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RESEARCH ARTICLE

Potential of Using Peanut Oil as Alternative to Fish Oil for European Seabass Diets (*Dicentrarchus labrax*) in Recirculated Systems

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ABSTRACT

The effects of diets containing peanut oil at different ratios on growth performance, biochemical and fatty acid compositions of juvenile European seabass, *Dicentrarchus labrax*, were evaluated under controlled conditions in the recirculated system for 12 weeks. The trial was planned as 6 groups (18 tanks) with three replicates and stocked as 12 fish (mean weight ~4.72±0.01 g) in each tank and was fed by one of six experimental diets. Each diet was formulated to replace 0% (FO, control), 20% (PNO20), 40% (PNO40), 60% (PNO60), 80% (PNO80) and 100% (PNO100) of the fish oil with peanut oil. Feed conversion ratio (FCR) was the best in the PNO80 group ($p<0.05$). The fatty acid composition of the fillet reflects the fatty acids in the test diets. The polyunsaturated fatty acids (PUFA) of PNO60, PNO80 and PNO100 groups were lower than other experimental groups. The saturated fatty acids (SFA) were the highest in the PNO100 group. Some fatty acids [C16:0, palmitic acid (PA); C20:0, arachidic acid (AA); C18:1n-9c, oleic acid (OA); C20:1, eicosenoic acid (ESA); C20:3n-6, dihomo γ-linolenic acid (γ-ALA); C20:5n-3, eicosapentaenoic acid (EPA) and C22:6n-3, docosapentaenoic acid (DHA)] were present higher proportion in fillet of all groups compared to experimental diets. EPA was lower in control and PNO20 compare to experimental diets. As a result, the use of peanut oil in different ratios in the diets had not a negative effect on the growth and proximate composition of European sea bass. This study also indicates that as long as fish meal is available in the diet, peanut oil can be considered a reasonably effective replacement for fish oil in sea bass feeds.

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Introduction

In almost all countries, aquaculture is evolving, expanding and intensifying and is today recognized as the world's fastest growing food industry. (Subasinghe et al., 2009). Due to the increase in aquaculture activities, the use of the fish oil and fish meal is restricted and it is necessary to determine alternative lipid and protein sources for the development of sustainable fish cultivation applications (Kazemi et al., 2016). Therefore, over the last two decades, researchers have focused on finding effective ways to substitute fish oil. (Turchini and Francis, 2009).

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Potential candidates for this substitution are vegetable oils (VO) produced in excess of fish oil and rich in C18 polyunsaturated fatty acids (PUFAs) (Gusstone, 2010; Kazemi et al., 2016).

The composition of fillet fatty acids in cultivated fish fed diets based on VO is typically characterized by a substantial decrease in the amount of 3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) compared to fish fed diets based on FO. In carnivorous marine fish, this condition is even worse, since marine fish are commonly recorded to have a restricted capacity to biosynthesize LC-PUFA from C18 PUFA precursors, unlike most freshwater species (Eroldoğan et al., 2013). Especially, marine fish have a very limited gene expression of Δ-6 and Δ-5 activity, and therefore are not able to synthesize PUFAs (Mourente and

Tocher, 1993) from linoleic (18:2n-6; LA) and linolenic acids (18:3n-3; ALA), abundant in many vegetable oils. Arachidonic (20:4n-6; ARA), eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic (22:6n-3; DHA) acids must be included in the marine fish diet. Long-chain polyunsaturated fatty acids (LC-PUFA) are important for marine fish (Izquierdo et al., 2003).

Fish, like all other vertebrates, require n3 LC-PUFAs for normal growth and development, including reproduction (Yıldız and Şener, 2004). Some vegetable oils are used in salmonids and freshwater fish feeds as good alternative lipid sources without affecting growth efficiency and feed conversion (Rosenlund et al., 2001; Bell et al., 2001; Caballero et al., 2002). In marine fish diets, the partial replacement of FO by VO would only be possible if the necessary fatty acid requirements are met, which can typically be done if the fish meal is included in the diet in the range of 30 to 50 percent (Izquierdo et al., 2005; Eroldoğan et al., 2013; Yılmaz and Eroldoğan, 2015). In European sea bass feed, the partial replacement of dietary fish oil with different VOs has been reported to have limited effects on growth efficiency and feed consistency, regardless of its bioconversion potential for PUFA (Yıldız and Şener, 2004; Izquierdo et al., 2003; Figueiredo-Silva et al., 2005; Mourente and Bell, 2006; Martins et al., 2006; Richard et al., 2006). European sea bass diets were researched their possible effects by adding to many vegetable oils, like soybean, cotton seed, palm, rapeseed, linseed, corn, olive, sunflower oils, etc. (Izquierdo et al., 2003; Yildiz and Şener, 2004; Richard et al., 2006; Eroldoğan et al., 2012; Özşahinoğlu et al., 2013; Sahan et al., 2016; Şahan et al., 2017). It can be argued that information is not yet available on the actual potential or limits of the inclusion of peanut oil (PNO) in feed for European sea bass. However, to our knowledge, it is surprising that only nine studies have been published so far regarding the inclusion of PNO in fish food. These are studies on Nile tilapia (*Oreochromis niloticus*), in which PNO was blended with soybean oil (Sagne et al., 2013); on pikeperch (*Sander lucioperca*), catfish (*Heterobranchus longifilis*), large yellow croaker (*Larmichhthys crocea*) in which PNO was compared with other VOs (Kowalska and Zakęś, 2009; Babalola and Apata, 2012; Qiu et al., 2017, respectively); on rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), African catfish (*Clarias gariepinus*), Mozambique tilapia (*Oreochromis mossambicus*), two-banded sea bream (*Diplodus vulgaris*) by adding at different rates (Acar and Türker, 2017; Yıldırım et al., 2013; Ochang, 2012; Demir et al., 2014; Kesbiç et al., 2016, respectively).

Peanuts are one of the most significant leguminous crops produced worldwide for both food and industrial use. Peanut oil is also an oil derived from the seeds of the Arachis hypogaea legume crop. Raw peanut beans contain up to 50% fat and are therefore food that can be consumed whole and/or processed with high energy. Peanuts are also commonly used for oil production and 45 percent of oleic acid (18:1n-9c; OA), 30 percent of linoleic acid (18:2n-6c; LA), and 10 percent of palmitic acid are the usual fatty acid composition of peanut oil (16:0; PA). The property of peanut oil is that it contains up to 6 percent long-chain saturated fatty acids, such as 20:0, 22:0, and 24:0 as opposed to other VOs (Turchini and Mailer, 2011). Babalola and Apata (2012) recorded that significant levels of n-3 PUFA were observed in soybean oil and groundnut oil, and soybean oil and groundnut oil were exceptionally high in vegetable oils (palm kernel, shea butter, palm, coconut, sunflower, melon seed oils), which are good sources of lipid for aquafeed processing, with an n-3 fatty acid content of 18:3n-3. Thus, the objective of this study was to determine the potential effects of diets containing peanut oil at different ratios on growth performance, biochemical and fatty acid compositions of juvenile European sea bass, *Dicentrarchus labrax*, under controlled conditions in the recirculated system.

