North American Journal of Aquaculture

ISSN: 1522-2055 (Print) 1548-8454 (Online) Journal homepage: http://www.tandfonline.com/loi/unaj20

Unrefined Peanut Oil as a Lipid Source in Diets for Juveniles of Two-banded Seabream Diplodus vulgaris

Osman Sabri Kesbiç, Ümit Acar, Murat Yigit, Musa Bulut, Nejdet Gültepe & Sevdan Yilmaz

To cite this article: Osman Sabri Kesbiç, Ümit Acar, Murat Yigit, Musa Bulut, Nejdet Gültepe & Sevdan Yilmaz (2016) Unrefined Peanut Oil as a Lipid Source in Diets for Juveniles of Two-banded Seabream Diplodus vulgaris, North American Journal of Aquaculture, 78:1, 64-71, DOI: 10.1080/15222055.2015.1095829

To link to this article: http://dx.doi.org/10.1080/15222055.2015.1095829

Published online: 21 Dec 2015.

North American Journal of Aquaculture 78:64–71, 2016

ISSN: 1522-2055 print / 1548-8454 online
DOI: 10.1080/15222055.2015.1095829

ARTICLE

Unrefined Peanut Oil as a Lipid Source in Diets for Juveniles of Two-banded Seabream *Diplodus vulgaris*

Osman Sabri Kesbiç
Inebolu Vocational School, Sea and Port Management Program, Kastamonu University, 37500-Inebolu, Turkey

Ümit Acar*
Faculty of Fisheries, Department of Aquaculture, Mugla Sıtkı Kocman University, 48000-Mugla, Turkey

Murat Yigit and Musa Bulut
Faculty of Marine Science and Technology, Departments of Aquaculture and Marine Technology, Çanakkale Onsekiz Mart University, 17100-Canakkale, Turkey

Nejdet Gültepe
Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Kastamonu University, 37200-Kastamonu, Turkey

Sevdan Yilmaz
Faculty of Marine Science and Technology, Department of Aquaculture, Canakkale Onsekiz Mart University, 17100-Canakkale, Turkey

Abstract

A feeding study with Two-banded Seabream *Diplodus vulgaris* was conducted to determine the effects of replacement of fish oil (FO) by unrefined peanut oil (PO) on growth performance, feed utilization, body composition, fatty acid composition, and serum biochemical and hematological variables. Three isonitrogenous (35.8%) and isoenergetic (21.15 kJ/g) diets were formulated by replacing dietary FO with PO at levels of level 0% (PO_0), 50% (PO_{50}), or 100% (PO_{100}). Fish were fed twice a day until satiation for an experimental period of 8 weeks. The best growth performance was observed in fish fed with the PO_0 and PO_{50} diets. A significant increase was observed in hematocrit and mean corpuscular volume levels of fish fed with PO_{50} diet compared with the other groups. Hemoglobin, mean corpuscular hemoglobin, total protein, albumin, and globulin were not affected by dietary PO treatment and did not differ among experimental groups. The glucose level was highest in the PO_{100} group. Triglyceride and cholesterol levels were lower in fish fed diets with PO inclusions than in those fed the control diet. The fatty acid composition of fish was significantly affected by the experimental diets. Glutamic oxaloacetic transaminase, glutamic pyruvictransaminase, lactate dehydrogenase, and alkaline phosphatase were not affected by dietary PO treatment. The n-3:n-6 ratio in fish fed the PO_0 diet was also higher than in fish fed the PO-supplemented diets. The results of the present study showed that FO could be substituted by PO up to 50% in Two-banded Seabream diets without any negative effect on growth performance or serum biochemical and hematological features.

The marine aquaculture industry in the Mediterranean region is mainly concentrated on two fish species, namely the Gilthead Seabream *Sparus aurata* and European Seabass *Dicentrarchus labrax*. The intensive production of only these two species has caused a decline in the market price, which has encouraged researchers to focus on alternative species to enlarge the market

*Corresponding author: umitacar@mu.edu.tr
Received July 20, 2015; accepted September 14, 2015

prospect of aquaculture products. The Two-banded Seabream *Diplodus vulgaris* (also known as Twoband Bream), belonging to the family Sparidae, has a high market value and is reported to adapt well to culture conditions (Jug-Dujakovic and Glamuzina 1988). With its high tolerance to a wide range of salinity compared with other marine species (Horta et al. 2004), the omnivorous Two-banded Seabream is considered to be a good candidate for the aquaculture industry (Ozório et al. 2009). Previous studies focused on feeding habits of Two-banded Seabream showed that the favorite food items for the species are algae and sea urchins (Guidetti 2004; Pallaoro et al. 2006). The production of omnivorous fish species is more cost effective for the aquaculture industry due to their lower requirements for animal-based protein in their diets. For this reason, the Two-banded Seabream could be a promising candidate species for the Mediterranean aquaculture industry.

