Impacts of different dietary lipid sources on growth performance, fatty acid composition and antioxidant enzyme activity of juvenile Black Sea bream, *Acanthopagrus schlegeli*

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Abstract

The aim of the present study was to evaluate the impacts of diets supplemented with different lipid sources on growth performance, body composition, fatty acid profile, and hepatic antioxidant enzyme activity of juvenile black sea bream, *Acanthopagrus schlegeli* (initial mean weight, 1.1±0.02g). Four isonitrogenous and isolipidic diets were formulated with either fish oil (FO), soybean oil (SO), linseed oil (LO) or a mixture of SO and LO (SO+LO). The results showed that survival, weight gain, feed efficiency and protein efficiency ratios of black sea bream were not affected by dietary lipid sources ($p>0.05$). Liver and muscle of fish fed the SO diet had high concentration of linoleic acid, while those of fish fed the LO diet were high in linolenic acid. Liver and muscle of fish fed the FO diet had significantly ($p<0.05$) higher levels of eicosapentaenoic acid and docosahexaenoic acid, compared to fishes fed the SO and LO diets. Superoxide dismutase and glutathione peroxidase enzymes activities in liver of black sea bream were not influenced by dietary lipid sources. Our findings suggest that SO and/or LO can be used as a substitute for FO in black sea bream diets without adverse effects on growth performance and antioxidant enzyme activity, when the essential fatty acid requirements are present in diets for black sea bream.

Keywords: *Acanthopagrus schlegeli*, Lipid sources, Growth performance, Fatty acids, Superoxide dismutase, Glutathione peroxidase

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Introduction

Fish oil (FO) is the main lipid source in compound aquafeeds, which has high digestibility and n-3 highly unsaturated fatty acids (HUFAs) content (Montero *et al*., 2003). In the last decade, the increase in demand, price and world supply fluctuations of FO have emphasized the need to find alternative lipid sources in aquaculture feeds (Bell *et al*., 1996; Mourente and Bell, 2006; Kowalska *et al*., 2010). In the recent years much attention has been focused on vegetable oils, which have lower price and larger production volume than FO (Bell *et al*., 2003; Regost *et al*., 2003; Fountoulaki *et al*., 2009). A number of studies have shown that plant oils could replace substantial levels of FO without affecting the survival and growth of fishes (Bell *et al*., 2003; 2006; Francis *et al*., 2006; Piedecausa *et al*., 2007). However, the evidence across different studies have shown that utilization of vegetable oils can change the fatty acids composition of tissue, especially in marine fish (Ganga *et al*., 2005; Montero *et al*., 2008; Peng *et al*., 2008;; Cho, 2012). Fish fed with the vegetable oils diets have higher levels of linoleic acid (C18:2n-6), linolenic acid (C18:3n-3) and lack of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) compared to FO diet (Montero *et al*., 2003; Izquierdo *et al*., 2005; Kim *et al*., 2012). This is attributed to the limited capacity of marine fish for bioconversion of C_{18} vegetable oils into HUFAs such as arachidonic acid (AA), EPA and DHA (Pratoomyot *et al*., 2008; Bouraoui *et al*., 2011; Ganga *et al*., 2011). The studies cited above suggest that it is important to ensure that the contents of EPA and DHA are sufficient in diets supplemented with vegetable oils. Since deficits of these fatty acids can disturb lipid metabolism and lead to poor fish health.

Reactive oxygen species (ROS), such as super oxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are formed in the body during normal metabolic processes, and are removed by antioxidant defense system (Brand-Williams *et al*., 1995). Superoxide dismutase (SOD) and glutathione peroxidase (GP_X) are common antioxidant enzymes, and they constitute the first line of antioxidant enzymatic defense. The superoxide radical (O_2^-) is decomposed to H_2O_2 by SOD, and GP_X metabolize H_2O_2 to H_2O (Lygren *et al*., 2000). An imbalance between ROS and antioxidant defenses has been described as oxidative stress (Homblad and Soderhall, 1999; Erdogan *et al*., 2004). The fatty acid composition of food are considered to have very contradictory role in oxidant–antioxidant system of fish (Kiron *et al*., 2004). The polyunsaturated fatty acids (PUFAs) are causative agents in oxidative stress, and protective agents in the antioxidant defense against stress. Since they are susceptible to oxidation and targets for oxygen radicals, the resulting products can be toxic to the cells (Lygren *et al*., 2000; Kiron *et al*., 2011; Olsvik *et al*., 2011). On the other side, there is evidence that long-chain PUFAs such as AA and EPA scavenge free radical directly or they also increase antioxidant enzyme gene expression (Wang *et al*., 2004; Kusunoki *et al*., 2013). Therefore, it is important to provide an

adequate amount of fatty acids in aquatic feeds to maintain optimal health for fish.

The black sea bream, *A. schlegeli*, is an important commercial marine fish species in the coastal waters of western Pacific Ocean. This fish is a promising fish with many qualities which make it an excellent candidate for culture (Nip *et al*., 2003). The purpose of present study was to determine the impacts of the replacement of fish oil with soybean and/or linseed oil on the growth performance, proximate muscle composition, liver lipid content, fatty acids composition of liver and muscle tissues and the activities of SOD and GPx enzymes in juvenile Black Sea bream.

