acid level in fish tissues is synthesized from linoleic acid by desaturase and elongase enzymes. Several studies have shown that freshwater species are capable of producing arachidonic acid from linoleic acid (Jonasson, 2008; Ling *et al.* 2006; Sargent, Bell, McEvoy, Tocher & Estevez, 1999; Tocher, Bell, MacGlaughlin, McGhee & Dick, 2001). It was reported that replacement of fish oil by blends of vegetable oils (rapeseed, linseed and palm oils) was increased the percentege of linolenic acid (18:3n-3) in diet and in tissues of fish (Mourente and Bell, 2006; Mourente *et al.* 2005), and linolenic acid increased especially with the increasing

in addition of linseed oil in diet (Montero *et al.* 2005). Similar observations were made in our study. Fish oil is rich in EPA and DHA, and diet containing fish oil contains EPA and DHA at high level (Guler and Yıldız, 2011). This is reflected in the fish tissues. Previous studies have reported that the percentages of EPA and DHA were higher in fish fed with the fish oil diet, compared with vegetable oil diets (Aminikhoei *et al*. 2013; Masiha, Ebrahimi, Soofiani & Kadivar, 2013). In our study, it was determined that EPA (20:5n-3) and DHA (22:6n-3) levels in the tissues (muscle and liver) of fish fed with fish oil were higher than those fed with vegetable oil diets. These results obtained in present study are also supported by the previous studies (Jonasson, 2008; Codabaccus, 2011; Peng *et al*. 2008; Jiang *et al.* 2013; Hung and Mao, 2009; Guler and Yıldız, 2011; Yones *et al.* 2013; Piedecausa *et al*. 2007). In our study, EPA levels in the fish tissues (muscle and liver) of fish fed with fish oil was lower although EPA levels were high in the fish oil diet, where DHA was higher in tissues. On the other hand, EPA and DHA levels in the both tissues of fish fed with vegetable oils were higher where the EPA and DHA levels were lower in the vegetable oil diets, but linolenic acid in the tissues were lower. This can be explained by a greater amount of DHA requirement of fish species used in the experiment, and attended to EPA and DHA synthesis of linolenic acid. It can be said that this result obtained with regard to DHA is similar to results obtained from different species grown in culture conditions such as Gilthead sea bream (Fountoulaki *et al.* 2009; Diaz-Lopez *et al.* 2010), Sea bass (Hunt and Tekelioglu, 2004; Dedeler, 2013; Eroldogan *et al.* 2013; Ozsahinoglu *et al.* 2013), Rainbow trout (Pettersson, Johnsson, Brannas & Pickova, 2009; Dernekbası, 2012) and Atlantic salmon (Bell, Henderson, Tocher & Sargen, 2004; Karalazos, 2007; Nanton *et al*. 2007). Fish have high nutritional needs for EPA and DHA due to they contain these fatty acids at high amount in their body tissues (Sargent, McEvoy & Bell, 1997). In a previous study, Abouel-Yazeed (2013) revealed fatty acid levels of four different freshwater fish and eight different seawater fish which ranged from 2.17%-19.22% of DHA in the seawater fish, 1.60%-2.58% of DHA in the freshwater fish, and consequently reported that the proportions of n-3 PUFA of marine water fish was higher than those of freshwater fish, where the proportions of n-6 PUFA was lower. In our study, DHA levels in the muscle and liver tissues were 9.18-28.19% and 9.53-31.23% respectively. It was reported that fish oil is a rich source in EPA and DHA (Zheng, Tocher, Dickson, Bell & Teale, 2004b; Dubey, Jayasooriya & Cheema, 2011), in contrast to vegetable oils (Costa-Pierce *et al.* 2011), and thus EPA and DHA levels of the fish fed with vegetable oils decrease significantly (Pickova and Morkore, 2007). Similar observations were made in our study. Besides, compared with freshwater fish species, Marine fish have lower levels of n-6 PUFA, and have higher levels of n-3 PUFA (Abouel-Yazeed, 2013). But levels of n-6 PUFA (particularly linoleic and arachidonic acids) of freshwater fish have higher than those of marine fish species, and have lower total n-3/n-6 ratio (Steffens, 1997). Fatty acid profile of fish may be affected by alteration in n-3/n-6 ratio of diet (Yones *et al.* 2013). Our study showed that n-3/n-6 ratio of muscle and liver reflected n-3/n-6 ratio of diet, and n-3/n-6 ratio of muscle and liver tissues of fish fed with fish oil was higher than those fed with vegetable oil. This is supported by previous studies (Pettersson, 2010; Guler and Yıldız, 2011; Böhm, 2012; Dadras, 2013; Yones *et al*., 2013). It was also reported that DHA in the muscle and liver tissues of wild Black Sea trout (*Salmo trutta labrax* Pallas, 1811) in the stream was respectively 21.42 and 18.04% (Aras, Haliloglu, Ayık & Yetim, 2003). This can change in culture form individuals and with diets given to these individuals. In our study it was found that DHA in the muscle and liver tissues of juvenile Black Sea trout (*Salmo trutta labrax* Pallas, 1811) were respectively 23.39% and 27.74% in case of feeding with fish oil, 9.54-15.55% and 9.53-17.02% in case of feeding with vegetable oil.