Materials and Methods

Experimental Conditions and Design

The European sea bass (400 fish) were brought from a trading company (Kızılırmak Su Ürünleri Inc., Samsun/Turkey). They were fed commercial feed for four weeks and adapted to the environment after the fish were taken to the testing and application center. Following adaptation, fish (mean weight ~4.22±0.01 g) were fasted for a day, weighed and randomly scattered in triplicates in rectangular glass aquariums (approximately 150 L water volume) in a recirculation seawater system with a density of 20 fish per tank.

System water quality variables were measured daily; on average, the water temperature was 21.74±0.05°C, dissolved oxygen (DO) was 5.76±0.01 mg/L, pH was 8.66±0.05 and salinity was 17.31±0.06 ‰. The European sea bass was kept under a natural light regime, and the air pump was provided the aeration of the aquariums. During the experimental period, at a rate of about 10% of the total amount, water was exchanged daily. At the beginning of the experiment, At the beginning (50 fish) of the study were killed with overdose anesthetics (clove oil), weighed and homogenized. At the end of the study, all of the fish in the recirculation seawater system were sampled. All samples taken were stored at -80°C for biochemical and fatty acid analysis.

Experimental Diets and Feeding

The peanut oil (PNO) was purchased from commercial companies (Başpınar Toprak Mah. ve Nak. Ltd. Şti., Osmaniye, Turkey) to produce feed for the replacement trial. PNO substitution levels were 0% (FO-Control), 20% (PNO20), 40% (PNO40), 60% (PNO60), 80% (PNO80), and 100% (PNO100). The experimental diets contained 49% protein and 18% lipid and were isonitrogenic and isolipidic (Table 1). For diet preparation, all dry ingredients and oils were carefully blended with a laboratory food mixer. In order to yield a suitable pulp, the blends were primed with tap water. Wet diets were assembled into 1 mm pellets, dried at 50°C in a drying cabinet, and stored at +4°C before use.

Table 1. Formulation (g/kg) and biochemical composition (%) of the experimental diets

			Experimental diets			
	FO	**PNO20**	**PNO40**	**PNO60**	**PNO80**	**PNO100**
Nutrients (g kg^{-1})						
Fish meal	380	380	380	380	380	380
Extracted soybean meal	230	230	230	230	230	230
Wheat flour	126	126	126	126	126	126
Corn protein	140	140	140	140	140	140
Fish oil	120	96	72	48	24	0
Peanut oil	0	24	48	72	96	120
Vitamin premix(*)	2	2	2	2	2	2
Mineral premix(*)	2	2	2	2	2	2
Proximate Composition (%)						
Moisture	10.23	9.09	8.89	10.43	9.39	10.32
Protein	48.12	48.39	48.31	48.37	48.36	48.17
Lipid	19.51	19.49	19.37	19.87	19.62	19.52
Ash	9.05	9.23	9.28	8.90	9.32	9.00
NFE[1]	13.09	13.81	14.15	12.43	13.31	12.99
Gross energy (kJg^{-1})[2]	21.30	21.46	21.46	21.38	21.42	21.29

*Vitamin-mineral premix (mg/kg premix): vitamin A, 210000 IU; Vitamin D_3, 35000 IU; vitamin E, 7000 mg; vitamin K_3, 322 mg; vitamin B_1, 588 mg; vitamin B_2, 252 mg; vitamin B_6, 294 mg; vitamin B_{12}, 826 mcg; niacin, 1400 mg; biotin, 7583 mcg; 182 mg folic acid, pantothenic acid, 1722 mg; inositol, 17220 mg; vitamin C, 933.31 mg; Ca, 1414mg.

[1]NFE=100-(%protein+ %lipid+ %ash+ %moisture)

[2]Gross energy is calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid and 17 kJ g^{-1} NFE

Fish in all groups were fed *ad libitium* by hand twice a day (at 09:00 am and 15:30 pm) for 12 weeks. The feeding procedure was done carefully in order to be sure all fish took the food.

Biochemical Analysis

All feed and fish samples were analyzed according to the standard methods of the Association of Official Analytical Chemists for proximate composition (AOAC, 1995). Dry matter was detected by drying the samples at 105ºC until a constant weight was reached. Ash content was measured after samples were processed in a muffle furnace for 6 h at 550ºC. The amount of crude protein was measured by the Kjeldahl method, and crude lipid after extraction with petroleum ether was determined by the Soxhlet method. Both triplicate experiments were performed.

Indices of the Nutrition Quality of Muscle Lipids

Measurements of the impact of PNO on the lipid nutritional value of fillets after replacement of FO in the diet are referred to as the atherogenicity index (IA), thrombogenicity index (IT), and flesh lipid consistency (FLQ) (Garaffo et al., 2011; Dagtekin et al., 2017; Yu et al., 2018).

IA =[(4*C14:0) + C16:0 + C18:0] / [(ΣMUFA + n-6 PUFA + n-3 PUFA)

IT =(C14:0 + C16:0 + C18:0) / (0.5*ΣMUFA + 0.5*n-6 PUFA + 3*n-3 PUFA) + (n-3 PUFA/n-6 PUFA)

FLQ =C20:5 n3 + C22:6 n3 / Σtotal FA

Fatty Acid Analysis and Coefficient of Distance (Djh) Values

Total lipid was determined using the modified method of Bligh and Dyer (Hanson and Olley, 1963.). 0.25 g of extracted oil from fish fillets and diets was thawed by adding 4 ml of heptane and 0.4 ml of 2N KOH was added. This mixture was stirred for 2 minutes in vortex, then centrifuged for 5 minutes at 5000 rpm. 1.5-2 ml of the heptane phase was collected after centrifugation and transferred to glass tubes for GC/MS analysis. Autosampler AI 1310 was used to inject samples into the system and samples were analyzed by Thermo Scientific ISQ LT model GC/MS gas chromatography by a spectrometer at the SUBITAM (Sinop University Scientific and Technological Research Application and Research Center).

Total fatty acid profiles were compared between diet and experimental groups by calculating the Coefficient of Distance (D_{jh}) values by the following formula (Rombenso et al., 2018):

$$D_{jh} = [\sum_{i}^{n} (P_{ij} - P_{ih})^2]^{1/2}$$

P_{ij}=The percent content of fatty acid “i” in the control treatment.

P_{ih}=The percent content of fatty acid “i” in an experimental treatment.

Fatty acids such as SFAs, MUFAs, Σn-3 and Σn-6 fatty acids, LC PUFA and PUFAs were not included in Djh calculations.

Statistical Analysis

Anderson-Darling and Levene's measures were used for homogeneity of variances and equality of variance of groups, respectively. Using one-way variance analysis (ANOVA) was performed to test for significant differences among treatment groups, followed by multiple comparison methods

from Tukey. Arcsine square root transformations of percentage data were conducted for homogeneity of variances prior to statistical analysis. When $p < 0.05$, the differences were considered important. Analyses for Windows applications is performed using Minitab 17.