Despite earlier studies on this species, knowledge on the nutritional requirements of Two-banded Seabream is still scarce and the production of nutritionally balanced diets is crucial to attain high growth performances. The globally increasing demand for fish oil (FO) has triggered a price increase of this indispensable ingredient, and production of fish oil in the world is limited. Hence, a supply of alternative resources is worth investigating to ensure the sustainability of the aquaculture industry. Since FO is a source of n-3 long-chain polyunsaturated fatty acids, the global need for FO for the aquaculture feed industry is still increasing. Therefore, oils from plant sources are considered to be a good alternative to FO to support sustainable aquaculture production; production of plant source oils is increasing and prices are lower than those for FO (Gunstone 2010). Some studies on FO replacement by vegetable oils have been reported with promising results (Montero et al. 2003; Lin and Shiau 2007; Yıldız et al. 2013; Yildirim et al. 2013). The most commonly studied vegetable oils are soybean, palm, linseed, and rapeseed oils (Wassef et al. 2007), and a few studies have looked at replacing FO with peanut oil (PO). Recent studies on dietary PO substitution for African Catfish *Clarias gariepinus* and Common Carp *Cyprinus carpio* have been reported (Aderolu and Akinremi 2009; Yildirim et al. 2013). Globally, peanuts are the fourth largest oilseed crop; they are cultivated in more than 60 countries and have an annual production of about 35 million tons (Liu et al. 2012). In the USA a PO production of 5.58 million tons was recorded in 2013 (USDA–NASS 2014), and the actual PO price was US$1,000/ton compared with $2,000/ton for FO.

The aim of this study was to evaluate the effects of replacing dietary FO with PO on growth performance, feed utilization, fatty acid composition, fish hematology, and serum biochemical variables for Two-banded Seabream fingerlings.

METHODS

Experimental conditions, diets, and proximate composition.—Unrefined PO was obtained from a local factory (Başpınar Fıstık, Osmaniye, Turkey) to produce feed for the replacement trial. A total of 135 wild Two-banded Seabream were captured in the Strait of Çanakkale (formerly Dardanelles), Çanakkale, Turkey, and transferred and randomly distributed into 80-L glass aquaria in a recirculation seawater system (15 fish per aquarium).

The trial was performed with triplicate groups, which were acclimated for 15 d. System water quality variables were measured daily; on average, water temperature was 17.8 ± 0.6°C (mean ± SD), dissolved oxygen (DO) was 7.6 ± 1.01 mg/L, pH was 7.8 ± 0.4, and salinity was 30.2 ± 0.9‰. The Two-banded Seabream, (average weight, 10.17 ± 0.21 g) were kept under a constant photoperiod (12 h light : 12 h dark), and aeration of aquaria was facilitated with an air pump. During the course of the experiment, water was exchanged daily at a rate of about 10% of the total volume.

The peanut oil substitution levels were 0% (PO_0), 50% (PO_{50}), and 100% (PO_{100}). The experimental diets used were isonitrogenous and isolipidic, containing 35% protein and 15% lipid, which are considered optimum levels for Two-banded Seabream as reported by Bulut et al. (2014) (Table 1). All dry ingredients (Agromey, İzmir, Turkey) were carefully mixed with a laboratory food mixer for the diet preparation. The mixtures were primed with tap water to yield a suitable pulp. Wet diets were assembled into 1-mm pellets, dried at 40°C in a drying cabinet, and stored at −20°C until use. During the trial fish were fed twice daily (1000 and 1700 hours) to apparent satiation for 8 weeks and fish were weighed every 20 d until the end of the experiment. Feed and fish samples were analyzed for proximate composition according to methods of the Association of Official Analytical Chemists (AOAC 1998) at the end of trial. All the samples were frozen at −20°C until analyzed. Dry matter was detected by drying the samples at 105°C until a constant weight was achieved. Ash content was measured after samples were treated in a muffle furnace at 525°C for 12 h. The amount of crude protein was analyzed by the Kjeldahl method, and total lipids in the experimental diets and muscles were determined with a mixture of chloroform and methanol (2:1, v/v) that contained 0.01% butylated hydroxytoluene as an antioxidant as described by Folch et al. (1957).

Fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% boron trifluoride-methanol (AOAC 1998). Fatty acid methyl esters (FAMEs) were analyzed by using a flame ionization gas chromatograph (Shimadzu GC-2014) equipped with an Omegawax 250 capillary column (30 mL × 0.25 mm internal diameter), a flame ionization detector, and a split injection system with nitrogen carrier gas. The injector port and detector temperatures were maintained at 250°C and 260°C, respectively. The column temperature program was held at 140°C for 5 min and then increased at a rate of 3°C/min to 200°C. Total run time was 60 min per sample.

TABLE 1. Ingredients and analytical composition (% dry matter) and fatty acid composition (% of total fatty acid) of experimental diets fed to juvenile Two-banded Seabream. "Fish oil" column refers to Glencross (2009); "peanut oil" column refers to the present study.

Variable	Fish oil	Peanut oil	Experimental diets PO_0	PO_{50}	PO_{100}
Ingredients (% dry matter)					
Fish meal			25	25	25
Soybean meal			32	32	32
Wheat meal			27	27	27
Fish oil			12	6	
Peanut oil				6	12
Vitamin mix[a]			2	2	2
Mineral mix[b]			2	2	2
Analytical composition (% dry matter)					
Protein			35.8	35.7	35.8
Lipids			15.2	15.3	15.1
Ash			5.76	5.82	5.66
NFE[c]			39.30	39.30	39.30
Energy (kJ/g)[d]			21.13	21.15	21.09
Fatty acid composition (% of total fatty acid)					
C12:0			0.10	0.09	0.07
C14:0	8		6.49	4.86	2.57
C15:0			0.96	0.72	0.63
C16:0	18	9.21	15.96	13.72	11.32
C16:1	11		6.37	5.73	4.96
C17:0			1.12	0.93	0.87
C18:0	6	3.4	6.38	6.11	6.02
C18:1(n-9)	15	57.2	24.12	28.74	35.41
C18:2(n-6)	1	27.9	15.61	16.02	16.32
C18:3(n-6)			1.14	0.92	0.86
C20:0	4		0.32	0.56	0.60
C20:1(n-9)	3		0.19	0.35	0.42
C20:2			0.30	0.28	0.36
C20:4(n-6)	1		0.87	0.75	0.68
C20:5(n-3)	12		6.81	5.63	5.69
C22:1(n-9)			1.02	0.98	0.96
C22:5(n-3)	2		2.20	1.87	1.69
C22:6(n-3)	12		9.19	9.22	7.30
Σ(n-3)	30		19.34	17.64	15.54
Σ(n-6)	2	27.9	16.87	17.05	17.36
n-3:n-6 ratio	15		1.15	1.03	0.90

