Partial Replacement of Fishmeal by Defatted Soybean Meal in Diets for Black Sea Turbo *(Psetta maeotica)*: Growth and Nutrient Utilization in Winter

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Abstract

The objective of the present study was to evaluate replacement of white fishmeal by soybean meal in practical diets for Black Sea turbot (*Psetta maeotica*) at levels of 0, 10%, and 20%. The diets were fed to triplicate groups of juvenile Black Sea turbot (initial body weight 18 g) for 60 days. At the end of the trial, there were no differences in growth performance, feed utilization, and nitrogen retention between the control group fed 100% fishmeal and the experimental groups fed 10% or 20% soybean meal. In conclusion, 20% inclusion of soybean meal can allow reduction of white fishmeal by up to 14%, leading to savings on fishmeal protein.

Introduction

Recent studies in fish nutrition seek to develop cost-effective alternative protein sources due to the increasingly high costs and limited supply of fishmeal. Soy products are the most nutritious vegetable alternative because of their protein and amino acid composition, availability, and reasonable price. Reduction of the growth rate by anti-nutritional factors (Rackis, 1974) and poor palatability of soy products can be alleviated by heat treatment or addition of enzymes (Francis et al., 2001; Forster 2002).

Turbot is often divided into two subspecies, *Psetta maxima maxima* and *Psetta maxima maeotica*. The latter is the Black Sea representative and an endemic subspecies (Nielsen, 1986). In the present study, we evaluated the effect of dietary incorporation of defatted soybean meal on growth performance, nutrient utilization, and nitrogen budget of Black Sea turbot (*Psetta maeotica*).

Materials and Methods

Experimental diets. Three iso-nitrogenous (55% crude protein) and iso-caloric (20.5 kJ/g diet) diets were formulated with a protein to energy ratio of about 26 mg/kJ (Table 1). The

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Table 1. Formulation and nutrient composition of diets.

	Diet		
	0	*10*	*20*
Ingredient (g/100 g)			
Fishmeal	77.3	70.6	63.7
Soybean meal	0	10	20
Fish oil	7.9	8.2	8.5
Corn starch	9.0	5.4	2.0
Vitamin mix[a]	3.5	3.5	3.5
Attractant	0.3	0.3	0.3
Binder (guar gum)	2.03	2.03	2.03
Proximate composition (g/100 g air dry basis)			
Dry matter	92.03	91.36	92.34
Crude protein	54.93	54.63	54.79
Crude lipid	15.26	16.19	16.04
Crude ash	13.09	13.21	12.94
Nitrogen free extracts[b]	8.75	7.33	8.57
Gross energy (kJ/g diet)[c]	20.52	20.57	20.76
Protein:energy (mg/kJ)	26.77	26.55	26.39
Protein energy: gross energy	0.63	0.63	0.62

[a] Vitamin/mineral mix from Kadai, Riken Vitamin, Tokyo, Japan
[b] Nitrogen free extracts calculated by difference
[c] Gross energy determined according to 23.6 kJ/g protein, 39.5 kJ/g lipid, 17 kJ/g nitrogen free extract

control diet contained high quality whiting meal (710 g crude protein/kg) as the sole protein source as suggested for Black Sea turbot (Yigit et al., 2003). Diets were produced at the Central Fisheries Research Institute (CFRI), Trabzon, Turkey, with commercially available ingredients. The dry ingredients and oil were mixed in a food mixer for 15 min, then tap water was blended into the mixture to attain a consistency appropriate for passing through a meat grinder with a 3-mm die. After pelleting, the diets were dried to a moisture content of 8-10% and stored frozen prior to feeding. The amino acid profiles of the diets were estimated according to Kaushik (1998; Table 2) from amino acid profiles of protein sources and turbot (Table 3). Total n-3 HUFA contents were calculated as the total fish oil in the diet x % n-3 HUFA in fish oil (Yigit et al., 2006; Table 4).