Elongase and desaturase (Δ6 and Δ5) enzymes are very important enzymes responsible for long chain C20 and C22 HUFA synthesis from short chain C18 PUFA (Zheng *et al.* 2004a). Marine fish species have a very limited gene expression of activity of these desaturase enzymes (Montero *et al.* 2005), and ability of these species to bioconvert LA and LNA, into AA, EPA and DHA is poor (Fountoulaki *et al.* 2009). But freshwater fish are capable of producing arachidonic acid from linoleic acid and EPA and DHA from linolenic acid by elongase and desaturase enzymes (Jonasson, 2008; Santigosa *et al*. 2011). In our study, Δ6 desaturase gene expression of muscle and liver tissues of fish fed with 33-67SO-LO diet rich in polyunsatureted fatty acids with C18 was higher than those fed with fish oil rich in long-chain highly unsatureted fatty acids, and elongase level of muscle tissue of fish fed 67-33SO-LO and 50-50SO-LO diets and of liver tissue of fish fed with 33-67SO-LO and 50-50SO-LO diets were higher than those fed with fish oil. In previous studies, it was determined that Δ6 desaturase gene expression of fish fed with vegetable oil diets was higher than those fed with fish oil diet (Zheng *et al*. 2005; Ling *et al*. 2006; Miller *et al*. 2008; Pratoomyot *et al*. 2008; Pratoomyot, 2010). It was also found that elongase gene expression of muscle tissue of Atlantic salmon fed with rapeseed oil in the freshwater and seawater was higher than those fed with fish oil (Codabaccus, 2011), and elongase gene expression of liver tissue of Atlantic salmon fed with linseed oil 100% instead of fish oil increased after trial for 20 weeks, but not influenced at the end of the trial (40 weeks) (Zheng *et al.* 2004a).

Conclusion

Present study suggests that complete replacement of fish oil with vegetable oil (especially, 50-50 SO-LO diet) can be used in diets of Black Sea trout without any negative effect on growth. Besides it was found that the use of vegetable oil diets stimulated the desaturase and elongase gene expression in the muscle and liver tissues of Black Sea trout. But it is understood that synthesis capabilities of DHA from linolenic acid is limited for Black Sea trout. For this reason, detailed genetic studies on how to increase the mRNA levels of enzymes involved in EPA and DHA synthesis are needed. Moreover, in order to increase EPA and DHA levels which have a very important role in meat quality and human health, and to obtain more economic production, the Black Sea trout in farming conditions can be suggested feeding with vegetable oil diets at the begining, and switching to the completely fish oil diet close to the harvest. In this way, the desired quality can be attained by replacing fish oil with vegetable oil, reducing of cost and dependence on fish oil.

Acknowledgement

This study was adapted from a doctoral thesis with the same name, and supported by the project titled "Determination of nutrition requirements of Black Sea trout (*Salmo trutta labrax* Pallas, 1811)". Besides, we would like to thank the researchers that support this project.

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