[a]Vitamin mix (IU/kg or mg/kg): vitamin A, 18,000 IU; vitamin D3, 2,500 IU; vitamin E, 250 mg; vitamin K3, 12 mg; vitamin B1, 25 mg; vitamin B2, 50 mg; vitamin B3, 270 mg; vitamin B6, 20 mg; vitamin B12, 0.06 mg; vitamin C 200 mg; folic acid, 10 mg; calcium D-pantothenate, 50 mg; biotin, 1 mg; inositol, 120 mg; choline chloride, 2,000 mg.

[b]Mineral mix (mg/kg): Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg.

[c]Nitrogen free extracts (NFE) = dry matter − (crude lipid + crude ash + crude protein).

[d]Energy calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, and 17.0 kJ/g nitrogen free extracts.

Fatty acids were identified by comparing their retention times to authentic standard fatty acid standards (Sigma-Aldrich, St. Louis, Missouri).

Growth performance as specific growth rate (SGR), feed utilization as feed conversion ratio (FCR), and feed profitability as relative growth rate (RGR) were calculated using the following equations as described by Watanabe et al. (1987a, 1987b), Burel et al. (2000), Yigit et al. (2006, 2010), and Piedecausa et al. (2007):

$$\text{SGR }(\%/\text{d}) = \{[\ln(\text{final wet weight}) - \ln(\text{initial wet weight})]/\text{d}\} \times 100$$

$$\text{FCR} = \text{feed consumed}/\text{weight gain}$$

$$\text{RGR}(\%) = [(\text{final wet weight} - \text{initial wet weight})/\text{initial wet weight}] \times 100$$

Blood collection and analyses.—After a growth period of 8 weeks, four fish were captured from each aquarium (12 fish per group) for blood collection. No anesthetic was used in order to avoid any possible effects of anesthesia on blood variables. Fish handling time was less than 1 min for each fish. Thus, the total capture time was less than 8 min for all fish in each tank. About 4 mL of blood was drawn from the caudal vein and immediately transferred to test tubes. The extracted blood was then centrifuged at 4,000 rpm for 10 min to separate the serum for biochemical analyses (Gültepe et al. 2015). Total protein, albumin, globulin, glucose, triglyceride, cholesterol, alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactate dehydrogenase (LDH) in serum were analyzed by using bioanalytical test kits (Bioanalytic Diagnostic Industry, Neuss, Germany) and measured by a Shimadzu spectrophotometer (PG Instruments, Lutterworth, UK). A few blood samples were allocated for the hematological assays and the rest were added to tubes containing heparin for the other hematological analyses (Bain et al. 2006; Gültepe et al. 2014).

Statistical analyses.—Values of all measured variables are expressed as mean ± SD. The data obtained from blood analyses and growth indices were examined by ANOVA with the Minitab Statistical Software. Levels of significance were determined using Tukey's post hoc honestly significantly different (HSD) test, in which critical limits were set at $P < 0.05$.

RESULTS

At the end of the experiment survival of Two-banded Seabream was 100% in all experimental groups. Fish growth performances are shown in Table 2. Values for SGR, RGR, and FCR were significantly affected by dietary PO levels. No significant differences in values for FCR, SGR, and RGR were obtained between the control (PO_0) and PO_{50} diet group. Feed utilization decreased as PO level increased in the diets ($P <$ 0.05).

The replacement of FO by PO did not show any significant effect on fillet protein lipid or ash content (Table 3). According to the results of the analyses, the incorporation of dietary PO increased the content of oleic acid (C18:1[n-9]) and linoleic acid (C18:2[n-6]) in the muscle tissues.

The effects of PO on the hematological and serum biochemical variables of Two-banded Seabream are shown in Table 4. Compared with the values for the control group, hematocrit (Hct) and mean corpuscular volume (MCV) were significantly higher in the experimental fish fed the PO_{50} diet ($P < 0.05$). At the end of the feeding trial levels of total protein, albumin, and globulin showed no significant difference among the experimental groups, but levels of glucose, cholesterol, and triglyceride were significantly different. The Hb and MCH values in the PO_{50} and PO_{100} treatment groups did not vary significantly from the values observed in the PO_0 control group.

Serum triglyceride and cholesterol levels in fish fed the PO_{50} diet were significantly lower than control values ($P <$ 0.05). Serum liver enzymes activities (GOT, GPT, LDH, and ALP) were not significantly different among the experimental groups.