Results

Growth Performance, Feeding Efficiency and Survival

During the 12-weeks feeding trial, fish weights increased by 5-6 times (Table 2). At the end of the study, whilst the highest final body weight (FBW), weight gain (WG) and specific growth rate (SGR) were determined in PNO60, PNO80 and PNO100 groups, these values were lower in FO, PNO20 and PNO40 groups. The differences between FO-PNO20-PNO40 and PNO60-PNO80-PNO100 groups were significant ($p<0.05$) in terms of WG, FBW and SGR. Feed conversion ratio (FCR) was the best in the PNO80 group ($p<0.05$). The highest hepatosomatic index (HSI) and viserosomatic index (VSI) were seen in the PNO80 and PNO100 groups compared to other groups, respectively ($p<0.05$). VSI was significantly increased with increasing inclusion of PNO.

Table 2. Growth and performance of European seabass fed the six experimental diets for 12 weeks

	Diet groups					
	FO (Control)	**PNO20**	**PNO40**	**PNO60**	**PNO80**	**PNO100**
IBW (g)	4.70±0.13	4.70±0.02	4.74±0.06	4.73±0.01	4.73±0.05	4.74±0.03
FBW (g)	22.95±3.56^{b}	22.59±2.81^{b}	22.89±1.40^{b}	24.36±2.13^{a}	24.50±2.39^{a}	24.48±3.74^{a}
WG (g)[1]	18.25±1.98^{b}	17.89±1.64^{b}	18.15±0.87^{b}	19.63±1.24^{a}	19.77±1.34^{a}	19.75±2.19^{a}
SGR(%)[2]	1.81±0.09^{b}	1.80±0.09^{b}	1.81±0.05^{b}	1.88±0.06^{a}	1.89±0.05^{a}	1.86±0.11^{a}
FCR[3]	1.37±0.09^{b}	1.36±0.16^{b}	1.44±0.11^{a}	1.37±0.10^{b}	1.28±0.03^{c}	1.32±0.04^{bc}
HSI (%)[4]	1.63±0.05^{a}	1.64±0.23^{a}	1.26±0.19^{c}	1.49±0.07^{b}	1.69±0.17^{a}	1.49±0.06^{b}
VSI (%)[5]	8.12±0.92^{a}	7.18±0.30^{b}	7.64±0.49^{ab}	8.17±0.38^{a}	8.41±0.18^{a}	9.32±0.76^{a}
Survival (%)	63.89±7.35^{d}	63.89±10.02^{d}	83.33±0.00^{a}	75±8.33^{c}	75±9.62^{c}	80.56±2.78^{b}

All values are mean ± SE, (n=3). There is no major difference in row values with the same superscript or no superscript ($p>0.05$). IBW: Initial body weight, FBW: Final body weight. replicates.

[1]Weight gain (WG, g)= Final body weight - initial body weight

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

[3]Feed conversion rate (FCR)= total feed intake/weight gain

[4]Hepatosomatic index (HSI) = (liver weight / body weight) x 100

[5]Viserosomatic index (VSI)= (viscera weight/body weight) x 100

Survival was different in all groups except PNO60-PNO80 and PNO20-FO groups. The lowest survival was in the PNO20 and FO groups, the highest survival was in the PNO40 group. In this case, it can be said that the production performance and fatty acid compositions are affected by dietary lipid source.

Biochemical Composition of Fillets

The biochemical composition of fish fillet was not significantly affected by the dietary treatment (Table 3). In the highest protein was in the PNO80 group, the lowest lipid was determined in the PNO40 group. The difference between the other groups and PNO20-PNO40 groups was no significant with regard to protein and lipid contents, respectively. However, no significant differences in moisture and ash contents of fillet were observed among trials over the course of the study ($p<0.05$).

Table 3. Chemical composition of European seabass fillets fed the six experimental diets for 12 weeks (% wet weight)

	Moisture (%)	**Protein (%)**	**Lipid (%)**	**Ash (%)**
Initial	80.81±0.18	19.79±0.35	1.47±0.21	2.28±0.14
FO	75.19±0.70^{a}	22.86±0.73^{a}	4.81±0.92^{a}	2.78±0.36^{a}
PNO20	75.50±0.24^{a}	21.44±0.15^{a}	4.98±0.46^{a}	3.09±0.25^{a}
PNO40	75.87±0.24^{a}	23.23±0.75^{a}	3.51±0.09^{b}	2.26±0.08^{a}
PNO60	75.62±0.19^{a}	23.02±0.85^{a}	4.09±0.31^{b}	2.62±0.12^{a}
PNO80	75.33±0.23^{a}	23.94±0.20^{a}	4.90±0.06^{a}	2.30±0.19^{a}
PNO100	75.35±0.39^{a}	22.53±0.40^{a}	4.68±0.27^{a}	2.34±0.12^{a}

Data are mean ± SE. Columns values with the same superscript or no superscript are not significantly different ($p> 0.05$).

Fatty Acid Composition of the Diets and Fillets

All the fatty acids necessary for growth were found in diets. Fatty acid compositions detected in all diets were quite good (Table 4). The fatty acid composition of all dietary groups was usually adequate to fulfill the requirements of the European sea bass for fatty acids.

Table 4. Composition of fatty acid in experimental diets (% total fatty acids)

