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Effects of safflower oil supplementation in diet on growth performance and body fatty acid composition of turbot (*Psetta maxima*)

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Abstract The aim of the study was to investigate the effects of the diets that contain safflower oil and fish oil as lipid sources, on growth, feed conversion and body composition in turbot (*Psetta maxima*). Two *iso*-nitrogenous and *iso*-lipidic diets (55 % protein and 14 % lipid) were prepared that include 100 % fish oil (FO group) and 100 % safflower oil (SFO group) for turbots with average weight of 62.21 ± 1.28 g, and fish were fed with these diets for 104 days. At the end of the experiment, the weight gain, specific growth rate and feed conversion ratio were the highest in SFO group than in FO group ($p < 0.05$). Fatty acid composition of fish body reflected the fatty acid composition determined in the experimental diets. The amounts of palmitic acid (PA; C16:0), oleic acid (OA; C18:1n-9), linoleic acid (LA; C18:2n-6) and docosahexaenoic acid (DHA; C22:6n-3) were dominant fatty acids in fish body. It was confirmed that the usage of safflower oil instead of fish oil in turbot feed did not generate any negative effects on growth, feed conversion and the values regarding the growth performance.

Keywords Turbot · *Psetta maxima* · Safflower oil · Growth · Body composition

Introduction

One of the basic dietary ingredients containing high energy in fish feeds for carnivorous marine fish is fish oil, because of its high digestibility and sufficient content of essential fatty acids (EFA), in particular long-chain polyunsaturated fatty acids (PUFA) (Nasopoulou and Zabetakis 2012). The supply of fish oil is limited and their cost is continuously increasing,

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affecting feeding costs and consequently total production costs in aquaculture. For this reason, many researches have been carried out in order to evaluate the alternative lipid sources as potential substitutes in fish diets (Tacon and Jackson 1985; Richard et al. 2006).

In order to reduce dependence on fish oil, significant advances have occurred over the past few years in replacing of fish oil with plant oils. By substituting feeds with plant oils, it also serves to reduce costs due to the fact that vegetable oil sources have continuously increasing production and better economic value. Several studies are carried out to investigate certain vegetable oils as possible sustainable partial or total substitutes for fish oil in fish feeds. The most common vegetable oils used for fish feeds are soybean, linseed, rapeseed, sunflower, palm and olive oil. Soybean and rapeseed oil are considered most possible alternative lipid sources for fresh water and marine fish since they are rich in PUFAs, especially linoleic acid (18:2n-6), but devoid of n-3 PUFA (Mourente et al. 2005).

The essential fatty acid requirement of fish varies between species qualitatively and quantitatively. In contrast to freshwater species, juvenile and sub-adult marine fish species cannot be met the EFA requirements by C18 PUFA and are required the n-3 highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). For juveniles of several marine species including turbot, red sea bream and European sea bass, the EFA requirements can be met by levels of n-3 HUFA of less than or up to 1 % of the dry weight of the diet (Tocher 2010). Otherwise, partially or totally substitution of fish oil by vegetable oils, e.g., olive oil, soybean oil or canola oil, was investigated for these species (Alkistis and Alexis 2001; Martins et al. 2006; Huang et al. 2007). Alkistis and Alexis (2001) informed that the growth parameters and fish body composition were not significantly affected by the use of olive oil, soybean oil and fish oil singly or in combination in diets for sea bass. Huang et al. (2007) also not found any adverse effect by the partially or totally use of canola oil in feeds for red sea bream.

In the present study, the nutritive value of safflower oil for turbot (*Psetta maxima*) was evaluated and the effects of safflower oil on growth performance, feed conversion, body and fatty acid composition of turbot were investigated.

Materials and methods

Fish and maintenance

The study was conducted at the Central Fisheries Research Institute (CFRI), Trabzon, Turkey, in a seawater recirculation system. The experimental fish were obtained from the same research institute. Experimental fish (avg 62 g) were fasted for a day; weighed; and randomly stocked in six rectangle fiberglass tanks (water volume of 500−l; 1 × 1.15 × 0.5 m) at 50 fish per tank. Water inflow was adjusted to 5 l/min, and supplemental aeration was provided via airstone diffusers. The natural seawater from the Blacksea (salinity was about 18 gL^{-1}) was used in the experiment. Temperature was measured twice in a day (18.35 ± 2.9 °C), and dissolved oxygen (8.00 ± 0.1 mgL^{-1}) and pH (7.76 ± 0.13) were measured once a day. At the beginning of the experiment, 10 fish from the stock tank and at the end of the experiment 8 fish from each tank were sampled and analyzed for muscle tissue composition. At the end of the trial, all fish were individually weighed to assess the growth performances.