Materials and methods

Experimental diets

Ingredients and nutrient contents of the experimental diets are presented in Table 1. Four isonitrogenous and isolipidic diets were formulated to contain fish oil (FO), soybean oil (SO), linseed oil (LO) and a mixture of SO and LO (SO+LO), each at an additive level of 8%. Pollack fish meal was used as the primary protein source and wheat flour was used as carbohydrate source. The crude protein and lipid in experimental diets were maintained at 48% and 12%, respectively. The experimental diets were pelletized by a laboratory pellet machine after 400 g water was mixed with 1 kg of ingredients and dried overnight at room temperature. All diets were stored at -30ºC until used. The fatty acid composition of the experimental diets are summarized in Table 2.

Table 1: Ingredients and nutrient contents of the experimental diets.

	Diets			
	FO	**SO**	**LO**	**SO+LO**
Ingredients (%)				
Pollack fish meal	52.0	52.0	52.0	52.0
Fermented soybean meal	8.0	8.0	8.0	8.0
Corn gluten meal	5.0	5.0	5.0	5.0
Wheat flour	22.5	22.5	22.5	22.5
Brewer's yeast	2.0	2.0	2.0	2.0
Cod liver oil	8.0			
Soybean oil		8.0		4.0
Linseed oil			8.0	4.0
Vitamin premix[1]	1.0	1.0	1.0	1.0
Mineral premix[2]	1.0	1.0	1.0	1.0
Vitamin C (50%)[3]	0.3	0.3	0.3	0.3
Chorine salt (50%)	0.2	0.2	0.2	0.2
Nutrient contents (%, dry matter)				
Crude protein	48.4	48.1	48.6	49.0
Crude lipid	12.2	12.1	12.1	12.1
Ash	12.5	12.8	12.6	12.3
C20:5n-3	1.18	0.69	0.7	0.61
C22:6n-3	1.1	0.75	0.76	0.73
C20:5n-3 + C22:6n-3	2.28	1.44	1.46	1.34

[1] Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

[2] Mineral premix contained the following ingredients (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.

[3] ROVIMIX® STAY-C® 35. DSM Nutrition Ltd. Seoul, Korea.

Table 2: Major fatty acid composition (% of the total fatty acids) of the experimental diets.

	Diets			
	FO	**SO**	**LO**	**SO+LO**
Fatty acids				
C14:0	3.0	0.9	0.8	0.8
C14:1	0.3	0.1	0.1	0.1
C16:0	18.5	16.2	12.7	12.2
C16:1	4.1	1.6	1.4	1.5
C18:0	4.7	4.2	3.8	4.1
C18:1n-9	20.6	19.3	18.4	19.6
C18:2n-6	15.5	33.2	14.4	25.4
C18:3n-3	2.1	4.3	29.4	19.0
C20:0	0.1	0.3	0.1	0.2
C20:1n-9	3.5	2.6	2.3	2.6
C20:3n-6	0.4	0.1	0.1	0.1
C20:4n-6	0.5	0.2	0.2	0.1
C20:5n-3	11.0	6.4	6.5	5.7
C22:5n-3	2.1	1.0	0.9	0.7
C22:6n-3	10.2	7.0	7.1	6.8

Experimental fish and feeding trial

Juvenile black sea bream were obtained from a local farm (Namhae, Korea). The fish were acclimated to the laboratory conditions for 2 weeks prior to the start of the feeding trial. Juvenile fish (initial mean weight, 1.1±0.02 g) were allocated randomly into 12 plastic tanks, with 40 fish per tank (50L water volume) for the feeding trial after being collectively weighted. Three replicate groups of fish were hand-fed to apparent satiation three times per day (09:00, 13:00, and 17:00 hours for 6 days per week) for 8 weeks. Water temperature was 19.6±1.5ºC and the photoperiod was left under natural conditions (12h:12h/dark:night) during the feeding trail. Records were kept of daily feed consumption, mortalities and feeding behavior of each tank.

Sampling procedures and chemical analysis

At the end of the feeding trial, all of the fish in each tank were collectively weighed and counted after anesthetizing with tricaine methanesulfonate (MS222, Sigma, St. Louis, MO, USA) at a concentration of 100 ppm, and after starvation for 24 h. All surviving fish at the termination were sacrificed and stored at -75ºC in a freezer. Samples of liver and dorsal muscle of ten fish fromeach tank were removed and pooled for analyses of proximate composition. The crude protein content was measured by the Kjeldahl method with an Auto Kjeldahl System (Buchi, Flawil, Switzerland), and the crude lipid content was determined by the diethylether-extraction method, using a Soxhlet extractor (VELP Scientifica, Milano, Italy). The moisture content was calculated with a

dry oven (105ºC for 6h), and the ash content was determined using a muffler furnace (550ºC for 4h). Lipid for fatty acid analyses was extracted by a mixture of chloroform and methanol (2:l v/v) according to the Folch *et al.* (1957) method, and fatty acid methyl esters were prepared by trans-esterification with 14% BF_3-MeOH (Sigma, St. Louis, MO, USA). Fatty acid methyl esters were analyzed using a gas chromatography (PerkinElmer, Clarus 600, GC, USA) with a flame ionization detector, equipped with SP^{TM}-2560 capillary column (100m×0.25mm i.d., film thickness 0.20 µm; Supelco, Bellefonte, PA, USA). Injector and detector temperatures were both 240ºC. The column temperature was programmed from 140 to 240ºC at a rate of 5ºC min^{-1}. Helium was used as the carrier gas. Fatty acids were identified by comparison with retention times of the standard fatty acid methyl esters (PUFA 37 component FAME Mix; Supelco).