Diets						
	FO	**PNO20**	**PNO40**	**PNO60**	**PNO80**	**PNO100**
C14:0	2.61±0.01	2.41±0.01	2.27±0.00	2.11±0.01	1.97±0.02	1.85±0.03
C15:0	0.66±0.03	0.58±0.01	0.58±0.00	0.52±0.01	0.50±0.01	0.48±0.01
C16:0	12.09±0.05	11.88±0.03	11.85±0.02	11.75±0.05	11.89±0.04	12.10±0.06
C17:0	1.04±0.02	0.93±0.01	0.90±0.00	0.82±0.02	0.78±0.01	0.74±0.01
C18:0	8.21±0.16	7.94±0.10	8.38±0.03	8.16±0.19	8.34±0.07	8.80±0.17
C20:0	0.51±0.01	0.43±0.01	0.33±0.04	0.24±0.01	0.19±0.01	0.15±0.00
C22:0	1.13±0.02	2.04±0.01	2.78±0.01	3.37±0.02	3.95±0.02	4.51±0.03
C24:0	0.62±0.03	0.32±0.01	0.50±0.00	0.43±0.02	0.38±0.01	0.35±0.01
ΣSFA	**26.86±0.0.33**	**26.73±0.19**	**27.58±0.04**	**27.38±0.33**	**28.01±0.16**	**28.99±0.27**
C14:1	0.16±0.01	0.13±0.01	0.13±0.00	0.11±0.00	0.09±0.00	0.08±0.00
C15:1	0.10±0.00	0.10±0.00	0.09±0.00	0.07±0.01	0.06±0.00	0.06±0.00
C16:1	3.98±0.01	3.74±0.01	3.56±0.01	3.30±0.05	3.10±0.01	2.92±0.03
C17:1	0.62±0.02	0.57±0.01	0.57±0.01	0.52±0.02	0.52±0.01	0.52±0.01
C18:1 n-9c	16.04±0.05	17.90±0.06	21.57±0.19	23.05±0.21	24.80±0.16	26.03±0.51
C18:1 n-9t	4.08±0.14	3.86±0.06	2.18±0.52	2.97±0.14	2.39±0.45	1.48±0.56
C20:1	2.57±0.00	2.59±0.01	2.62±0.01	2.66±0.02	2.76±0.02	2.83±0.03
C20:1 n-9	2.29±0.02	2.01±0.01	1.74±0.01	1.44±0.03	1.13±0.02	0.82±0.02
C24:1	1.15±0.01	1.09±0.02	1.12±0.00	1.08±0.03	1.06±0.01	1.02±0.01
ΣMUFA	**30.98±0.09**	**31.99±0.03**	**33.58±0.35**	**35.17±0.19**	**35.92±0.27**	**35.78±0.86**
C18:2 n-6c	18.18±0.22	18.07±0.11	17.00±0.18	16.65±0.12	16.44±0.11	16.03±0.29
C18:2 n-6t	0.16±0.01	0.14±0.01	0.19±0.02	0.17±0.02	0.15±0.01	0.19±0.02
C18:3 n-3	1.37±0.03	1.73±0.02	2.17±0.01	2.50±0.03	2.86±0.02	3.21±0.02
C18:3 n-6	5.74±0.03	5.22±0.02	4.52±0.04	3.88±0.02	3.13±0.01	2.13±0.02
C20:2	0.31±0.01	0.27±0.01	0.27±0.00	0.23±0.01	0.19±0.02	1.03±0.88
C20:3 n-3	0.56±0.01	0.44±0.01	0.40±0.01	0.32±0.01	0.25±0.01	0.20±0.00
C20:3 n-6	0.89±0.02	0.74±0.01	0.66±0.00	0.51±0.03	0.38±0.00	0.26±0.01
C20:4 n-6	1.75±0.02	1.62±0.02	1.56±0.01	1.44±0.03	1.38±0.01	1.34±0.02
C20:5n-3	5.53±0.09	5.47±0.04	5.06±0.05	4.91±0.06	4.69±0.06	4.51±0.06
C22:2	0.29±0.04	0.22±0.01	0.24±0.00	0.24±0.01	0.25±0.00	0.27±0.01
C22:6 n-3	7.12±0.11	7.18±0.11	6.60±0.06	6.43±0.07	6.20±0.03	5.75±0.10
ΣPUFA	**39.60±2.59**	**41.10±0.17**	**38.66±0.31**	**37.27±0.13**	**35.91±0.13**	**34.92±0.47**
ΣC18 UFA	**25.76±0.22**	**25.42±0.10**	**24.15±0.21**	**23.43±0.08**	**22.76±0.14**	**22.59±0.59**
Σn-3PUFA	**14.58±0.16**	**14.82±0.12**	**14.22±0.11**	**14.15±0.09**	**13.99±0.05**	**13.67±0.16**
Σn-6PUFA	**26.73±0.21**	**25.79±0.10**	**23.93±0.21**	**22.65±0.05**	**21.48±0.14**	**19.94±0.27**
n-3/n-6	**0.55±0.00**	**0.57±0.00**	**0.59±0.00**	**0.62±0.00**	**0.65±0.01**	**0.69±0.00**
DHA/EPA	**1.29±0.01**	**1.31±0.01**	**1.30±0.00**	**1.31±0.01**	**1.32±0.02**	**1.27±0.01**
Djh	**0.0**	**0.37**	**0.43**	**0.51**	**0.67**	**0.23**

Values are means±SE of three determinations. FO: Fish oil, PNO: Peanut oil

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids,

DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, Djh: Coefficient of Distance.

The monounsaturated fatty acids (MUFA) were significantly higher in the fillet of PNO60, PNO80 and PNO100 groups compared to other groups ($p<0.05$), but not polyunsaturated fatty acids (PUFA). The saturated fatty acids (SFA) were the highest in the PNO100 group. Some fatty acids [C16:0, palmitic acid (PA); C20:0, arachidic acid (AA); C18:1n-9c, oleic acid (OA); C20:1, eicosenoic acid (ESA); C20:3n-6, dihomo γ-linolenic acid (γ-ALA); C22:6n-3, docosapentaenoic acid (DHA)] were present in higher proportion in the fillet of all groups compared to trial diets. EPA was lower in control and PNO20 compare to experimental diets (Table 5). Whereas the PA, OA, alpha-ALA and n3/n6 content of fish fillets increased significantly with the growing inclusion of PNO, the content of ESA, gamma-ALA, EPA and n-6 PUFA decreased significantly ($P<0.05$). The n-3/n-6 PUFA ratio has increasingly increased from 0.59 for fish fed with FO to 0.73 for fish fed with PNO100. N-3 PUFA was significantly comprised of all n-3 LC-PUFAs in all therapies, and EPA and DHA were the two most

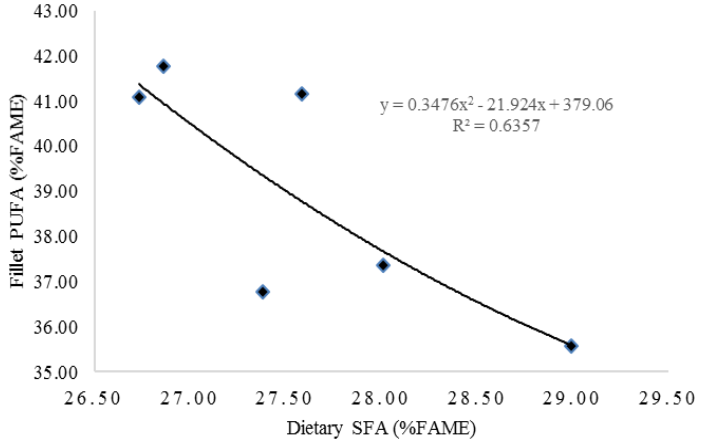
abundant in particular. The highest n-3 PUFA (16.28%) in European sea bass fillets were in the PNO40 group (P<0.05). The IA, IT and FLQ values, the indices of the nutritional quality of the fillet lipids, did not decrease with the increase of the PNO substitution level. Differences were between groups (P<0.05). Among other groups except the PNO20 group, there was no difference in the IT values. There were differences in the FLQ and IA values. The highest IA and FLQ were in the FO and PNO20 groups, respectively.