Experimental diets

Diet ingredients were obtained from a local fish feed manufacturer (Sibal Inc., Sinop, Turkey). Safflower oil was obtained from a commercial feed firm (Gülce Yağ/Kurtuluş

Yağ San.Tic.A.Ş., Manisa, Turkey). Two experimental diets of equivalent protein (55 %) and lipid (14 %) contents were prepared containing 100 % fish oil or 100 % safflower oil (Table 1). Both feeds contained approximately 4 % fish oil from the fish meal. Therefore, the 100 % safflower oil group also contained 4 % fish oil from the fish meal. The ingredients were thoroughly mixed, homogenized, moistened by the addition of 40 % water and pelleted (3.0 mm) in a mincer. The pellets were dried at 70 °C for 12 h and in room temperature for 5 h and then cut into pieces approximately 5 mm in length. All feeds were stored at −40 °C in plastic bags until need for feeding.

Feeding

The experiment was conducted in triplicate in randomly assigned tanks. During experimental period, fish were fed diets by hand to apparent satiation twice a day (at 09:00 and 16:00), 6 days a week. The feeding procedure was done carefully in order to be sure all fish took the feed. The experiment lasted 104 days.

Chemical analysis

Chemical composition of wet samples of fish and diets were analyzed by standard methods (AOAC 1995). Samples were dried to a constant weight at 105 °C to determine moisture level. Crude protein was determined by measuring nitrogen (Nx6.25) using the Kjeldahl method. Crude lipid was extracted with petroleum ether by the Soxhlet method, and the ash was determined by incineration at 550 °C in a muffle furnace. All analyses were performed in triplicate.

Table 1 Formulation and proximate compositions of the FO and SFO diets

	Experimental diets	
	FO	SFO
Ingredients (g kg^{-1})		
Fish meal	600	600
Extracted soybean meal	115	115
Corn protein	101.5	101.5
Wheat flour	80	80
Fish oil	100	–
Safflower oil	–	100
Vitamin premix (*)	2	2
Mineral premix (*)	1.5	1.5
Proximate composition (%)		
Moisture	9.72	5.84
Protein	54.21	55.88
Lipid	14.45	13.48
Ash	7.67	7.24
NFE+Crude fiber[a]	23.67	23.40
Gross energy kJg^{-1}	20.8	20.6

* Per kg feed: 12,500 IU vitamin A; 2,500 IU vitamin D3; 10 mg vitamin K3; 10 mg vitamin B1; 20 mg vitamin B2; 15 mg vitamin B6; 0.03 mg vitamin B12; 250 mg vitamin C; 200 mg niacin; 1 mg biotin; 10 mg folic acid; 60 mg pantothenic acid; 1,000 mg Ca; 130 mg ethoxyquin; 600 mg magnesium; 450 mg potassium; 90 mg zinc; 12 mg manganese; 5 mg Cu

[a] NFE + Crude fiber = 100 − (% protein + % lipid + % ash)

Fatty acid analysis

Lipid extraction of the fish and diet samples was performed according to the method of Bligh and Dyer (1959), using chloroform/methanol (2:1,v/v). Methyl esters were prepared by transmethylation using 2 M potassium hydroxide in methanol and n-hexane according to the method described by Ichihara et al. (1996), and extracted oil sample was dissolved in 2 ml hexane, followed by 4 ml of 2 M methanolic KOH. After centrifugation at 4,000 rpm and at 4 °C for 10 min, the upper phase was taking in a separate tube and covered with parafilm. A total of 1 ml from samples was taken and injected into the GC. Fatty acid peaks were identified from standard fatty acid mixtures, and the percent of individual fatty acids was calculated.

Statistical analysis

All statistical analyses were performed using the program Statistica 7.0 for Windows. To assess normality of distributions, Kolmogorov–Smirnov test was used, and homogeneity of variances was tested using the Levene's *F* test. One-way ANOVA was applied, followed by Tukey's test to locate any differences among treatments. A significance level of 5 % was used in all tests.