DISCUSSION

Several studies have evaluated the replacement of FO with vegetable oil in finfish diets with successful results; however, to our knowledge there are no data for Two-banded Seabream. The present study was performed to determine the feasibility of replacing dietary FO with PO in practical diets for Two-banded Seabream. Growth performance results were similar to other marine finfish species such as Gilthead Seabream (Silvia et al. 2007), Black Seabream *Acanthopagrus schlegeli* (Peng et al. 2008), Sharpsnout Seabream *Diplodus puntazzo* (Piedecausa et al. 2007), and Red Seabream *Pagrus major* (Huang et al. 2007). Two-banded Seabream is an omnivorous sparid species that has a higher ability than other carnivorous species to digest plant sources (Ozório et al. 2009). Our results indicate that FO can be successfully replaced with PO by up to 50% in Two-banded Seabream diets without affecting fish growth or the nutritive utilization of the diet. Similarly, Yıldırım et al. (2013) and Demir et al. (2014) reported that the incorporation of PO up to 50% in the diet did not show any adverse effect on growth performance in Common Carp or Mozambique Tilapia *Oreochromis mossambicus*, respectively. However, to-

TABLE 2. Growth performance and feed utilization (mean ± SD) of juvenile Two-banded Seabream fed different levels of dietary PO as a replacement for FO. Diets are defined in Table 1.. Within a row mean values with different letters are significantly different ($P < 0.05$). RGR = relative growth rate; SGR = specific growth rate; FCR = feed conversion ratio.

	Experimental diets		
Performance metric	PO_0	PO_{50}	PO_{100}
Initial weight (g)	10.37 ± 0.25	10.25 ± 0.16	10.45 ± 0.53
Final weight (g)	28.81 ± 0.74 zy	29.43 ± 1.18 y	26.95 ± 0.27 z
RGR (%)	177.81 ± 2.75 zy	187.49 ± 15.69 y	157.98 ± 2.05 z
SGR (%/d)	1.70 ± 0.01 zy	1.75 ± 0.09 y	1.57 ± 0.013 z
FCR	1.67 ± 0.02 z	1.67 ± 0.02 z	1.92 ± 0.01 y

TABLE 3. Proximate (% wet weight basis) and fatty acid (% of total fatty acid) composition (mean ± SD) of muscle lipids in Two-banded Seabream fed PO diets for 8 weeks. Within a row mean values with different letters are significantly different ($P < 0.05$).

	Experimental diets		
Variable	PO_0	PO_{50}	PO_{100}
Proximate composition (% wet weight basis)			
Protein	18.77 ± 1.82 z	18.26 ± 2.08 z	18.92 ± 1.96 z
Lipids	4.16 ± 0.51 z	4.40 ± 0.16 z	4.21 ± 0.45 z
Ash	2.31 ± 0.56 z	2.22 ± 0.31 z	2.52 ± 0.15 z
Fatty acid (% of total)			
C12:0	0.15 ± 0.01 z	0.13 ± 0.02 z	0.13 ± 0.01 z
C14:0	2.65 ± 0.19 y	2.50 ± 0.21 z	2.69 ± 0.23 y
C15:0	0.36 ± 0.02 z	0.29 ± 0.05 z	0.27 ± 0.03 z
C16:0	17.24 ± 0.61 z	16.42 ± 0.32 z	16.86 ± 0.80 z
C16:1	3.88 ± 0.38 z	4.06 ± 0.42 z	3.85 ± 0.51 z
C17:0	0.32 ± 0.04 z	0.29 ± 0.01 z	0.28 ± 0.02 z
C18:0	5.90 ± 0.22 y	5.19 ± 0.41 z	4.69 ± 0.35 z
C18:1(n-9)	25.37 ± 1.12 z	31.11 ± 2.01 y	33.49 ± 1.96 y
C18:2(n-6)	11.33 ± 0.36 z	14.20 ± 0.41 y	14.63 ± 0.72 y
C18:3(n-6)	2.16 ± 0.10 z	1.90 ± 0.15 z	1.79 ± 0.22 z
C20:0	0.62 ± 0.02 z	0.71 ± 0.08 z	0.65 ± 0.05 z
C20:1(n-9)	3.32 ± 0.05 z	3.41 ± 0.08 z	3.38 ± 0.04 z
C20:2	0.91 ± 0.02 y	0.85 ± 0.06 zy	0.80 ± 0.03 z
C20:4(n-6)	0.96 ± 0.03 y	0.88 ± 0.06 zy	0.83 ± 0.05 z
C20:5(n-3)	5.09 ± 0.10 y	4.65 ± 0.08 z	4.55 ± 0.08 z
C22:1(n-9)	0.49 ± 0.05 z	0.43 ± 0.04 z	0.31 ± 0.08 z
C22:5(n-3)	1.5 ± 0.01 x	1.3 ± 0.03 y	0.6 ± 0.08 z
C22:6(n-3)	14.97 ± 0.97 x	10.76 ± 0.82 y	7.46 ± 0.71 z
Σ(n-3)	23.72	18.61	14.4
Σ(n-6)	13.02	15.93	16.26
n-3:n-6 ratio	1.82	1.17	0.89

tal replacement of FO by PO significantly decreased growth performance and feed utilization parameters. The poor growth performance of Twobanded Seabream fed the diet containing high levels of PO could be attributed to the relatively lower fatty acid profiles that did not meet the requirements of this species. Previous studies reported that body fatty acid compositions of fish generally reflects the fatty acid profiles of the diet supplied to fish (Montero et al. 2004; Huang et al. 2007). Generally, dietary PO caused a decrease in saturated fatty acids and an increase in n-6 polyunsaturated fatty acids. In this regard, PO is a rich source of unsaturated fatty acids, especially oleic and linoleic acids. Consequently, in the present study, the n3:n6 ratio of Twobanded Sea-bream fillets showed a decreasing trend when the PO level increased in the diet. Similar results have been reported in marine finfish species in terms of a lower n3:n6 ratio of fish fed diets containing plant-based oil compared with those fed FO-based diets (Trushenski et al. 2011; Deng et al. 2014). Furthermore, marine finfish species exhibit limited or no ability to desaturate and elongate 18:3(n-3) to n-3 highly unsaturated fatty acids and sometimes eicosapentaenoic acid to docosahexaenoic acid as well as 18:2(n-6) to 20:4(n-6) due to low or absent delta-6-desaturase activity (Bell et al. 1994, 2001; Montero et al. 2004; Izquierdo et al. 2005); therefore, vegetable and FO mixtures seem to be the best source of oil for omnivore fishes.