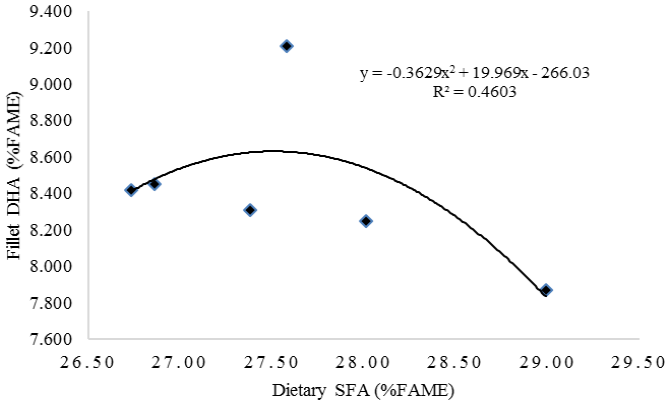
Table 5. Fatty acid composition (percentage of total fatty acids), correlation coefficients (R2) and nutritional quality index of fillet lipids of European sea bass fed experimental diets for 12 weeks

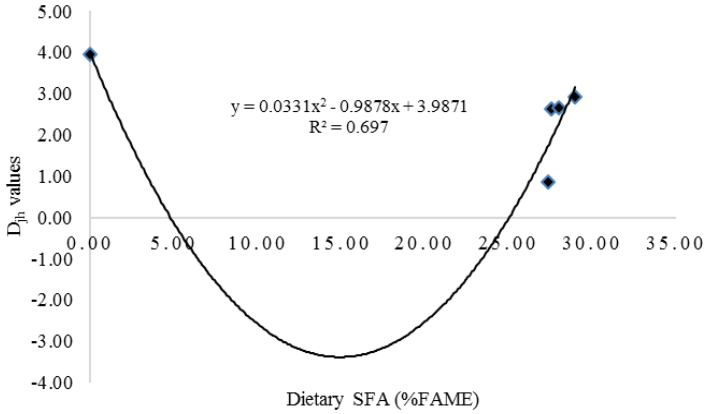
			Diet Groups				
	FO	**PNO20**	**PNO40**	**PNO60**	**PNO80**	**PNO100**	**R^2**
C14:0	2.31±0.03^{a}	2.26±0.04^{a}	1.97±0.04^{b}	1.87±0.03^{bc}	1.73±0.02^{c}	1.75±0.02^{c}	0.92
C15:0	0.68±0.03^{a}	0.67±0.02^{a}	0.57±0.01^{b}	0.54±0.01^{b}	0.49±0.02^{b}	0.49±0.01^{b}	0.82
C16:0	12.60±0.03^{a}	12.69±0.09^{a}	12.59±0.02^{a}	12.16±0.04^{b}	12.06±0.07^{b}	12.54±0.02^{a}	0.21
C17:0	1.19±0.04^{a}	1.17±0.03^{a}	1.10±0.01^{a}	1.00±0.01^{b}	0.91±0.02^{bc}	0.89±0.02^{c}	0.87
C18:0	7.14±0.24^{abc}	7.45±0.22^{abc}	6.84±0.09^{c}	6.93±0.03^{bc}	7.59±0.12^{ab}	7.71±0.12^{a}	0.14
C20:0	1.20±0.03^{a}	1.19±0.02^{a}	1.06±0.01^{b}	1.07±0.01^{b}	1.04±0.02^{b}	1.09±0.01^{b}	0.71
C22:0	0.60±0.02^{f}	1.07±0.02^{e}	1.22±0.01^{d}	1.69±0.00^{c}	1.92±0.02^{b}	2.26±0.01^{a}	0.98
C24:0	0.51±0.02^{a}	0.46±0.02^{a}	0.38±0.01^{b}	0.35±0.01^{b}	0.30±0.01^{c}	0.28±0.01^{c}	0.31
ΣSFA	**26.23±0.42^{ab}**	**26.95±0.41^{ab}**	**25.72±0.16^{ab}**	**25.60±0.10^{b}**	**26.05±0.28^{ab}**	**27.01±0.19^{a}**	**0.05**
C14:1	0.15±0.01^{a}	0.14±0.01^{a}	0.10±0.01^{b}	0.10±0.01^{b}	0.08±0.00^{b}	0.08±0.00^{b}	0.81
C15:1	0.09±0.01^{a}	0.09±0.01^{a}	0.08±0.01^{ab}	0.07±0.00^{abc}	0.05±0.00^{bc}	0.05±0.01^{c}	0.95
C16:1	3.94±0.04^{a}	3.80±0.03^{b}	3.47±0.01^{c}	3.27±0.02^{d}	3.07±0.03^{e}	3.00±0.02^{e}	0.97
C17:1	0.73±0.04^{a}	0.70±0.02^{a}	0.60±0.00^{b}	0.59±0.00^{b}	0.55±0.01^{b}	0.56±0.02^{b}	0.79
C18:1 n-9c	16.02±0.24^{f}	17.53±0.18^{e}	18.69±0.04^{d}	22.37±0.06^{c}	23.92±0.17^{b}	25.00±0.13^{a}	0.94
C18:1n-9t	3.76±0.41^{a}	3.29±0.45^{a}	3.64±0.04^{a}	3.25±0.04^{a}	3.14±0.13^{a}	2.96±0.08^{a}	0.32
C20:1	2.91±0.02^{d}	3.05±0.02^{c}	2.99±0.01^{cd}	3.09±0.01^{c}	3.20±0.04^{b}	3.35±0.02^{a}	0.93
C20:1 n-9	1.92±0.02^{a}	1.73±0.02^{b}	1.42±0.00^{c}	1.22±0.00^{d}	0.96±0.03^{e}	0.74±0.00^{f}	0.99
C24:1	1.48±0.02^{ab}	1.47±0.03^{b}	1.58±0.03^{a}	1.42±0.02^{b}	1.40±0.03^{b}	1.40±0.02^{b}	0.51
ΣMUFA	**30.99±0.59^{d}**	**31.81±0.28^{cd}**	**32.57±0.05^{c}**	**35.37±0.08^{b}**	**36.36±0.12^{ab}**	**37.14±0.03^{a}**	**0.93**
C18:2 n-6c	16.63±0.38^{a}	16.52±0.18^{a}	16.57±0.05^{a}	15.90±0.08^{ab}	15.70±0.15^{ab}	15.01±0.14^{b}	0.75
C18:2 n-6t	0.22±0.02^{a}	0.15±0.02^{a}	0.14±0.02^{a}	0.15±0.02^{a}	0.15±0.01^{a}	0.15±0.02^{a}	0.05
C18:3 n-3	0.97±0.03^{e}	1.22±0.03^{d}	1.24±0.01^{d}	1.50±0.02^{c}	1.65±0.03^{b}	1.85±0.02^{a}	0.97
C18:3 n-6	4.83±0.07^{a}	4.46±0.02^{b}	3.99±0.02^{c}	3.39±0.0.1^{d}	2.71±0.02^{e}	1.89±0.01^{f}	0.99
C20:2	0.28±0.00^{a}	0.28±0.01^{a}	0.23±0.01^{b}	0.24±0.01^{b}	0.14±0.01^{c}	0.13±0.00^{c}	0.30
C20:3 n-3	0.68±0.03^{a}	0.61±0.02^{a}	0.50±0.00^{b}	0.45±0.01^{b}	0.36±0.01^{c}	0.31±0.01^{c}	0.97
C20:3 n-6	2.00±0.01^{a}	1.92±0.02^{a}	1.40±0.35^{a}	1.52±0.01^{a}	1.42±0.01^{a}	1.34±0.01^{a}	0.67
C20:4 n-6	2.19±0.01^{b}	2.16±0.01^{b}	2.38±0.01^{a}	2.09±0.00^{c}	1.93±0.01^{d}	2.04±0.01^{e}	0.54
C20:5n-3	5.26±0.14^{a}	5.10±0.04^{ab}	5.33±0.02^{a}	5.07±0.03^{abc}	4.85±0.01^{bc}	4.80±0.04^{c}	0.64
C22:2	0.27±0.01^{a}	0.23±0.01^{ab}	0.18±0.00^{cd}	0.15±0.00^{d}	0.19±0.01^{bcd}	0.20±0.01^{bc}	0.24
C22:6 n-3	8.45±0.20^{b}	8.42±0.08^{b}	9.21±0.11^{a}	8.31±0.10^{b}	8.25±0.11^{b}	7.87±0.12^{b}	0.57
ΣPUFA	**41.77±0.67^{a}**	**41.08±0.25^{a}**	**41.17±0.34^{a}**	**38.76±0.18^{b}**	**37.35±0.19^{b}**	**35.57±0.23^{c}**	**0.86**
ΣC18 UFA	**22.64±0.41**	**22.35±0.16**	**21.93±0.08**	**20.94±0.08**	**20.21±0.11**	**18.89±0.09**	**0.86**
Σn-3 PUFA	**15.36±0.28^{b}**	**15.36±0.08^{b}**	**16.28±0.10^{a}**	**15.33±0.14^{b}**	**15.11±0.09^{b}**	**14.83±0.15^{b}**	**0.12**
Σn-6 PUFA	**25.87±0.41^{a}**	**25.21±0.19^{ab}**	**24.47±0.37^{b}**	**23.05±0.06^{c}**	**21.91±0.12^{c}**	**20.42±0.09^{d}**	**0.96**
n-3/n-6	**0.59±0.00^{c}**	**0.61±0.00^{c}**	**0.67±0.01^{b}**	**0.67±0.01^{b}**	**0.69±0.00^{b}**	**0.73±0.00^{a}**	**0.91**
DHA/EPA	**1.61±0.01^{c}**	**1.65±0.00^{bc}**	**1.73±0.03^{a}**	**1.67±0.01^{bc}**	**1.70±0.02^{ab}**	**1.64±0.01^{bc}**	**0.24**
Djh	**0.0**	**2.54**	**1.42**	**2.25**	**2.30**	**2.21**	
IA	**0.40±0.01^{a}**	**0.40±0.01^{a}**	**0.37±0.01^{b}**	**0.36±0.00^{bc}**	**0.36±0.01^{c}**	**0.38±0.00^{bc}**	
IT	**0.29±0.01^{a}**	**0.30±0.01^{a}**	**0.27±0.00^{b}**	**0.28±0.00^{ab}**	**0.28±0.00^{ab}**	**0.30±0.00^{a}**	
FLQ	**13.85±0.23^{b}**	**13.55±0.12^{b}**	**14.62±0.13^{a}**	**13.41±0.13^{bc}**	**13.13±0.12^{bc}**	**12.70±0.17^{c}**	