To determine the activity of hepatic SOD and GPx, liver from five fish per each tank were pooled, and 0.1 g of liver homogenate was mixed with in 9 volumes of 5 mM Tris and 35 mM glycine (pH 7.6). The activities of SOD and GPx in liver were assayed according to the methods as described in previous paper (Azarm and Lee, 2012).

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Significant differences (p<0.05) among the means were determined using a Duncan's multiple range test (Duncan, 1955). Data are presented as the mean±SE of three replicate groups.

Results

Growth performance and morphological parameters of juvenile black sea bream fed the experimental diets are presented in Table 3. There were no significant differences in weight gain, specific growth rate, feed efficiency, protein efficiency ratio, daily feed intake and daily protein intake among the treatments (p>0.05). Morphological parameters, such as condition factor, hepatosomatic index and viscerasomatic index were not affected by dietary treatments. Also, dietary lipid source did not affect the crude lipid, moisture and ash contents of fish muscle and lipid content of liver (Table 4).

Table 3: Growth performance and morphological parameters of juvenile black sea bream fed with the experimental diets for 8 weeks.

	Diets			
	FO	**SO**	**LO**	**SO+LO**
Initial body weight (g/fish)	1.12 ± 0.01^{ns}	1.11 ± 0.01	1.13 ± 0.03	1.12 ± 0.02
Weight gain[1] (%)	378 ± 3.35^{ns}	370 ± 3.5	401 ± 3.18	400 ± 1.2
Specific growth rate[2] (%)	2.8 ± 0.04^{ns}	2.7 ± 0.01	2.9 ± 0.09	2.9 ± 0.00
Feed efficiency[3] (%)	95 ± 3.7^{ns}	80 ± 1.0	81 ± 5.1	89 ± 3.0
Daily feed intake[4] (%)	2.3 ± 0.01^{ns}	2.5 ± 0.6	2.4 ± 0.1	2.5 ± 0.14

Table 3 continued				
Daily protein intake[5] (%)	1.1 ± 0.05[ns]	1.2 ± 0.02	1.2 ± 0.05	1.2 ± 0.07
Protein efficiency ratio[6]	1.9 ± 0.07[ns]	1.7 ± 0.02	1.7 ± 0.1	1.6 ± 0.16
Condition factor[7]	1.83 ± 0.04 [ns]	1.95 ± 0.02	2.05 ± 0.03	2.04 ± 0.13
Hepatosomatic index[8]	2.87 ± 0.38 [ns]	3.27 ± 0.27	2.54 ± 0.18	2.88 ± 0.30
Viscerasomatic index[9]	6.43 ± 0.18 [ns]	7.21 ± 0.21	6.22 ± 0.41	6.62 ± 0.22

Values are means ± SE (n = 3).
ns= values are not significant ($P > 0.05$).
[1] (Final fish wt. - initial fish wt.) × 100/initial fish wt.
[2] [ln (final fish wt.) - ln (initial fish wt.)] × 100/days of feeding.
[3] Wet weight gain × 100/feed intake.
[4] Feed intake × 100/ [(initial fish wt. + final fish wt. + dead fish wt.) × days reared/2].
[5] Protein intake × 100/ [(initial fish wt. + final fish wt. + dead fish wt.) × days reared/2].
[6] Wet weight gain × 100/ protein intake.
[7] [Fish weight (g)/fish length (cm)^3] × 100.
[8] (Liver weight/body weight) × 100.
[9] (Viscera weight/body weight) × 100.

Table 4: Proximate composition (%) of the dorsal muscle and liver of juvenile black sea bream fed with the experimental diets for 8 weeks.

	Diets			
	FO	**SO**	**LO**	**SO+LO**
Muscle				
Crude lipid	1.4 ± 0.11[ns]	1.1 ± 0.21	1.3 ± 0.20	1.1 ± 0.08
Crude protein	19.0 ±0.07 [ns]	18.2± 0.27	18.6 ± 0.25	19.0 ± 0.22
Moisture	78.1 ± 0.32 [ns]	77.5 ± 0.41	78.3 ± 0.33	78.3 ±0.12
Ash	1.4 ± 0.04[ns]	1.5 ± 0.10	1.6 ± 0.07	1.5 ± 0.02
Liver				
Crude lipid	13.0 ± 0.64[ns]	13.0 ± 0.73	14.2 ± 0.20	14.4 ± 0.51

Values are means ± SE (n = 3).
ns= values are not significant ($p>0.05$).