Hematological variables have generally been convenient as indicators of stress or disease in fish (Campbell 2004; Harikrishnan et al. 2011; Yılmaz and Ergün 2012). Changes in red blood cell (RBC) count, Hct value, Hb concentration, and erythrocyte index are important hematological variables that provide information about the health of the organs (Başusta 2005). In the present study some of the hematological variables such as Hct, MCH concentration (MCHC), MCV, and RBC count are significantly affected by PO replacement level. Fackjouri et al. (2011) reported decreased Hct and Hb values in Beluga *Huso huso* fed with diets based on soybean oil. Similarly, Guroy et al. (2012) indicated that Hct level can decrease with the use of canola oil in diets. Ochang et al. (2007) reported an increase in Hct value when the level of dietary palm oil was increased for Nile Tilapia *O. niloticus*.

TABLE 4. Hematological and serum biochemical values (mean ± SD) of juvenile Two-banded Seabream fed diets with different PO inclusion levels. Within a row the means with different letters are significantly different ($P < 0.05$). WBC = white blood cell count; RBC = red blood cell count; Hct = hematocrit; Hb = hemoglobin concentration; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; GLU = glucose; TPROT = total protein; ALB = albumin; GLO = globulin; TRI = triglyceride; CHOL = cholesterol; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase.

	Experimental diets		
Variable	PO_0	PO_{50}	PO_{100}
WBC ($\times 10^3/mm^3$)	66.14 ± 1.66 y	65.02 ± 2.72 y	60.56 ± 1.03 z
RBC ($\times 10^6/mm^3$)	4.01 ± 0.31 y	3.66 ± 0.15 y	3.20 ± 0.11 z
Hct (%)	37.18 ± 1.88 y	40.78 ± 0.84 x	34.64 ± 1.14 z
Hb (g/dL)	11.42 ± 0.53 z	10.78 ± 0.45 z	10.16 ± 1.41 z
MCV (μm^3)	93.01 ± 5.42 z	111.62 ± 4.83 y	108.15 ± 3.07 y
MCH (pg)	28.56 ± 1.45 z	29.47 ± 0.55 z	31.67 ± 3.93 z
MCHC (%)	30.74 ± 1.19 y	26.43 ± 0.99 z	29.26 ± 3.26 zy
GLU (mg/dL)	119.90 ± 3.3 z	126.50 ± 5.9 z	161.14 ± 2.50 y
TPROT (g/dL)	7.25 ± 0.72 z	6.22 ± 0.49 z	6.82 ± 0.45 z
ALB (g/dL)	0.12 ± 0.072 z	0.15 ± 0.026 z	0.14 ± 0.09 z
GLO (g/dL)	7.14 ± 0.69 z	6.09 ± 0.49 z	6.66 ± 0.54 z
TRI (mg/dL)	172.0 ± 6.10 y	96.30 ± 4.20 z	122.0 ± 3.70 zy
CHOL (mg/dL)	159.58 ± 4.40 y	126.30 ± 3.07 z	134.68 ± 4.37 zy
GOT (U/L)	50.35 ± 8.14 z	54.50 ± 1.06 z	47.33 ± 6.43 z
GPT (U/L)	22.09 ± 3.55 z	21.84 ± 4.22 z	17.72 ± 1.41 z
LDH (U/L)	77.02 ± 2.24 z	81.37 ± 4.27 z	80.67 ± 2.39 z
ALP (U/L)	79.93 ± 3.31 z	77.82 ± 4.70 z	75.25 ± 2.19 z

Blood indices (MCV, MCH, and MCHC) are important for evaluations of anemia in most animals (Coles 1986). In the present study, no symptoms of anemia were observed. These results are different from Ochang et al. (2007) who used palm oil as a replacement for FO in the diets of African Catfish.

Biochemical variables can be useful indicators to determine stress in fish (Yılmaz and Ergün 2012); in particular, glucose levels are an important indicator of ammonia-related stress (Morgan and Iwama 1997; Başusta 2005). Previous studies have reported that blood glucose can be reduced with dietary plant oils (Kenari et al. 2011; Han et al. 2012). We obtained no significant difference in glucose levels of fish fed the PO_0 and PO_{50} diets; however, the glucose levels were significantly affected when the PO replacement level increased to 100%. Similar results were reported by Demir et al. (2014) in Mozambique Tilapia. Serum total protein, albumin, and globulin levels are also considered to contribute to a strong immune response of fish (Wiegertjes et al. 1996; Yildirim et al. 2013). In the present study, the dietary inclusion of PO did not show any significantly adverse effects on serum protein levels. Earlier studies reported similar findings in terms of no immune suppression in Malabar Grouper *Epinephelus malabaricus* (Lin and Shiau 2003), Largemouth Bass *Micropterus salmoides* (Subhadra et al. 2006), and Caspian Brown Trout *Salmo trutta caspius* (Kenari et al. 2011) fed diets containing vegetable oil. Triglyceride and cholesterol are important lipids, and plasma lipid levels are affected by diet types and stress levels (McDonald and Milligan 1992). In our study cholesterol and triglyceride levels were lowest in fish fed the PO_{50} diet. This may be explained by the cholesterol-lowering effects of plants (Lin et al. 2007). Similar results were obtained in Common Carp and Mozambique Tilapia when PO was used in diets as a replacement for FO (Yıldırım et al. 2013; Demir et al. 2014). The activities of serum enzymes (GPT, GOT, LDH, and ALP) are also used as important stress indicators. In the present study, the GPT, GOT, LDH, and ALP levels in experimental groups were not significantly affected by the substitution of FO with PO. Similar results were reported by Díaz-López et al. (2009) for Gilthead Seabream when dietary FO replaced *Echium* oil. Lee et al. (2003) reported a decrease in GOT levels in juvenile Starry Flounder *Platichthys stellatus* when the FO substitution level with vegetable oils reached 80%. Increased hepatic enzymes may indicate hepatic lesions, possibly a result of induced stress as reported in tilapia (Chen et al. 2003).