Fish and rearing conditions. Hatchery-reared Black Sea turbot (mean wt 18.07±0.04 g) were obtained from CFRI and transported to the marine facility of the Faculty of Fisheries, Ondokuz Mayis University in Sinop, Turkey. Fish were acclimated for one month during which they were fed a commercial fishmeal based diet consisting of 55% crude protein, 16% crude lipid, 9% nitrogen-free extract, 21 kJ gross energy/g diet, and 26.2 mg protein per kJ energy to satiation once a day.

After acclimation, the turbot were randomly stocked into nine (three replicates per treatment) identical rectangular polypropylene tanks of 60 l at 15 fish per tank. Tanks were supplied with seawater (17 g/l salinity) at a flow rate of 1.5 l/min and air-stones for continuous aeration. Water temperature was seawater ambient and ranged 6-8°C (6.56±0.69°C). Fish were exposed to the natural light regime (42º01'N 35º09'E) during the 60-day trial from January 10 to March 13, 2004. Fish were hand fed twice daily at 9:00 and 16:00. Feeding activity was monitored carefully to ensure even distribution of feed to all fish in the tank. Tanks were cleaned daily to remove uneaten feed and fecal material. Fish were individually weighed at the start of the experiment and every 15 days.

Sampling, analytical methods, and calculations. At the beginning of the experiment, 15 fish from the initial pool were sacrificed by lowering the body temperature in a freezer, stored in polyethylene bags, and frozen (-20°C) until analysis of whole body composition. At the end of the trial, the same method was followed for three randomly-sampled fish

Table 2. Essential amino acid contents (% dry matter) in experimental diets**.**

	Turbot requirement	*Diet*		
		0	*10*	*20*
Arg	3.59	3.41	3.45	3.50
His	1.47	1.14	1.16	1.19
Ile	1.84	2.30	2.40	2.49
Leu	3.25	3.69	3.78	3.85
Lys	3.42	3.83	3.81	3.78
Met+Cys	2.27	2.06	2.01	1.97
Phe+Tyr	3.90	3.48	3.59	3.71
Thr	2.12	2.18	2.18	2.18
Trp	N/A	0.56	0.58	0.60
Val	2.11	2.56	2.59	2.62

Essential amino acid contents calculated from Table 3.

from each tank. For comparative analysis of whole body dry matter, protein, lipid, and ash, whole carcasses were homogenized in a blender. Fish and diets were analyzed as follows: dry matter after drying in an oven at 105ºC for 24 h to a constant weight, protein (N x 6.25) by the Kjeldahl method after acid digestion, lipids by ethyl ether extraction in a Soxhlet System, and ash by incineration in a muffle furnace at 550ºC for 12 h (AOAC, 1984). Nitrogen-free extract was calculated by difference. Specific growth rate (SGR), feed conversion rate (FCR), protein efficiency rate (PER), and total nitrogen excretion and retention rates were calculated.

Statistical analysis. Statistical analysis of significant differences among treatment means was performed by analysis of variance (ANOVA) using SPSS for Windows, Version 10.0. Duncan's multiple range test was used to compare differences among individual means. Probability values less than 0.05 were considered significant. Results are presented as means±SD.

Table 3. Proximate analysis (%) and essential amino acid composition (%) of turbot and protein sources in diets.

	Turbot[a]	*White fishmeal*[b]	*Soybean meal*[b]
Proximate analysis			
Moisture	-	8.0	11.0
Protein	-	71.4	47.7
Lipid	-	5.5	1.6
Ash	-	12.0	6.4
Essential amino acids			
Arg	6.53	4.41	3.41
Cys	N/A	0.82	0.63
His	2.67	1.47	1.26
Ile	3.35	2.98	2.92
Leu	5.91	4.78	4.02
Lys	6.22	4.96	3.10
Met	N/A	1.84	0.72
Met+Cys	4.12	2.66	1.35
Phe	N/A	2.50	2.45
Phe+Tyr	7.09	4.50	4.17
Thr	3.86	2.82	1.92
Trp	N/A	0.73	0.68
Tyr	N/A	2.00	1.72
Val	3.84	3.31	2.53

[a] according to Kaushik (1998)
[b] according to Halver (1991)

Results

The highest net weight gain and specific growth rate were obtained in the 100% fishmeal group but results did not statistically differ from groups fed the soybean diets (Table 5). No mortality was observed. All diets were well accepted and feed intake, measured as a percentage of the initial body weight, was similar in all treatments. FCR and PER were slightly better in the 100% fishmeal group but

Table 4. Estimated n-3 HUFA content (%) in the experimental diets.