All values are mean ± SE (n=3). Columns values with the same superscript or no superscript are not significantly different (p> 0.05). SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid. Djh: Coefficient of Distance. IA: The index of atherogenicity, IT: The index of thrombogenicity, FLQ: Flesh lipid quality.

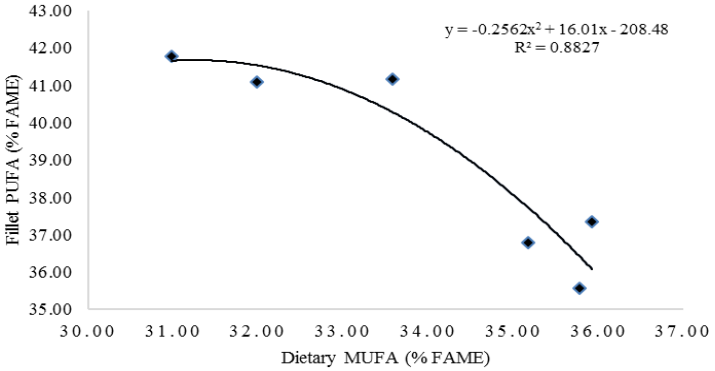
Regression analysis showed a positive correlation between diet SFA and fillet PUFA accumulation (r = 0.64), while there was a negative relationship between diet SFA-fillet PUFA, diet MUFA-fillet PUFA, diet MUFA-fillet DHA, diet C18 PUFA-fillet PUFA, diet C18 PUFA-fillet DHA accumulation (r=0.64, r=0.88, r=0.65, r= 0.85, r=0.61, respectively). In addition, regression analysis showed a positive relationship between diet SFA, MUFA and C18 PUFAs and Djh values (r=0.70, r= 0.77, r=0.70, respectively) (Figure 1. A, B, C).