The fatty acid composition of liver and muscle of black sea bream reflected the fatty acid composition of the dietary lipid sources (Table 5). Statistically, significant differences among the four feeding treatments were noticed in the content of saturated fatty acids ($p<0.05$). The saturated fatty acids such as C14:0 and C16:0 in muscle were higher in fish fed FO diet than fish fed with vegetable oils diets. Percentages of C16:1 of liver and muscle in fish fed FO diet were significantly higher than those in fish fed other diets ($p<0.05$). Percentages of linoleic acid were significantly highest in liver and muscle of fish fed SO diet ($p<0.05$). While, the value of the linolenic acid of liver and muscle of fish in LO group were statistically the highest among the treatments ($p<0.05$). Fish fed the diet supplemented with FO had higher content of EPA and DHA compared to vegetable oils diets ($p<0.05$).

Table 5: Major fatty acid composition (% of the total fatty acids) of the liver and muscle of fish fed with the experimental diets for 8 weeks.

	Diets			
	FO	**SO**	**LO**	**SO+LO**
Liver				
C14:0	2.7 ± 0.3^{b}	2.0 ± 0.3^{a}	1.8 ± 0.1^{a}	1.8 ± 0.15^{a}
C16:0	16.5 ± 0.25^{b}	15.3 ± 0.05^{ab}	12.9 ± 0.8^{a}	14.4 ± 0.72^{ab}
C16:1	7.0 ± 0.05^{b}	4.5 ± 0.85^{a}	3.8 ± 0.37^{a}	4.1 ± 0.23^{a}
C18:0	7.0 ± 0.02^{ns}	6.6 ± 0.01	7.0 ± 0.09	6.6 ± 0.06
C18:1n-9	30.0 ± 1.0^{b}	24.2 ± 0.9^{a}	25.1 ± 1.0^{ab}	24.3 ± 0.9^{a}
C18:2n-6	5.3 ± 0.7^{a}	22.5 ± 0.3^{c}	6.9 ± 0.08^{a}	14.0 ± 0.5^{b}
C18:3n-6	0.8 ± 0.05^{a}	2.5 ± 0.06^{b}	1.3 ± 0.05^{ab}	1.7 ± 0.06^{ab}
C18:3n-3	1.8 ± 0.05^{a}	2.8 ± 0.09^{a}	19.4 ± 1.0^{c}	10.0 ± 0.63^{b}
C21:0	0.5 ± 0.01^{b}	0.4 ± 0.05^{a}	0.4 ± 0.03^{a}	0.4 ± 0.04^{a}
C20:2n	2.0 ± 0.01^{b}	1.5 ± 0.05^{a}	1.6 ± 0.01^{a}	1.5 ± 0.03^{a}
C20:3n-6	0.8 ± 0.05^{b}	0.6 ± 0.07^{a}	0.6 ± 0.05^{a}	0.6 ± 0.05^{a}
C20:4n-6	0.8 ± 0.02^{ns}	1.0 ± 0.00	0.8 ± 0.00	0.9 ± 0.00
C23:0	1.3 ± 0.05^{ns}	1.1 ± 0.1	1.6 ± 0.05	0.9 ± 0.1
C22:2n	0.6 ± 0.05^{b}	0.3 ± 0.05^{a}	1.0 ± 0.03^{c}	0.9 ± 0.05^{b}
C20:5n-3	8.0 ± 0.07^{b}	4.2 ± 0.09^{a}	3.2 ± 0.01^{a}	$3.6.\pm 0.05^{a}$
C22:5n-3	2.8 ± 0.05^{b}	1.0 ± 0.04^{a}	1.3 ± 0.03^{a}	1.3 ± 0.05^{a}
C22:6n-3	10.0 ± 0.4^{b}	6.2 ± 0.6^{a}	6.0 ± 0.6^{a}	6.2 ± 0.4^{a}
Muscle				
C14:0	1.7 ± 0.1^{b}	1.0 ± 0.1^{a}	1.1 ± 0.03^{a}	1.0 ± 0.03^{a}
C16:0	17.6 ± 0.2^{c}	16.4 ± 0.3^{b}	15.0 ± 0.3^{a}	15.5 ± 0.3^{ab}
C16:1	3.6 ± 0.02^{b}	2.1 ± 0.02^{a}	2.2 ± 0.03^{a}	2.0 ± 0.06^{a}
C18:0	6.6 ± 0.1^{ns}	6.4 ± 0.1	6.5 ± 0.2	6.4 ± 0.1
C18:1n-9	17.8 ± 0.8^{ns}	16.0 ± 1.0	18.2 ± 0.3	17.4 ± 0.08
C18:2n-6	9.3 ± 0.2^{a}	22.5 ± 0.5^{c}	10.6 ± 0.4^{a}	17.7 ± 0.4^{b}
C18:3n-6	0.4 ± 0.00^{a}	1.1 ± 0.00^{c}	0.5 ± 0.05^{a}	0.8 ± 0.03^{b}
C18:3n-3	1.2 ± 0.1^{a}	2.6 ± 0.2^{a}	17.2 ± 0.1^{c}	10.0 ± 0.3^{b}
C21:0	0.4 ± 0.00^{b}	0.3 ± 0.03^{a}	0.3 ± 0.00^{a}	0.3 ± 0.00^{a}
C20:2n	0.8 ± 0.03^{a}	0.7 ± 0.00^{a}	1.1 ± 0.08^{b}	0.8 ± 0.03^{a}
C20:3n-6	0.7 ± 0.03^{b}	1.4 ± 0.11^{c}	0.5 ± 0.00^{a}	0.9 ± 0.03^{b}
C20:4n-6	0.3 ± 0.15^{ns}	0.4 ± 0.15	0.5 ± 0.03	0.3 ± 0.00
C23:0	1.2 ± 0.03^{b}	0.5 ± 0.28^{a}	0.8 ± 0.06^{ab}	0.9 ± 0.00^{ab}
C22:2n	0.7 ± 0.00^{b}	0.3 ± 0.16^{a}	1.2 ± 0.05^{c}	0.9 ± 0.00^{b}
C20:5n-3	9.8 ± 0.7^{b}	6.7 ± 0.5^{a}	6.2 ± 0.55^{a}	6.5 ± 0.21^{a}
C22:5n-3	3.2 ± 0.05^{b}	1.5 ± 0.03^{a}	1.5 ± 0.00^{a}	1.3 ± 0.04^{a}
C22:6n-3	17.0 ± 0.5^{b}	13.4 ± 1.4^{a}	13.1 ± 1.1^{a}	12.0 ± 0.3^{a}