	Diet		
	0	*10*	*20*
Fishmeal content in diet	77.30	70.60	63.70
Crude fat content in fishmeal	5.50	5.50	5.50
Fat from fishmeal in diet	4.25	3.88	3.51
Fish oil in diet	7.90	8.20	8.45
Total fish oils in diet	12.15	12.08	11.96
n-3 HUFA in fish oil[a]	29.76	29.76	29.76
Total n-3 HUFA in diet	3.62	3.60	3.56
n-3 HUFA requirement of turbot: 0.8%[b] to 0.6%[c]			

[a] According to Guner et al. (1998)
[b] According to Gatesoupe et al. (1977)
[c] According to Leger et al. (1979)

did not significantly differ from the soybean groups. Nitrogen excretion was slightly lower and nitrogen retention slightly higher in the 100% fishmeal treatment than in the soybean treatments. Proximate analyses of the fish body were similar among experimental groups (Table 6).

Discussion

Results suggest that inclusion of defatted soybean meal up to the tested levels does not affect growth performance, feed utilization, or nitrogen retention in Black Sea turbot, in accordance with findings reported by Yigit et al. (2007). Growth, feed utilization, and nitrogen budget values of Black Sea turbot in the present study are comparable to those of previous studies on turbot nutrition (Burel et al., 2000; Day and Plascencia Gonzalez, 2000; Fournier et al., 2003, 2004; Turker et al., 2005; Hasimoglu et al., 2007; Yigit et al., 2006, 2007; Ergun et al., 2008).

The fish readily accepted all diets, showing no palatability problems. Our feed intakes agree with those of Robaina et al. (1995) who reported similar feed consumption by rainbow trout of all soybean meal levels used in their study.

Digestibility experiments were not performed in the present study; however, the similar nitrogen retention in all experimental groups suggests that the available protein in the 20% replacement diet was similar to that of the 100% fishmeal control. The ammonia excretion level was slightly but insignificantly higher in the 20% diet. Similarly, nitrogen retention was slightly but insignificantly lower, possibly indicating a higher rate of protein catabolism in turbot fed diets containing 20% soybean meal. Total ammonia nitrogen (TAN) excretion rates were not affected by the inclusion of 20% soybean meal, supporting the growth results.

Soybean contains anti-nutritional factors that can negatively affect fish growth and long-term health (Rackis, 1974; Storebakken et al., 2000; Hendricks, 2002). The inhibition of trypsin and basic proteases in fish by protease inhibitors varies according to fish species. Generally, inhibitor activity can be reduced in defatted soybean meal during the oil extracting process by steaming in toasters. It can be further reduced during pelleting when high pressure and moisture are applied. In the present study, since defatted soybean meal was used, it is likely that some of the anti-nutritional factors were reduced as the experimental fish showed no adverse response to either soybean diet.

We found that soybean meal can replace up to 20% of fishmeal in diets for turbot juveniles without amino acid supplementation. Even without supplementation, almost all the essential amino acid requirements of turbot were provided by the experimental diets, indicating that the sub-optimal amino acid balance of soybean meal did not negatively affect the fish when the soybean content was increased to 20%. Replacement of up to 25% of fishmeal by soy protein concentrate without

Table 5. Growth, feed utilization, and nitrogen excretion of Black Sea turbot juveniles fed test diets for 60 days (means±SD for triplicate groups).

