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Evaluation of Anchovy Meal and Soybean Meal as Dietary Protein Sources for the Black Sea Turbot, *Psetta maxima*

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Abstract

Soybean meal and locally-produced anchovy (*Engraulis encrasicolus*) fishmeal were evaluated as protein sources in the intensive culture of Black Sea turbot, *Psetta maxima*, by comparing them with imported Alaskan fishmeal. Three diets were prepared. One contained imported fishmeal and the second anchovy fishmeal. In the third diet, part of the anchovy fishmeal was replaced by soybean meal. The dietary protein levels were adjusted to approximately 50%. Juveniles (avg wt 8.2 g) were reared for 61 days in triplicate tanks. Growth and feed efficiency in fish fed the anchovy fishmeal diet were inferior to those of fish fed the imported fishmeal diet, indicating the need to improve the quality of the anchovy fishmeal. Partial replacement of the anchovy fishmeal by soybean meal resulted in worse growth performance. The low growth rates obtained with the anchovy fishmeal diet and the anchovy plus soybean diet were accompanied by low lipid and glycogen accumulation. Fatty acid compositions of the muscle and liver were not influenced by the dietary protein source. The inferior results with the soybean meal diet might have been due to effects of anti-nutrients on the digestive system.

Introduction

The main aquaculture species in Turkey are rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*), and sea bream (*Sparus aurata*). Although production of these species fully meets market demand, there is a consumer trend towards consumption of new species. Black Sea turbot, a commercially important fish on the Black Sea coast of

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Turkey, is a new candidate for aquaculture. The project of the Central Fisheries Research Institute in Trabzon, Turkey, to develop production of turbot seed has reached an average yearly production of 1.96 million hatched larvae, with a fertilization rate of 39.5% and a mean hatching rate of 32.5%. Although seed production techniques for Black Sea turbot were established in 1998-2001 (Kohno et al., 2001; Moteki et al., 2001; Suzuki et al., 2001; Hara et al., 2002; Ustundag et al., 2002), these techniques have not yet been applied to commercial fish culture. Improvements in factors such as feed manufacture, feedstuffs, and cost performance are required before establishing intensive turbot culture in Turkey.

There are two turbot species in the area, *Psetta maxima* in the Black Sea (Black Sea turbot) and *Scophthalmus maximus* (Atlantic turbot) in the Atlantic Ocean. Nutritional studies have been carried out for Atlantic turbot but the nutritional requirements of Atlantic turbot should be evaluated before applying them to Black Sea turbot. The nutritional requirements of adult Black Sea turbot are almost unknown. Erteken and Nezaki (2002) and Yigit et al. (2003) examined the nutritional requirements of Black Sea turbot with allowances for intensive aquaculture.

Local fishmeal produced from the anchovy, *Engraulis encrasicolus*, is available from autumn to spring, mainly in the Black Sea region. It is the cheapest protein source produced in Turkey. It is used for human consumption as well as for domestic animals, but has not been evaluated as a protein source for aquaculture. In this study the nutritional quality of local anchovy fishmeal was compared with that of imported Alaskan fishmeal for growth in Black Sea turbot.

The inclusion of plant proteins in formulated diets reduces production costs. Experiments involving partial replacement of fishmeal by plant protein have been carried out on Japanese flounder (*Paralichthys olivaceus*; Kikuchi et al., 1994; Kikuchi, 1999) and turbot (Regost et al., 1999; Burel et al., 2000). Day and Gonzalez (2000) examined the nutritional quality of soybean protein concentrate as an alternative protein source in turbot culture. In this study, we also examined the effectiveness of partial replacement of anchovy fishmeal by soybean meal as a protein source for Black Sea turbot in terms of growth performance, biology, and biochemical parameters.

Materials and Methods

Fish and rearing conditions. Juvenile turbot were produced at the Central Fisheries Research Institute in Trabzon, Turkey. Two hundred and seventy juveniles (avg wt 8.23± 1.27 g and length 65.8±3.15) were stocked into nine indoor 160-l experimental tanks at 30 fish per tank, with three tanks for each treatment. The fish were reared 61 days (October 2-December 3, 2003). Fish were hand-fed three times a day (8:30, 11:30, 14:30) with the same quantity of diet for each tank. Water temperature ranged 13.6-18.9ºC, salinity was 18.3 ppt, DO was 8.6 mg/l, and the water flow 160 l/h.

Diets. Diet 1 was prepared with imported Alaskan white fishmeal (Kodiak Fishmeal Co., USA; 65% protein), Diet 2 with anchovy fishmeal (*Engraulis encrasicolus*) prepared by a fishmeal factory in Turkey, and Diet 3 with 5% of the anchovy fishmeal replaced by soybean meal (Hayat Gida Sanayi Co., Trabzon, Turkey). The crude protein content in each diet was adjusted to approximately 50% (Table 1). Diets were formed by a pellet machine and dried in a warm air dryer at 50ºC. Other feed requirements were adjusted according to Regost et al. (2001). The diameter of the pellets ranged 2.6-2.9 mm.

Biometric measurements. At the end of the experiment, some of the fish were sacrificed, measured, and put into a -40ºC freezer for further study. Biomass gain, feed efficiency, protein efficiency rate, and daily growth rate were used as growth performance indicators. Biological parameters (wet basis) included: daily growth rate = 100(ln final wt - ln initial wt)/days reared, feed efficiency (in %) = 100(body wt gain/diet given), protein efficiency ratio = body wt gain/dietary protein given, muscle ratio (in %) = 100(muscle wt/body wt), and hepatosomatic index (HSI; in %) = 100(liver wt/body wt).