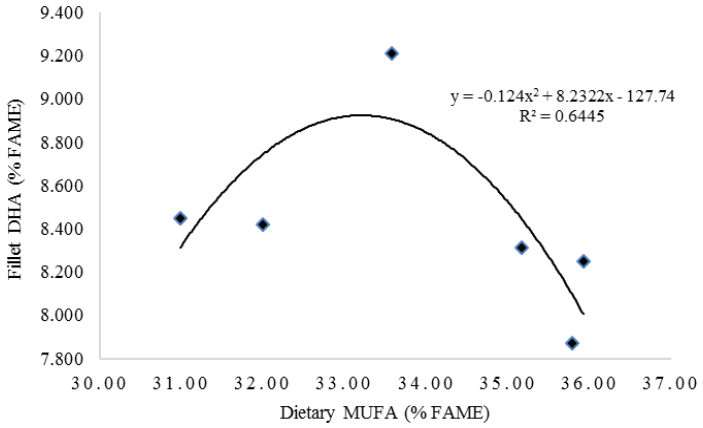


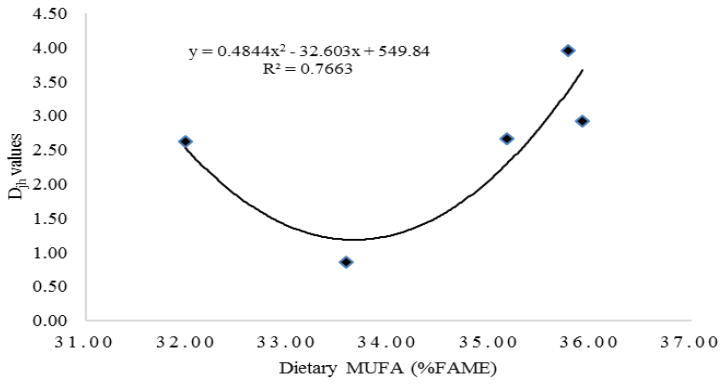




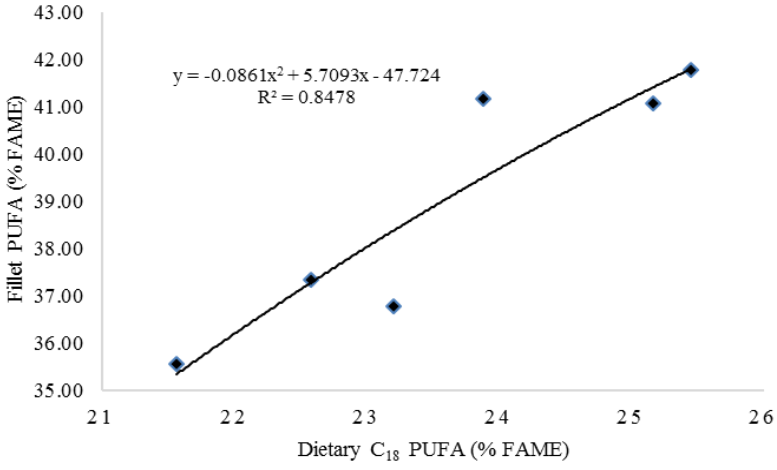
A

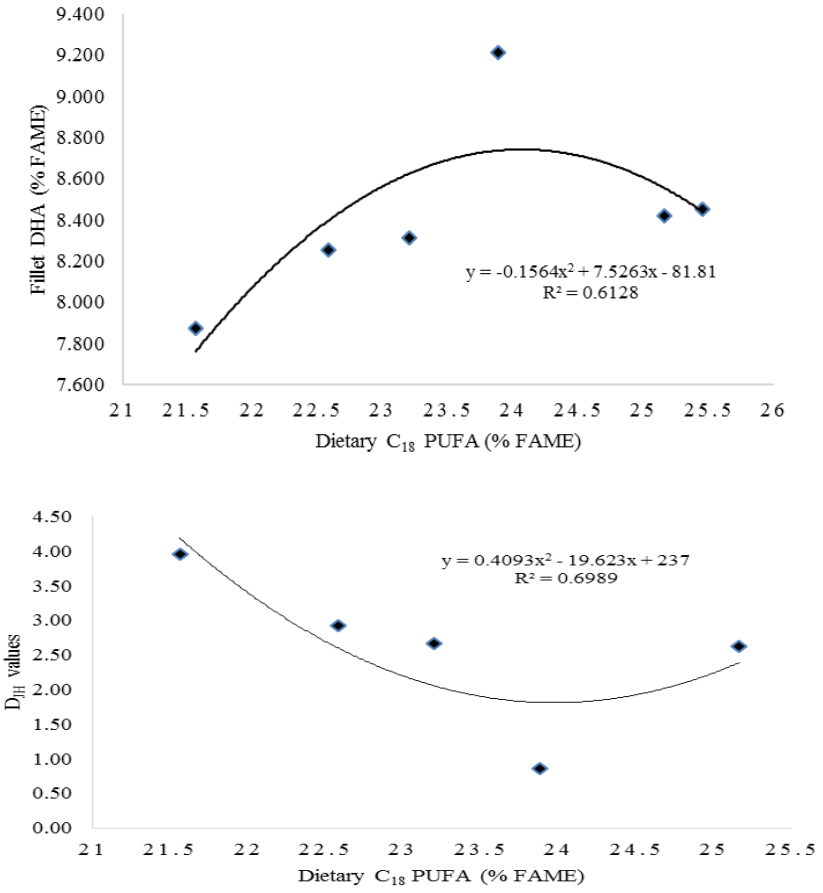

y = -0.2562x² + 16.01x - 208.48
R² = 0.8827
Fillet PUFA (% FAME)
Dietary MUFA (% FAME)


y = -0.124x² + 8.2322x - 127.74
R² = 0.6445
Fillet DHA (% FAME)
Dietary MUFA (% FAME)


y = 0.4844x² - 32.603x + 549.84
R² = 0.7663
Dij values
Dietary MUFA (%FAME)

B


y = -0.0861x² + 5.7093x - 47.724
R² = 0.8478
Fillet PUFA (% FAME)
Dietary C18 PUFA (% FAME)



C

Figure 1. Regression analysis between the groupings of dietary fatty acids [SFA(a), MUFA(b), C18 PUFA(c)] and the quality of tissue fatty acids (i.e. PUFA and DHA) and distance coefficient (Djh values)

Discussion

Prior to this study, potential PNO additive in the European sea bass diet did not come upon any information available. However, there are many studies on the replacement of fish oil and different vegetable oils in European sea bass diets. The present study indicates that peanut oil can be substituted for the fish oil part of the realistic diet for European sea bass without any adverse effects on growth and chemical composition. This result is similar to a number of other earlier studies on European sea bass and salmonids, in which the full replacement of substitute vegetable oils with dietary fish oil did not impact the quality of fish production (Torstensen et al., 2000; Bell et al., 2001, 2002; Yıldız and Şener, 2004; Mourente et al., 2005; Mourente and Bell, 2006; Eroldoğan et al., 2012; Yılmaz and Eroldoğan, 2015). In the present study, growth retardation in treatments containing the highest PNO inclusion was expected to occur, but no significant effects were observed. Also, it was slightly higher the growth performances of European seabass, for this species, compared with previously published results, (Izquierdo et al., 2003; Person-Le Ruyet et al., 2004; Montero et al., 2005).

In the present study, the final weight, weight gain and specific growth rate of the fish fed by PNO60, PNO80 and PNO100 diets were significantly higher than those fish fed the FO, PNO20 and PNO40 diets. Even though all diets contained the same quantity and amount of the fish meal and other materials, the PNO20 and PNO40 diets exhibited the same growth performance by the FO diet. Kesbiç et al. (2016) reported that peanut oil can be used instead of 50% FO in the two-banded seabream (*Diplodus vulgaris*) diets without any negative effects on growth efficiency, FCR and SGR. Yılmaz and Eroldoğan (2015) reported that in their work with 100% fish oil (FO), rapeseed oil (RO) and cottonseed oil (CSO) supplemented diets, while all diets contained the same quantity of fish meal, the CSO diet displayed a significantly lower growth rate than other diets and was therefore not ideal for the replacement of European sea bass with total fish oil. It is well known that through the ß-oxidation process, MUFA-rich diets are more effectively transformed into energy than n-6 PUFA-rich oils since MUFA-rich oils are more digestible than n-6 PUFA-rich oils (Yılmaz and Eroldoğan, 2015). Thus, this situation could partly explain the higher growth output of the PNO60, PNO80 and PNO100 groups in the current study. Studies in which fish oil is partially or completely replaced by PNO in different fish species were shown a reduction in growth for rainbow trout (Acar and Türker, 2017), common carp (Yıldırım et al., 2013) compared to control groups, were identified better growth for Nile tilapia (Sagne et al., 2013), pikeperch (Kowalska and Zakęś, 2009), African catfish (Ochang, 2012). Babalola and Apata (2012) determined that the growth rate of

Heterobranchus longifilis fed with different vegetable oils was quite high in the groups fed with peanut oil. Again, Qiu et al. (2017) used different vegetable oils in large yellow croaker (*Larmichthys crocea*) diets and the best growth, SGR, WG and survival was determined in the group fed with peanut oil.