The results of this study showed that in Two-banded Seabream diets, peanut oil at a level of 50% of the total oil content can be used instead of 100% FO without any negative effects on growth performance, feed conversion rate, specific growth rate, and serum hematological and biochemical variables.

ACKNOWLEDGMENTS

The authors thank Nail Üçyol and Hakan Baki for their help.

REFERENCES

Aderolu, A. Z., and O. A. Akinremi. 2009. Dietary effects of coconut oil and peanut oil in improving biochemical characteristics of *Clarias gariepinus* juvenile. Turkish Journal of Fisheries and Aquatic Sciences 9: 105–110.

AOAC. 1998. Official methods of analysis of AOAC international. AOAC, Gaithersburg, Maryland.

Bain, B. J., S. M. Lewis, and I. Bates. 2006. Basic haematological techniques. Pages 26–54 *in* S. M. Lewis, B. J. Bain, I. Bates, editors. Dacie and Lewis practical haemotology, 10th edition. Churchill Livingstone Elsevier, Philadelphia.

Başusta, G. A. 2005. Fish hematology and hematological techniques. Pages 275–300 *in* M. Karatas, editor. Research techniques in fish biology. Nobel Publications, Ankara, Turkey.

Bell, J. G., J. McEvoy, D. R. Tocher, F. McGhee, P. J. Campbell, and J. R. Sargent. 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic Salmon (*Salmo salar*) affects tissue lipid composition and hepatocyte fatty acid metabolism. American Society for Nutritional Sciences 131:1535–1543.

Bell, J. G., D. R. Tocher, F. M. MacDonald, and J. R. Sargent. 1994. Effects of diets rich in linoleic (18:2n-6) and alpha-linolenic (18:3n-3) acids on the growth, lipid class and fatty acid compositions and eicosanoid production in juvenile turbot (*Scophthalmus maximus* L.). Fish Physiology Biochemistry 13:105–118.

Bulut, M., M. Yigit, S. Ergun, O. S. Kesbic, U. Acar, N. Gultepe, M. Karga, S. Yilmaz, and D. Guroy. 2014. Evaluation of dietary protein and lipid requirements of Two-banded Seabream (*Diplodus vulgaris*) cultured in a recirculating aquaculture system. Aquaculture International 22:965–973.

Burel, C., T. Boujard, S. J. Kaushik, G. Boeuf, S. Van Der Geyten, K. A. Mol, E. R. Kühn, A. Quinsac, M. Krouti, and D. Ribaillier. 2000. Potential of plant-protein sources as fish meal substitutes in diets for turbot (*Psetta maxima*) growth, nutrient utilization and thyroid status. Aquaculture 188:363–382.

Campbell, T. W. 2004. Clinical chemistry of fish and amphibians. Pages 499–517 *in* M. A. Thrall, D. C. Baker, T. W. Campbell, D. DeNicola, M. J. Fettman, E. D. Lassen, A. Rebar, and G. Weiser, editors. Veterinary hematology and clinical chemistry: text and clinical case presentations. Lippincott Williams and Wilkins, Philadelphia.

Chen, C. H., G. A. Wooster, R. G. Getchell, P. R. Bowser, and M. B. Timmons. 2003. Blood chemistry of healthy, nephrocalcinosisaffected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. 218:89–102.

Coles, E. H. 1986. Veterinary clinical pathology. Saunders, Philadelphia.

Demir, O., A. Turker, U. Acar, and O. S. Kesbic. 2014. Effects of dietary fish oil replacement by unrefined peanut oil on the growth, serum biochemical and hematological parameters of Mozambique Tilapia juveniles (*Oreochromis mossambicus*). Turkish Journal of Fisheries and Aquatic Sciences 14:887–892.

Deng, D. F., Z. Y. Ju, W. G. Dominy, L. Conquest, S. Smiley, and P. J. Bechtel. 2014. Effect of replacing dietary menhaden oil with Pollock or soybean oil on muscle fatty acid composition and growth performance of juvenile Pacific Threadfin (*Polydactylus sexfilis*). Aquaculture 422:91–97.

Díaz-López, M., M. J. Pérez, N. G. Acosta, D. R. Tocher, S. Jerez, A. Lorenzo, and C. Rodríguez. 2009. Effect of dietary substitution of fish oil by *Echium* oil on growth, plasma parameters and body lipid composition in Gilthead Seabream (*Sparus aurata* L.). Aquaculture Nutrition 15:500–512.