	Diet		
	0	*10*	*20*
Initial wt (g)	18.09±0.04	18.11±0.02	18.03±0.02
Final wt (g)	30.09±0.90	29.84±0.65	29.29±0.24
Net growth (%)	66.36±4.67	64.78±3.49	62.44±1.15
SGR (%)	0.85±0.05	0.83±0.04	0.81±0.01
Survival (%)	100±0.00	100±0.00	100±0.00
Total FI (%/day)	0.67±0.01	0.67±0.01	0.66±0.02
FCR	0.88±0.04	0.90±0.04	0.91±0.02
PER	2.25±0.09	2.24±0.10	2.18±0.04
N intake (mg N/fish)	853.87±27.67	838.46±16.27	825.71±30.05
Total N intake/wt gain (mg/g)	71.27±3.01	71.67±3.12	73.31±1.25
Nitrogen in diet (%)	8.79	8.74	8.77
Nitrogen in fish (%)	2.70±0.02	2.68±0.03	2.66±0.02
Total N retention (mg/g)	28.40±2.24	27.96±1.03	27.22±0.98
Total N excretion (mg/g)	42.87±1.55	43.72±2.38	46.09±2.17
Total N excretion (% intake)	60.19±1.93	60.98±1.05	62.85±1.95

No significant differences ($p>0.05$).
Net growth = [(final wt - initial wt)/initial weight] x 100
SGR = specific growth rate = [(ln final wt - ln initial wt)/days] x 100
FI = feed intake = 100 x (total food distributed)/average live wt/2/days
FCR = feed conversion ratio = feed/wt gain
PER = protein efficiency ratio = wet wt gain/protein intake
N intake (mg N/fish, for 60 days) = Total mg protein intake/6.25
N intake per production (mg/g net wet gain) = (total mg protein intake/6.25)/total wet weight gain(g)
Total N retention = (total g protein retained in fish/6.25)/total wt gain
Total N excretion = (total nitrogen intake - total nitrogen retained)/net wt gain

amino acid supplementation did not significantly reduce fish growth or feed utilization, while methionine and lysine supplementation of the soy protein concentrate improved utilization, although not significantly (Day and Plascencia-Gonzalez, 2000). The slightly but insignificantly lower results in the 20% replacement treatment indicate that higher levels of soybean meal inclusion could affect growth performance or nutrient utilization.

The chemical composition of the body of the fish was not affected by the soybean inclusion level in agreement with studies on rainbow trout (Pongmaneerat and Watanabe, 1993; Kaushik et al., 1995; Davies and Morris, 1997), sea bream (Robaina et al., 1995;

Table 6. Chemical composition of Black Sea turbot fed experimental diets for 60 days.

	Diet			
	Initial	*0*	*10*	*20*
Moisture (% wet wt)	79.62±0.07^{b}	79.23±0.13^{a}	79.26±0.12^{a}	79.37±0.11^{a}
Crude protein (% dry basis)	80.05±0.19	81.21±1.02	80.78±0.93	80.55±0.64
Crude lipid (% dry basis)	13.66±0.32^{b}	12.40±0.08^{a}	12.31±0.49^{a}	12.14±0.32^{a}
Crude ash (% dry basis)	5.70±0.11	5.51±0.23	5.44±0.46	5.71±0.36
Crude protein (% wet basis)	16.32±0.09^{a}	16.87±0.12^{b}	16.76±0.16^{b}	16.62±0.15^{b}

Means in a row with different superscripts differ significantly (p<0.05).

Nengas et al., 1996), Atlantic salmon (Carter and Hauler, 2000; Refstie et al., 2000), Atlantic halibut (Grisdale-Helland et al., 2002), and Black Sea turbot (Yigit et al., 2007).

In conclusion, soybean meal can replace up to 20% of fishmeal in diets for Black Sea turbot juveniles with no adverse effects on growth performance, nutrient utilization, or nitrogen retention. Further studies with higher inclusion levels of defatted soybean meal and soybean products with higher protein contents such as soy protein concentrate should be studied on other size groups to optimize fishmeal replacement in Black Sea turbot diets.

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