Results

Diet proximate and fatty acid composition

All test diets contained similar concentrations of the proximate constituents (Table 1). The diet of SFO group was characterized by having increased proportions of 18:1n-9, and reduced proportions of saturated fatty acids (SFA), monounsaturated (MUFA; 24:1) and n-3 PUFA (22:6n-3) compared to diet of FO group (Table 2). However, linolenic acid (18:3n-3, LNA) and eicosapentaenoic acid (EPA, 20:5n-3), in the SFO diet, were the highest ($p < 0.05$). The FO diet had the highest concentrations of palmitoleic acid (16:1, POA), linoleic acid (18:2n-6c: LA) and docosahexaenoic acid (22:6n-3, DHA) with values of 6.03, 18.89 and 20.34 % lipid, respectively. The n-3/n-6 ratio was 1.49 and 1.50 % for the FO and SFO diets, respectively.

Growth performance and feed conversion

The experimental diets were well accepted by the fish, and no pathological signs were observed during the study. The survival rate was 100 %. Diet treatment affected the growth performance of the turbot and weight gain (WG), and specific growth rate (SGR) and feed conversion ratio (FCR) were significantly affected by diets (Table 3). The weight gain (62.34 g) and SGR (0.65 %) of SFO group were higher than in FO group (52.23 g; 0.58 %, respectively) ($p < 0.05$). In point of final condition factor, there was significant difference between FO and SFO groups ($p < 0.05$). Feed conversion was significantly more efficient in SFO group (0.90) than in FO group (1.07) ($p < 0.05$).

Proximate composition of muscle tissue

At the end of the experiment, protein, lipid and ash contents of muscle tissue were statistically different between groups (Table 4) ($p < 0.05$). The protein, lipid and ash contents of SFO group were higher than those of FO group.

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

Fatty acids	Groups	
	FO	SFO
C14:0	4.45 ± 0.02[a]	1.33 ± 0.09[b]
C16:0	18.87 ± 0.11[a]	10.49 ± 0.26[b]
C18:0	3.63 ± 0.01[a]	2.86 ± 0.09[b]
C20:0	0.28 ± 0.00[a]	0.28 ± 0.01[a]
ΣSFA	27.23 ± 0.15[a]	14.96 ± 0.43[b]
C16:1	6.03 ± 0.31[a]	1.83 ± 0.18[b]
C18:1 n-9	18.26 ± 0.04[a]	24.18 ± 0.37[b]
C20:1	1.41 ± 0.01[a]	1.40 ± 0.01[a]
C24:1	0.53 ± 0.06[a]	0.14 ± 0.01[b]
ΣMUFA	26.23 ± 0.32[a]	27.55 ± 0.21[b]
C18:3 n-3	1.23 ± 0.00[a]	1.49 ± 0.00[b]
C20:5 n-3	9.16 ± 0.02[a]	9.20 ± 0.02[b]
C22:6 n-3	20.34 ± 0.15[a]	17.21 ± 0.55[b]
Σn-3 PUFA	30.73 ± 0.18[a]	27.89 ± 0.56[b]
DHA/EPA	2.22	1.87
C18:2 n-6c	18.89 ± 0.00[a]	17.62 ± 0.17[b]
C18:2 n-6t	0.28 ± 0.00[a]	0.29 ± 0.02[a]
C20:3 n-6ϒ	0.28 ± 0.01[a]	0.26 ± 0.01[b]
C18:3 n-6	0.17 ± 0.00[a]	0.12 ± 0.00[b]
C20:4 n-6	0.87 ± 0.01[a]	0.26 ± 0.00[b]
Σn-6 PUFA	20.49 ± 0.00[a]	18.55 ± 0.19[b]
Σn3/n6	1.49	1.50

Values are mean ± SEM ($n = 3$)

Values within the same row with different superscripts denote significant differences ($p < 0.05$)

Fatty acid composition of muscle tissue

Muscle fatty acid compositions of turbot were significantly influenced by dietary lipid source. The SFO group was quite low SFA, MUFA and n-6 PUFA, but there was no difference between groups in terms of n-3 PUFA. Fish fed with SFO diet showed increased proportions of n-3 PUFA in combination with reduced amounts of 18:1n-9 and 18:2n-6, except for 18:3n-3, as compared with fish fed with FO diet (Table 5). The greatest concentration of DHA was identified in the FO group, but did not differ from SFO group ($p > 0.05$), whereas the greatest concentration of EPA was determined in the SFO group ($p < 0.05$). The n-3/n-6 ratio observed as 1.20, 0.99 and 1.09 % for the groups, respectively.