Different levels of inclusion in the diet of peanut oil resulted in a higher survival rate than the diet of fish oil. With the exception of the PNO20 group, fish mortality during the feeding trial was particularly low for fish fed diets containing peanut oil. Throughout the study, mortality, which was very low, seemed to be independent of dietary care. Fish fed diets containing various amounts of replacement for peanut oil performed better than fish fed the control diet and PNO20 in terms of survival. Ochang (2012) determined a better survival rate in African catfish diets in which they used peanut oil in different proportions, diets using peanut oil than those using fish oil. Similar results have been reported by Qui et al. (2017) for large yellow croaker (*Larmichthys crocea*) and Babalola and Apata (2012) for *Heterobranchus longifilis*. Most of the fish deaths in the trial occurred as a result of the fish jumping out of the water. This suggests that the control group and the PNO group tend to jump out of the water as they are more stressed than the other groups. Vegetable oils have antioxidant properties, which are important as they reduce physiological stress in organs and cells. In the present study, we can say that the antioxidant properties of peanut oil have reduced the rate of jumping out of the water, resulting in less mortality.

Increasing PNO inclusion in the diet significantly increased viscerosomatic index (VSI), when hepatosomatic index (HSI) showed differences among all groups. The highest HSI was detected in the PNO80 group. Viscera was obviously the preferred lipid deposition tissue, and the liver was the second preferred lipid deposition tissue. Figueirdo-Silva et al (2005) showed that no major modification in the aggregation of hepatic lipid droplets in European sea bass was responsible for replacing dietary fish oil with soybean oil. On the other hand, Montero and Izquierdo (2010) reported that increased hepatic lipid deposition is frequently associated with a morphological modification known as steatosis due to increased triacylglycerol synthesis and deposition in hepatocyte vacuoles. The findings of this study indicated that hepatocyte vacuolation and lipid infiltration could not be affected by PNO, which is a rich source of OA and LA.

The fillet fatty acid compositions of fish generally reflect the fatty acid profiles of the diets (Kesbiç et al., 2016). Previous studies have shown that while dietary fatty acids are associated with fatty acids stored in fillets, these particular fatty acids are used or retained selectively. (Bell et al., 2001, 2002; Torstensen et al., 2000; Turchini and Francis, 2009). Rombenso et al. (2018) stated that in terms of LC-PUFA separation, diet SFAs and MUFAs interact antagonistically, MUFAs are lower than SFAs, and SFAs are the primary driving forces in the "protective effect" mechanism of LC-PUFA. In this experiment, dietary PNO caused an increase in saturated fatty acids and a decrease in n-6 polyunsaturated fatty acids. The relative composition of ALA and LA in the fillets was positively linearly correlated with the amount of the individual fatty acid in the diets. The deposition of ALA in fillets increased with the increase of PNO as a substitute for FO in the diet. It was confirmed by Sinclair et al. (2002) that the increased concentration of ALA in salmon species fillets is beneficial for human cardiovascular disease and certain cancers. In this study, the composition of ALA in the fillets was lower than the composition of the diets in this sample, suggesting that ALA was used during the phase of ß-oxidation (Turchini and Francis, 2009). However, ARA, DHA and EPA composition in fillets were higher than in diet, indicates that ARA, DHA and EPA are selectively retained in fillets. Higher levels of DHA in fish fillets were also found in European sea bass compared to the concentration present in the diet (Yılmaz and Eroldoğan, 2015; Mourente et al., 2005), turbot, *Psetta maxima* (Regost et al., 2003), kızıl çipura, *Pagrus major* (Huang et al., 2007) and Atlantic salmon (Bell et al., 2001: 2002). Again, the n3/n6 ratio of in the fish fillets tended to increase when the PNO level increased in the diet. In addition, SFA, MUFA, PUFA and Djh values in the fillet increased compared to the diets. When combined with SFA, MUFA, PUFAs in diets as a determinant variable, fillet contributed positively to Djh values.

To determine the nutritional quality of food lipids for human consumption, several FA ratios and indices have been identified. The PUFA/SFA ratio in human diets should be above 0.45 as per nutritional guidelines. (Wood et al., 2004). The incidence of cardiovascular disease can be increased by lower dietary PUFA/SFA ratios (WHO, 2003). In addition, lower atherogenicity (IA) and thrombogenicity (IT) fats have been reported to inhibit platelet aggregation and reduce esterified FA, cholesterol and phospholipid levels, thus preventing the occurrence of micro- and macrocoronary diseases (Turan et al., 2007). In the present study, the IA, IT and FLQ concentrations were calculated in fillets to measure the flesh nutrition content. Lower levels of IA and IT values are beneficial to human health, Monge-Ortiz et al (2018) reported. In this study, as the level of PNO in the diet was increased, the IA and IT values gradually decreased. Lipid quality of the flesh is directly related to EPA and DHA ratios (Dagtekin et al., 2017). A relatively high FLQ value was obtained in this study. It was reported that omega-3 fatty acids, which are beneficial for both healthy and people suffering from cardiovascular diseases, are lower the risk of arrhythmias, which can lead to sudden death (URL 2, 2016). Therefore, Yu et al. (2018) reported that consuming both the IA and IT indices together with high FLQ with low efficacy is an indication that it may help prevent the development of coronary heart disease, which is more favorable in terms of lipid quality for human consumption. However, Izquierdo et al. (2003) reported that partial replacement of fish oil by vegetable oils (rapeseed, soybean and linseed oils) for 3 months does not affect fillet taste and texture in gilthead seabream and European seabass. In this context, it can be said that the European seabass obtained after the present study have a good nutritional quality for human consumption.

Conclusion

The results of this study indicate that it is possible to use cheap and readily available peanut oil to replace expensive and scarce FO, without adversely affecting the growth, FCR and fatty acid composition of European sea bass. Therefore, for improved nutrient utilization and growth efficiency, it is recommended that peanut oil (PNO) be added to the European sea bass diet. While juvenile specimens were used in the current study and the final fish weight was not indicative of the fish found for consumption on the market, it is expected that the findings obtained may be useful in gaining a better understanding of fatty acid deposition.

Compliance with Ethical Standards

a) Authors' Contributions

SD: Designed the study, interpreted data and wrote the article.

İK: Designed the study and drafted the paper.

EK: Conducted the experimental work and performed the laboratory work.

APA: Performed the laboratory work.

b) Conflict of Interest

The authors declare that they have no conflict of interests.

c) Statement on the Welfare of Animals

In accordance with the Guides for the Care of Research Animals, the Animal Ethics Committee of Sinop University approved all animal management and experimental procedures according to the rules and regulations of experimental field management protocols (Decision No. 2018-03).

d) Statement of Human Rights

This study does not involve human participants.

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