Values (means±SE, n=3) with different superscripts in the same row are significantly different ($p<0.05$). ns= values are not significant ($p>0.05$).

The activities of liver superoxide dismutase (SOD) and glutathione peroxidase (GPx) are expressed as percentage of inhibition rate and U mg^{-1} protein, respectively in Table 6. SOD and GPx activities of the liver did not show significant differences among the treatments ($p>0.05$).

Table 6:Antioxidant enzyme activities in liver of juvenile black sea bream fed with the experimental diets for 8 weeks.

	Diets			
	FO	**SO**	**LO**	**SO+LO**
Superoxide dismutase (%)	70.6 ± 4.41^{ns}	64.5 ± 5.3	67.6 ± 4.0	66.5 ± 5.26
Glutathione peroxidase (U mg^{-1} protein)	8.7± 0.29^{ns}	6.7 ± 0.68	7.9 ± 0.83	6.4 ± 0.78

Values are means ± SE (n = 3).
ns= values are not significant (p>0.05).

Discussion

The present study showed that the inclusion of vegetable oils in the diet of black sea bream for 8 weeks did not have any adverse effect on survival, growth performance and feed utilization. Similarly, the results obtained in Atlantic salmon (Bell *et al*., 2003), Atlantic cod (Bell *et al*., 2006), Murray cod (Francis *et al*., 2006), red sea bream (Glencross *et al*., 2003) and sharp snout sea bream (Piedecausa *et al*., 2007) showed that total replacement of dietary FO by vegetable oils had no significant effect in growth rates. However, total FO substitution by vegetable oils diets in sea bass (Izquierdo *et al*., 2003) and gilthead sea bream (Montero *et al*., 2008) reduced fish growth. These variations may be related to the particular essential fatty acid requirements of the studied species, the dietary inclusion of fish meal or other fatty acid sources and the lipid content of diets assayed for each species and the ability to accept vegetable oils of the target fish (Bell *et al*., 2010). Peng *et al*. (2008) reported that total substitution of FO by SO produced a significant reduction in weight gain of black sea bream, while the current study does not show significant difference in weight gain between FO and SO diet. These differences may be related to less utilization of fish meal in this experimental feed compared to present study (35% vs. 52%). The SO and LO diets in this study contained some n-3 HUFA, as the fish meal itself contained 0.6% EPA and 0.7% DHA. These amounts might be sufficient to meet the n-3 HUFA requirement (1%) of black sea bream to maintain normal growth (Peng *et al*., 2008). According to a number of different studies, the proper dietary fish meal level could supply the essential nutrients, like amino acid and fatty acid, and consequently increase growth performance and feed efficiency in fish (Bell *et al*., 2002; Izquierdo *et al*., 2003; Kim *et al*., 2012).

The present study showed that the values of hepatosomatic index (HSI) and viscerasomatic index (VSI) were not influenced by dietary lipid sources. Peng e*t al*. (2008) also did not note the differences in the HSI value of black sea bream fed FO or SO diet. Similarly, previous studies have not demonstrated significant HSI differences on other fish species, including turbot (Regost *et al*., 2003), and European sea bass (Mourente *et al*., 2005). By contrast, increased VSI in pikeperch (*Sander lucioperca*) fed peanut oil diet (Kowalska *et al*., 2010) and increased HSI in black carp (*Mylopharyngodon piceus*) fed rapeseed oil have been reported. It is probably because of the high content of oleic acid in the feeds of these fish, because mainly monounsaturated fatty acids are the energy reserves and are stored in the fish

visceral adipose tissue (Caballero *et al*., 2002; Montero *et al*., 2008). The results of pervious researches showed that the reduction of dietary essential fatty acids due to the inclusion of vegetable oils, the type of non-essential fatty acid and the interaction of different fatty acids included in the diet, affects the hepatic morphology and lipid content of fish (Bell *et al*., 2010; Bouraoui *et al*., 2011). Moreover, no effect of dietary lipid source was observed in liver and muscle composition of black sea bream, suggesting that the oils were equally well utilized by the fish. The high lipid content of liver, regardless of dietary lipid sources, shows that this fish have the ability to store large amounts of lipid in the liver.