Discussion

The replacement of fish oil by some vegetable oils in marine fish diets has been studied in turbot (Regost et al. 2003), gilthead sea bream (Caballero et al. 2002; Izquierdo et al. 2005) and sea bass (Izquierdo et al. 2003) without negative effect on growth performances of fish. The results of this study have shown that it is possible to substitute up to 100 % fish oil by safflower oil in diets for turbot without negative effect on growth performance and feed utilization, confirming the similar results found in some experiments with vegetable oils

Table 3 Growth performance and feed efficiency in fish fed the experimental diets

	FO	SFO
Initial body weight (g)	62.28 ± 1.36[a]	62.13 ± 1.20[a]
Final body weight (g)	114.51 ± 3.23[a]	124.47 ± 3.28[b]
Weight gain (g)	52.23[a]	62.34[b]
FCR[1]	1.07 ± 0.03[a]	0.90 ± 0.02[b]
SGR (%)[2]	0.58 ± 0.03[a]	0.65 ± 0.04[b]
Initial condition factor	1.45 ± 0.02[a]	1.47 ± 0.01[b]
Final condition factor	1.45 ± 0.01[a]	1.49 ± 0.01[b]

Values are mean ± SEM ($n = 3$)

Values within the same row with different superscripts denote significant differences ($p < 0.05$)

[1] Feed conversion ratio (FCR) = total feed intake/total weight gain

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

Table 4 Proximate composition (% wet weight) of muscle tissue in fish fed the experimental diets

Proximate composition	Initial	FO	SFO
Moisture (%)	79.19 ± 0.02	78.48 ± 0.48[a]	77.55 ± 0.26[a]
Protein (%)	17.50 ± 0.50	17.56 ± 0.66[a]	17.96 ± 0.33[b]
Lipid (%)	1.78 ± 0.04	1.80 ± 0.09[a]	1.92 ± 0.04[b]
Ash (%)	1.67 ± 0.14	1.59 ± 0.19[a]	1.88 ± 0.15[b]

Values are mean ± SEM ($n = 3$)

Values within the same row with different superscripts denote significant differences ($p < 0.05$)

(canola oil, soybean oil, linseed oil; rapeseed oil, palm oil) in different fish species (brook charr, turbot, Atlantic salmon and European Sea Bass) (Guillou et al. 1995; Regost et al. 2003, Menoyo et al. 2005; Richard et al. 2006). Bell et al. (1994) and Regost et al. (2003) reported no negative effect on growth of turbot fed diets contained safflower oil and linseed oil. Bell et al. (1994) investigated three diets containing fish oil, safflower oil and linseed oil for juvenile turbot (1.2 g). No differences in final weight were found, and any pathological lesions were evident during the trial. Regost et al. (2003) also searched three diets containing fish oil, soybean oil and linseed oil for marketable size turbot (579 g). It was reported that the growth of turbot was high, but the incorporation of vegetable oils in the diets resulted in a slight decrease in growth as compared to those fed with fish oil-based diet. In the present study, no adverse effect on growth performance of turbot fed diet contained safflower oil. On the contrary, the weight gain and SGR of SFO group were higher than in FO group. FCR was not negatively affected by using SFO in feed. Our data showed that it is possible to replace fish oil by safflower oil in diets for turbot. The results denoted the higher ability of turbot to accept vegetable oils in comparison with other marine fish species such as gilthead seabream (*Sparus aurata)* (Izquierdo et al. 2005). In addition, previous studies have shown that the use of vegetable oils instead of fish oil in turbot diets depends on the n-3 content of the basal diet (Bell et al. 1994, 1999).

Little effect of dietary lipid source was observed in tissue composition of turbot, as reported for other fish species (rainbow trout, Atlantic salmon) (Greene and Selivonchick 1990; Dosanjh et al. 1998). In the present study, the crude protein, lipid and ash of fish tissue fed the SFO diet were significantly higher than those fed the FO diet. Though, the

Table 5 Fatty acid composition of muscle tissue in fish fed the experimental diets (% of total fatty acids)