Fackjouri, A. H., B. Falahatkar, and H. Ershad Langroudi. 2011. The influence of different lipid sources and levels on growth, body composition and haematology of *Huso huso*. Journal of Animal Physiology and Animal Nutrition 95:632–641.

Folch, J. M., S. M. Lee, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from the animal tissues. Journal of Biological Chemistry 226:497–509.

Glencross, B. D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture 1:71–124.

Guidetti, P. 2004. Consumers of sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, in shallow Mediterranean rocky reefs. Helgoland Marine Research 58:110–116.

Gültepe, N., Ü. Acar, O. S. Kesbiç, S. Yılmaz, Ö. Yıldırım, and A. Türker. 2014. Effects of dietary *Tribulus terrestris* extract supplementation on growth, feed utilization, hematological, immunological and biochemical variables of Nile Tilapia *Oreochromis niloticus*. Israeli Journal of Aquaculture Bamidgeh [online serial] 66:IJA_66.2014.1024.

Gültepe, N., O. S. Kesbiç, Ü. Acar, K. Gökkuş, M. İ. Gültepe, A. Y. Sönmez, S. Bilen, and S. Aydın 2015. Effects of prebiotic mannanoligosaccharides on histology and biochemical blood parameters of Gilthead Seabream, *Sparus aurata*. Israeli Journal of Aquaculture Bamidgeh [online serial] 67:IJA_66.1072.

Gunstone, F. D. 2010. 3 The world's oils and fats. Pages 61–94 *in* M. T. Giovanni, N. Wing-Keong, and R. D. Douglas, editors. Fish oil replacement and alternative lipid sources in aquaculture feeds. Taylor and Francis Group, Boca Raton, Florida.

Guroy, D., B. Guroy, D. L. Merrifield, A. A. Tekinay, S. J. Davies, and I. Sahin. 2012. Effects of fish oil and partial fish meal substitution with oilseed oils and meals on growth performance, nutrient utilization and health of the Rainbow Trout *Oncorhynchus mykiss*. Aquaculture International 20:481–497.

Han, Y. Z., T. J. Ren, Z. Q. Jiang, B. Q. Jiang, J. Gao, S. Koshio, and C. F. Komilus. 2012. Effects of palm oil blended with oxidized fish oil on growth performances, hematology, and several immune parameters in juvenile Japanese Sea Bass, *Lateolabrax japonicas*. Fish Physiology and Biochemistry 38:1785–1794.

Harikrishnan, R., C. Balasundaram, and M. S. Heo. 2011. Influence of diet enriched with green tea on innate humoral and cellular immune response of kelp grouper (*Epinephelus bruneus*) to *Vibrio carchariae* infection. Fish and Shellfish Immunology 30:972–979.

Horta, M., M. J. Costa, and H. Cabral. 2004. Spatial and trophic niche overlap between *Diplodus bellottii* and *Diplodus vulgaris* in the Tagus estuary, Portugal. Journal of the Marine Biological Association of the UK 84:837–842.

Huang, S. S. Y., A. N. Oo, D. A. Higgs, C. J. Brauner, and S. Satoh. 2007. Effect of dietary canola oil level on the growth performance and fatty acid composition of juvenile Red Sea Bream, *Pagrus major*. Aquaculture 271:420–431.

Izquierdo, M. S., D. Montero, L. Robaina, M. J. Caballero, G. Rosenlund, and R. Gines. 2005. Alterations in fillet fatty acid profile and flesh quality in Gilthead Seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250:431–444.

Jug-Dujakovic, J., and B. Glamuzina. 1988. Preliminary studies of reproduction and early life history of *Diplodus vulgaris* (E. Geoffroy Saint-Hilaire 1817) in captivity. Aquaculture 69:361–377.

Kenari, A. A., M. T. Mozanzadeh, and R. Pourgholam. 2011. Effects of total fish oil replacement to vegetable oils at two dietary lipid levels on the growth, body composition, haemato-immunological and serum biochemical parameters in Caspian Brown Trout (*Salmo trutta caspius* Kessler, 1877). Aquaculture Research 42:1131–1144.

Lee, S. M., J. H. Lee, and K. D. Kim. 2003. Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile Starry Flounder (*Platichthys stellatus*). Aquaculture 225:269–281.

Lin, T., H. Lin, C. Chen, M. Lin, M. Chou, and C. Wang. 2007. *Hibiscus sabdariffa* extract reduces serum cholesterol in men and women. Nutrition Research 27:140–145.

Lin, Y., and S. Shiau. 2007. Effects of dietary blend of fish oil with corn oil on growth and non-specific immune responses of grouper, *Epinephelus malabaricus*. Aquaculture Nutrition 13:137–144.

Lin, Y., and S. Shiau. 2003. Dietary lipid requirement of grouper, *Epinephelus malabaricus*, and effects on immune responses. Aquaculture 225: 243–250.

Liu, X., J. Ye, K. Wang, J. Kong, W. Yang, and L. Zhou. 2012. Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei.* Aquaculture Research 43:745–755.

McDonald, D. G., and C. L. Milligan. 1992. Chemical properties of the blood. Pages 55–133 *in* W. S. Hoar, D. J. Randall, and A. P. Farrell, editors. Fish physiology, volume 12, part B: the cardiovascular system. Academic Press, San Diego, California.

Montero, D., T. Kalinowski, A. Obach, L. Robania, L. Tort, M. J. Caballero, and M. S. Izquerdo. 2003. Vegetable lipid sources for Gilthead Sea Bream (*Sparus aurata*) effects on fish health. Aquaculture 225:353–370.