In this experiment, the liver and muscle fatty acid compositions reflected the dietary fatty acid profile. However, specific fatty acids were selectively retained in the muscle and liver of the fish, especially, DHA concentration in muscles were higher than that of diet concentration. The same results have also been obtainedin turbot (Regost *et al*., 2003) gilthead sea bream (Izquierdo *et al*., 2005) and European sea bass (Montero *et al*., 2005; Mourente and Bell, 2006). The selective deposition of DHA might be related to the high specificity of some synthesizing enzymes such as 1-lysophosphatidylacyl CoA transferase for DHA. The increased DHA/EPA ratio in muscles, also indicated a selective catabolism of EPA relative to DHA in fatty acid oxidative processes. The relative resistance of DHA to β-oxidation is stemming from the complex catabolic pathway of this fatty acid (Caballero, 2002; Mourente and Bell, 2006). The livers AA content were more than its respective level in diet, which was similar to Atlantic salmon (Torstensen *et al*., 2004). This indicates the importance of AA as an essential fatty acid for proper function of the liver cells. Besides, in this studya higher content of C18:1n-9 was observed in liver compared to diet. A similar finding was reported for European sea bass (Montero *et al*., 2005) and yellow croaker (Wang *et al*., 2012). The accumulation of oleic acid in liver of fish may be related to the low activity of mitochondrial fatty acid oxidation enzyme, which results in the reduction of metabolism and an increase in deposition of oleic acid in fish liver (Gavino and Gavino, 1991). This can also be attributed to the inhibitory effect of n-3 HUFA such as EPA on beta-oxidation of monounsaturated fatty acids such as 18: 1n-9 (Osmundsen and Bjornstand, 1985; Montero *et al*., 2005). On the other hand, there were a high correlation between thesaturated fatty acids (mainly C16:0) and monounsaturated fatty acids (mainly C18:1n-9) content of diets and muscles tissue, indicating that these fatty acids were used efficiently as an energy source. As reported previously for this species (Peng *et al*., 2008), and for many other fish species (Caballero *et al*., 2002; Montero *et al*., 2005; Piedecausa *et al*., 2007), fish fed diet containing SO showed a significantly higher 18:2n-6 compared to fishes fed diets containing FO or LO. By contrast, fish fed LO diet exhibited a significantly higher 18:3n-3 than the other fishes. However, the concentration of 18:2n-6 and 18:3n-3 in liver and muscle were always lower than diets.These information suggest that these fatty acids were readily oxidized and selectively utilized when present at high concentration in diet. The

content of EPA and DHA significantly decreased in fish fed with diets containing vegetable oils. Thismay be due to the inability of marine fish species to synthesize EPA and DHA from C18 (Izquierdo *et al*., 2005; Mourente and Bell, 2006; Peng *et al*., 2008).

There are some evidences showing that both humoral and cellular antioxidant enzyme activities may be modulated by consumption of different oils in rat and fish species (Ruiz-Gutie´rrez *et al*., 1999; Olsvik *et al*., 2011). Contradictory results were also found in different studies. Olsvik *et al*. (2011) reported that vegetable oil reduced the antioxidative defense system in Atlantic salmon, whereas no significant difference in SOD activity were observed in black carp (Sun *et al*., 2011). The results of our study proved that, SOD and GPx activities as indices of endogenous defense were not significantly affected by dietary lipid sources. These observations state the fact that antioxidant mechanisms in this fish are operating properly. Moreover gene expression and activity of antioxidant enzymes can be controlled by AA and n-3 HUFAs and their metabolites (Luo *et al*., 2012; Kusunoki *et al*., 2013). Similar antioxidant response in black sea bream may also be related to sufficient levels of AA and n-3 HUFAs in diets containing SO and/or LO.

In conclusion, the results of this study suggest that SO and LO can be used as a substitute for FO in the diets of juvenile black sea bream without any negative effects on growth, feed utilization and antioxidant enzymes activities. This will maintain and satisfy the dietary essential fatty acid requirements of black sea bream.

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References

Azarm, H. M. and Lee, S. M., 2012. Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquaculture Research,*45(6), 994-1003.

Bell, J. G., Ashton, I., Secombes, C. J., Weitzel, B. R., Dick, J. R. and Sargent, J. R., 1996. Dietary lipid affects phospholipid fatty acid compositions, eicosanoids production and immune function in Atlantic salmon (*Salmo salar*). *Prostaglandins, Leukotrienes and Essential Fatty Acids,*54, 173–182.

Bell, J. G., Henderson, R. J., Tocher, D. R., McGhee, F., Dick, J. R., Porter, A., Smullen, R. P. and Sargent, J. R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. *Journal of Nutrition,* 132, 222–230.

Bell, J. G., McGhee, F., Campbell, P. J. and Sargent, J. R., 2003. Rapeseed oil as an alternative to marine fish oil in diets of post-smolt Atlantic salmon (*Salmo salar*): changes in flesh fatty acid composition and effectiveness of subsequent fish oil "wash out". *Aquaculture,* 218, 515–528.

Bell, J. G., Strachan, F., Good, J. E. and Tocher, D. R., 2006. Effect of dietary echium oil on growth, fatty acid

composition and metabolism, gill prostaglandin production and macrophage activity in Atlantic cod (*Gadus morhua* L.). *Aquaculture Research*, 37, 606-617.