Fatty acids	Groups		
	Initial	FO	SFO
C14:0	4.55 ± 0.16	3.77 ± 0.26[a]	1.54 ± 0.12[b]
C16:0	13.50 ± 0.23	15.56 ± 0.38[a]	12.00 ± 0.15[b]
C18:0	2.37 ± 0.19	2.47 ± 0.16[a]	2.47 ± 0.16[a]
C20:0	0.25 ± 0.03	0.31 ± 0.02[a]	0.30 ± 0.07[a]
ΣSFA	20.67 ± 0.12	22.11 ± 0.53[a]	16.31 ± 0.24[b]
C16:1	6.07 ± 0.17	3.30 ± 0.23[a]	1.75 ± 0.08[b]
C18:1 n-9	18.22 ± 0.58	16.21 ± 0.30[a]	14.77 ± 0.55[b]
C20:1	1.90 ± 0.04	1.77 ± 0.04[a]	1.67 ± 0.07[b]
C24:1	0.23 ± 0.03	0.36 ± 0.03[a]	0.12 ± 0.01[b]
ΣMUFA	26.42 ± 0.74	21.64 ± 0.29[a]	18.31 ± 0.48[b]
C18:3 n-3	1.38 ± 0.04	1.20 ± 0.07[a]	1.40 ± 0.09[a]
C20:5 n-3	3.25 ± 0.14	2.22 ± 0.07[a]	2.65 ± 0.16[b]
C22:6 n-3	17.60 ± 0.76	17.63 ± 0.58[a]	17.31 ± 0.51[a]
Σn-3 PUFA	22.23 ± 0.60	21.05 ± 0.56[a]	21.36 ± 0.21[a]
DHA/EPA	5.41	7.94	6.53
C18:2 n-6c	15.67 ± 0.55	19.12 ± 0.28[a]	16.83 ± 0.37[b]
C18:2 n-6t	1.15 ± 0.03	1.49 ± 0.10[a]	1.89 ± 0.05[b]
C20:3 n-6ϒ	0.26 ± 0.01	0.25 ± 0.01[a]	0.23 ± 0.01[a]
C18:3 n-6	0.20 ± 0.01	0.18 ± 0.00[a]	0.23 ± 0.01[b]
C20:4 n-6	1.23 ± 0.00	0.20 ± 0.02[a]	0.45 ± 0.08[b]
Σn-6 PUFA	18.51 ± 0.52	21.24 ± 0.35[a]	19.63 ± 0.35[b]
Σn3/n6	1.20	0.99	1.09

Values are mean ± SEM ($n = 3$)

Values within the same row with different superscripts denote significant differences ($p < 0.05$)

lipid content of turbot was low (between 1.80 and 1.92 %), confirming earlier reports (Sérot et al. 1998).

Tocher (2010) stated, in contrast to freshwater species the studies on juvenile and sub-adult marine fish, that the EFA requirements cannot be met by C18 PUFA and that the n-3 PUFA, EPA and DHA are required. For juveniles of several species including turbot, red sea bream, European sea bass, red drum and Korean rockfish, the EFA requirements can be met by levels of n-3 HUFA of less than or up to 1 % of the dry weight of the diet (Tocher 2010). The ARA requirement was estimated a value of around 0.3 % of the dry weight of the diet in juvenile turbot (Castell et al. 1994; Bell et al. 1995). Additionally, optimum DHA/EPA ratios have been defined in diets for turbot larvae around 2 (Reitan et al. 1994). In the present study, ARA and DHA/EPA ratios were determined for FO and SFO groups between 0.87 and 0.26 and 2.22–1.87, respectively.

Although the ARA and DHA/EPA ratios were high in diet of FO group, the growth was better in SFO group.

In terms of the muscle fatty acid compositions of groups fed experimental diets, the SFO group has higher fatty acid composition than the FO group. Namely, in the SFO group, ΣSFA, oleic acid (8:1n-9), linoleic acid (18:2n-6), ΣMUFA and Σn-6 PUFA ratios were low, but linolenic acid (18:3n-3), EPA, ARA and Σn-3 PUFA ratios were high.

There were significant differences between groups ($p < 0.05$). Bell and Dick (1990) and Sargent et al. (1999) reported that DHA and EPA are the major PUFAs of cell membranes in fish and 20:4n-6 has an important physiological function in the membrane of fish since it

is known to be the main precursor fatty acid of eicosanoid. Castell et al. (1994) suggested also that dietary 20:4n-6 is essential for juvenile turbot.

Results of this study suggest that potential exists for replacing fish oil with safflower oil in the feeds of turbot without negative effects in growth performance and fatty acid composition in muscle tissue. Further studies are needed to determine the effects of mixture utilization of several oil sources and safflower oil on flesh quality and muscle fatty acid composition of turbot in long-term studies.

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