Montero, D., J. Socorro, L. Tort, M. J. Caballero, L. E. Robaina, J. M. Vergara, and M. S. Izquierdo. 2004. Glomerulonephritis and immunosuppression associated with dietary essential fatty acid deficiency in Gilthead Sea Bream, *Sparus aurata* L., juveniles. Journal of Fish Diseases 24:297–306.

Morgan, J. D., and G. K. Iwama. 1997. Measurements of stressed states in the field. Pages 247–270 *in* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, editors. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, UK.

Ochang, S., N. Oyedapo, A. Fabbenro, and T. A. Olabode. 2007. Growth performance, body composition, haematology and product quality of the African Catfish (*Clarias gariepinus*) fed diets with palm oil. Pakistan Journal Nutrition 6:452–459.

Ozório, R. O. A., L. M. P. Valente, S. Correia, P. Pousão-Ferreira, A. Damasceno-Oliveira, C. Escórcio, and A. Olivia-Teles. 2009. Protein requirement for maintenance and maximum growth of Two-banded Seabream (*Diplodus vulgaris*) juveniles. Aquaculture Nutrition 15:85–93.

Pallaoro, A., M. Santic, and I. Jardas. 2006. Feeding habits of the Common Two-banded Sea Bream, *Diplodus vulgaris* (Sparidae), in the eastern Adriatic Sea. Cybium 30:19–25.

Peng, S., L. Chen, J. G. Qin, J. Hou, N. Yu, Z. Long, J. Ye, and X. Sun. 2008. Effects of replacement of dietary fish oil by soybean oil on growth performance and liver biochemical composition in juvenile Black Seabream, *Acanthopagrus schlegeli*. Aquaculture 276:154–161.

Piedecausa, A., M. J. Mazón, B. García García, and M. D. Hernández. 2007. Effects of total replacement of fish oil by vegetable oils in Sharpsnout Seabream (*Diplodus puntazzo*) diets. Aquaculture 263:211–219.

Silvia, M. L., A. T. Vidal, A. V. Moñino, M. P. Torres, and M. J. Cerdá. 2007. Effects of dietary soybean oil concentration on growth, nutrient utilization and muscle fatty acid composition of Gilthead Sea Bream (*Sparus aurata* L.). Aquaculture Research 38:76–81.

Subhadra, B., R. Lochmann, S. Rawles, and R. Chen. 2006. Effect of dietary lipid source on the growth, tissue composition and hematological parameters of Largemouth Bass (*Micropterus salmoides*). Aquaculture 255:210–222.

Trushenski, J., M. Schwarz, H. Lewis, J. Laporte, B. Delbos, R. Takeuchiand, and L. A. Sampaio. 2011. Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile Cobia *Rachycentron canadum*. Aquaculture Nutrition 17:437–447.

USDA–NASS (U.S. Department of Agriculture–National Agricultural Statistics Service). 2014. Census of agriculture. USDA–NASS, Washington, D.C.

Wassef, E. A., O. M. Wahby, and E. M. Sakr. 2007. Effect of dietary vegetable oils on health and liver histology of Gilthead Seabream (*Sparus aurata*) growers. Aquaculture Research 38:852–861.

Watanabe, T., T. Takeuchi, S. Satoh, T. Ida, and M. Yaguchi. 1987a. Development of low protein-high energy diets for practical carp culture with special reference to reduction of total nitrogen excretion. Nippon Suisan Gakkaishi 53:1413–1423.

Watanabe, T., T. Takeuchi, S. Satoh, K. W. Wang, T. Ida, M. Yaguchi, M. Nakada, T. Amano, S. Yoshijima, and H. Aoe. 1987b. Development of practical carp diets for reduction of total nitrogen loading on water environment. Nippon Suisan Gakkaishi 53:2217–2225.

Wiegertjes, G. F., R. J. Stet, H. K. Parmentier, and W. B. Van Muiswinkel. 1996. Immunogenetics of disease resistance in fish: a comparative approach. Developmental and Comparative Immunology 20:365–381.

Yigit, M., M. Erdem, S. Koshio, S. Ergün, A. Türker, and B. Karaali. 2006. Substituting fish meal with poultry by-product meal in diets for Black Sea turbot *Psetta maeotica*. Aquaculture Nutrition 12:340–347.

Yigit, M., S. Ergün, A. Türker, B. Harmantepe, and A. Erteken. 2010. Evaluation of soybean meal as a protein source and its effect on growth and nitrogen utilization of Black Sea turbot (*Psetta maeotica*) juveniles. Journal of Marine Science and Technology 18:682–688.

Yildirim, O., U. Acar, A. Turker, M. C. Sunar, and S. Yilmaz. 2013. Effects of Partial or Total Replacement of Fish Oil by Unrefined Peanut Oil on Growth and Chemical Composition of Common Carp (*Cyprinus carpio*). Israeli Journal of Aquaculture Bamidgeh [online serial] 65:IJA_65.2013.919.

Yıldız, M., O. T. Eroldoğan, K. Engin, A. Gülçubuk, and M. A. Baltacı. 2013. Effects of dietary cottonseed and/or canola oil inclusion on the growth performance, FA composition and organ histology of the juvenile Rainbow Trout, *Oncorhynchus mykiss*. Turkish Journal of Fisheries and Aquatic Sciences 13:453–464.

Yılmaz, S., and S. Ergün. 2012. Effects of garlic and ginger oils on hematological and biochemical parameters of Sea Bass, *Dicentrarchus labrax*. Journal of Aquatic Animal Health 24:219 224.