Bell, J. G., Pratoomyot, J., Strachan, F., Henderson, R. J., Fontanillas, R., Hebard, A., Guy, D. R., Hunter, D. and Tocher, D. R., 2010. Growth, flesh adiposity and fatty acid composition of Atlantic salmon (*Salmo salar*) families with contrasting flesh adiposity: Effects of replacement of dietary fish oil with vegetable oils. *Aquaculture*, 306, 225-232.

Bouraoui, L., Sanchez-Gurmaches, J., Cruz-Garcia, L., Gutierrez, J., Benedito-Palos, L., Perez-Sanchez, J. and Navarro, I., 2011. Effect of dietary fish meal and fish oil replacement on lipogenic and lipoprotein lipase activities and plasma insulin in gilthead sea bream (*Sparus aurata*). *Aquaculture Nutrition,* 17, 54–63.

Brand-Williams, W., Cuvelier, M. E. and Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28, 25-30.

Caballero, M. J., Obach, A., Rosenlund, G., Montero, D., Gisvold, M. and Izquierdo, M. S., 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorrynchus mykiss*. *Aquaculture*, 214, 253-271.

Cho, S. H., 2012. Effects of dietary nutrient on the biological index and serum chemistry of juvenile olive flounder *Paralichthys olivaceus* achieving compensatory growth. *Fisheries and Aquatic Sciences*, 15, 69-72.

Duncan, D. B.,1955. Multiple-range and multiple F tests. *Biometrics*, 11, 1-42.

Erdogan, H., Fadillioglu, E., Ozgocmen, S., Sogut, S., Ozyurt, B., Akyol, O. and Ardicoglu, O., 2004. Effect of fish oil supplementation on plasma oxidant/antioxidant status in rats. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 71, 149–152.

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

Fountoulaki, E., Vasilaki, A., Hurtado, R., Grigorakis, K., Karacostas, I., Nengas, I., Rigos, G., Kotzamanis, Y., Venou, B. and Alexis, M. N., 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. *Aquaculture*, 289, 317–326.

Francis, D. S., Turchini, G. M., Jones, P. L. and De Silva, S. S., 2006. Effect of dietary oil on the growth and muscle fatty acid composition of Murray cod, *Maccullochella peelii. Aquaculture*, 253, 547 –556.

Ganga, R., Bell, J. G., Montero, D., Robaina, L., Caballero, M. J. and Izquierdo, M. S., 2005. Effect of dietary lipids on plasma fatty acid profiles and prostaglandin and leptin production in gilthead seabream (*Sparus aurata*).

Comparative Biochemistry and Physiology, 142, 410 – 418.

Ganga, R., Montero, D., Bell, J. G., Atalah, E., Ganuza, E., Vega-Orellana, O., Tort, L., Acerete, L., Afonso, J. M., Benitez-Sanatana, T., Vaquero, A. F. and Izquierdo, M., 2011. Stress response in sea bream (*Sparus aurata*) held under crowded conditions and fed diets containing linseed and/or soybean oil. *Aquaculture*, 311, 215-223.

Gavino, G. R. and Gavino, V. C., 1991. Rat liver outer mitochondrial carnitine palmitoyltransferase activity towards long-chain polyunsaturated fatty acids and their CoA esters. *Lipids*, 26, 266-270.

Glencross, B., Hawkins, W. and Curnow, J., 2003. Evaluation of canola oils as alternative lipid resources in diets for juvenile red seabream, *Pagrus auratus.Aquaculture Nutrition*, 9, 305-315.

Homblad, T. and Söderhäll, K., 1999. Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity. *Aquaculture*, 172, 111–123.

Izquierdo, M. S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L. and Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. *Aquaculture Nutrition*, 9, 397-407.

Izquierdo, M. S., Montero, D., Robaina, L., Caballero, M. J., Rosenlund, G. and Gines, R., 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.

Kim, D. K., Kim, K. D., Seo, J. Y. and Lee, S. M., 2012. Effects of dietary lipid source and level on growth performance, blood parameters and flesh quality of sub-adult olive flounder *(Paralichthys olivaceus). Asian-Australasian Association of Animal Societies*, 25, 869-879.

Kiron, V., Puangkaew, J., Ishizaka, K., Satoh, S. and Watanabe, T., 2004. Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources. *Aquaculture*, 234, 361–379.

Kiron, V., Thawonsuwan, J., Panigrahi, A., Scharsack, J. P. and Satoh, S., 2011. Antioxidant and immune defences of rainbow trout (*Oncorhynchus mykiss*) offered plant oils differing in fatty acid profiles from early stages. *Aquaculture Nutrition*, 17, 130-140.

Kowalska, A., Zakes, Z., Jankowska, B. and Siwicki, A., 2010. Impact of diets with vegetable oils on the growth, histological structure of internal organs, biochemical blood parameters, and proximate composition of pikeperch *Sander lucioperca* (L.). *Aquaculture*, 301, 69–77.

Kusunoki, C., Yang, L., Yoshizaki, T., Nakagawa, F., Ishikado, A., Kondo, M., Morino, K., Sekine, O., Ugi, S., Nishio, Y., Kashiwagi, A. and Maegawa, H., 2013. Omega-3 polyunsaturated fatty acid has an anti-oxidant effect via the Nrf-2/HO-1 pathway in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications*, 430, 225-230.

Luo, Z., Tan, X. Y., Li, X. D. and Yin, G. J., 2012. Effect of dietary arachidonic acid levels on growth performance, hepatic fatty acid profile, intermediary metabolism and antioxidant responses for juvenile *Synechogobius hasta*. *Aquaculture Nutrition*, 18, 340-348.

Lygren, B., Hamre, K. and WaagbØ, R., 2000. Effect of induced hyperoxia on the antioxidant status of Atlantic salmon *Salmo salar* L. fed three different levels of dietary vitamin E. *Aquaculture Research*, 31, 401- 407.

Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M. J. and Izquierdo, M. S., 2003. Vegetable lipid sources for gilthead seabream (*Sparus aurata*): effects on fish health. *Aquaculture*, 225, 353-370.

Montero, D., Robaina, L., Caballero, M. J., Gine´s, R. and Izquierdo, M. S., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet. *Aquaculture*, 248, 121– 134.

Montero, D., Grasso, V., Izquierdo, M. S., Ganga, R., Real, F., Tort, L., Caballero, M. J. and Acosta, F., 2008. Total substitution of fish oil by vegetable oils in gilthead sea bream (*Sparus aurata*) diets: Effects on hepatic Mx expression and some immune parameters. *Fish & Shellfish Immunology*, 24, 147-155.

Mourente, G., Good, J. E. and Bell J. G., 2005. Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L.): effects on flesh fatty acid composition, plasma prostaglandins E_2 and $F_{2\alpha}$, immune function and effectiveness of a fish oil finishing diet. *Aquaculture Nutrition*, 11, 25-40.

Mourente, G. and Bell, J. G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on flesh and liver fatty acid composition and effectiveness of a fish oil finishing diet. *Comparative Biochemistry and Physiology*, 145, 389–399.

Nip, T. H. M., Ho, W. Y. and Wong, C. K., 2003. Feeding ecology of larval and juvenile black seabream (*Acanthopagrus schlegeli*) and Japanese seaperch (*Lateolabrax japonicus*) in Tolo Harbour, Hong Kong. *Environmental Biology of Fishes*, 66, 197-209.

Olsvik, P. A., Torstensen, B. E., Hemre, G. I., Sanden, M. and WaagbØ, R., 2011. Hepatic oxidative stress in Atlantic salmon (*Salmo salar* L.) transferred from a diet based on marine feed ingredients to a diet based on plant ingredients. *Aquaculture Nutrition*, 17, 424-436.

Osmundsen, H. and Bjørnstand, K., 1985. Inhibitory effects of some long-chain unsaturated fatty acid on mitochondrial beta-oxidation. Effects of streptozotocin-induced diabetes on mitochondrial beta-oxidation of polyunsaturated fatty acid. *Biochemical Journal*, 230, 329-337.

Peng, S., Chen, L., Qin, J. G., Hou, J., Yu, N., Long, Z., Ye, J. and Sun, X., 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, M. A., Mazón, M. J., García García, B. and Hernández, M. D., 2007. Effects of total replacement of fish oil by vegetable oils in the diets of sharp snout sea bream (*Diplodus puntazzo).* *Aquaculture*, 263, 211–219.

Pratoomyot, J., Bendiksen, E. A., Bell, J. G. and Tocher, D. R., 2008. Comparison of effects of vegetable oils blended with southern hemisphere fish oil and decontaminated northern hemisphere fish oil on growth performance, composition and gene expression in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, 280, 170-178.

Regost, C., Arzel, J., Robin, J., Rosenlund, G. and Kaushik, S. J., 2003. Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*) 1. Growth performance, flesh fatty acid profile, and lipid metabolism. *Aquaculture,* 217, 465–482.

Ruiz-Gutie´rrez, V., Pe´rez-Espinosa, A., Vazquez, C. M. and Santa-Marı´a, C., 1999. Effects of dietary fats (fish, olive and high-oleic sunflower oils) on lipid composition and antioxidant enzymes in rat liver. *British Journal of Nutrition*, 82, 233–241.

Sun, S., Ye, J., Chen, J., Wang, Y. and Chen, L., 2011. Effect of dietary fish oil replacement by rapessed oil on the growth, fatty acid composition and serum non-specific immunity response of fingerling black carp, *Mylopharyngodon piceus*. *Aquaculture Nutrition,* 17, 441-450.

Torstensen, B. E., FrØyland, L. and Lie, Ø., 2004. Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil – effects on Atlantic salmon (*Salmo salar* L.) tissue and lipoprotein lipid composition and lipogenic enzyme activities. *Aquaculture Nutrition*, 10, 175–192.

Wang, H. H., Hunga, T. M., Wei, J. and Chiang, A. N., 2004. Fish oil increases antioxidant enzyme activities in macrophages and reduces atherosclerotic lesions in apoE-knockout mice. *Cardiovascular Research*, 61, 169– 176.

Wang, X. X., Li, Y. J., Hou, C. L., Gao, Y. and Wang, Y. Z., 2012. Influence of different dietary lipid sources on the growth, tissue fatty acid composition, histological changes and peroxisome proliferator-activated receptor γ gene expression in large yellow croaker (*Pseudosciaena crocea* R.). *Aquaculture Research*, 43, 281-291.