Biochemical measurements. The proxi-

Table 1. Dietary composition, proximate composition, and fatty acid contents of juvenile Black Sea turbot diets containing imported fishmeal (diet 1), local anchovy meal (diet 2), or anchovy plus soybean meals (diet 3).

	Diet		
	1	*2*	*3*
Ingredient (%)			
Imported fishmeal	72.4	0	0
Anchovy fishmeal	0	72.7	69.0
Soybean meal	0	0	5.0
Feed oil (anchovy)	5.0	5.0	5.0
α-Corn starch	13.6	13.3	12.0
Vitamin mixture	2.48	2.48	2.48
Mineral mixture	2.5	2.5	2.5
CMC-Na[1]	3.9	3.9	3.9
APM[2]	0.02	0.02	0.02
Attractants[3]	0.3	0.3	0.3
Proximate composition (%)			
Moisture	12.3	7.6	10.4
Ash	14.0	11.1	10.8
Crude protein	47.1	51.0	50.0
Lipid	10.3	9.8	8.8
Fatty acid (%)			
14:0	5.0	6.9	7.5
16:0	17.9	23.8	24.3
18:0	3.5	4.6	4.5
Total saturated	26.4	35.3	36.3
18:1 n-7	4.9	3.1	2.9
16:1 n-9	7.3	7.0	8.3
18:1 n-9	16.5	18.5	18.1
22:1 n-9	0.2	0.3	0.3
20:1 n-11	1.6	1.5	1.4
22:1 n-11	1.4	1.4	1.3
Total monoenes	31.9	31.8	32.3
18:3 n-3	0.8	1.0	1.0
18:4 n-3	1.3	1.2	1.2
20:4 n-3	0.4	0.3	0.3
20:5 n-3	12.8	6.1	6.1
22:5 n-3	1.1	0.5	0.5
22:6 n-3	14.4	10.8	10.8
Total n-3	30.8	19.9	19.9
18:2 n-6	1.3	1.7	2.0
20:2 n-6	0.2	0.2	0.2
20:4 n-6	0.9	0.6	0.5
22:5 n-6	0.3	0.3	0.3
Total n-6	2.7	2.8	3.0
Total unsaturated	65.4	54.5	55.2

[1] Sodium carboxy methylcellulose
[2] L-ascorbyl 2-monophosphate Mg
[3] Inosine 3, alanine 1.6, glutamic acid 1.1

mate composition of the diet, muscle, and liver were analyzed. The muscle and liver from five fish from each tank were mixed. Moisture and ash of the diet and muscle were analyzed according to standard methods. Crude protein was determined by the Kjeldahl method. Lipid was extracted with a mixture of methanol and chloroform (Bligh and Dyer, 1959). Glycogen of the muscle and liver was analyzed by the anthrone method (Morris, 1948). Fatty acids were converted into methyl esters by dichloromethane. Fatty acid composition was analyzed at 200°C with a Shimadzu Gas Chromatograph GC-17A with a FID detector (Shimadzu Co. Ltd., Kyoto, Japan). A 30-m capillary column packed with Omegawax 320 (Supelco Co. Ltd., Bellefonte, PA, USA) was used. Fatty acid methyl esters were identified by comparison with authentic standards in Funakoshi's FAME mixture (Tokyo, Japan).

Starvation test. At the end of the feed experiment, the rest of the fish were starved for 14 days and their body weight, hepatosomatic index, muscle ratio, muscle lipid, and muscle protein were determined.

Statistical analysis. One-way analysis of variance (ANOVA) was used to determine significant differences between group means. Probabilities of 0.05 or less were considered statistically different.

Results

There were no diseases during the experiment and survival was 100%. Appetite and feeding activity were best with the imported fishmeal (group 1) and lowest with the anchovy/soybean diet (group 3). The feces of group 1 were yellowish brown and those of groups 2 and 3 brown grey. There were significant differences between groups in biomass gain, feed efficiency, protein efficiency and daily growth rate as well as in muscle and liver protein and lipid, before and after starvation (Table 2). The lipid and protein contents per 100 g fish were higher in group 1, indicating that high growth rate accompanies high lipid accumulation (Fig. 1). After starvation, loss of body weight and whole lipid was markedly lower in group 1, loss of body weight

Table 2. Growth, biometric parameters, and proximate composition of muscle and liver of Black Sea turbot juveniles fed a diet containing imported fishmeal (diet 1), local anchovy meal (diet 2), or anchovy and soybean meals (diet 3).

	Diet		
	1	*2*	*3*
Final body wt (g)[1]	23.5±2.9^{a}	18.4±2.7^{b}	11.6±1.7^{c}
CV of body wt[2]	0.13	0.15	0.23
Daily growth rate (%)	1.75^{a}	1.40^{b}	0.66^{c}
Survival (%)	100	100	100
Biomass gain (g)	457^{a}	325^{b}	121^{c}
Total length (mm)	112±5.2^{a}	108±4.9^{b}	94±4.7^{c}
Body length (mm)[3]	88.1±4.4^{a}	84.2±4.1^{b}	73.3±3.6^{c}
Body height (mm)	62.4±3.4^{a}	59.9±3.3^{b}	52.0±3.0^{c}
Body depth (mm)	8.7±0.6^{a}	8.3±0.6^{a}	6.7±0.6^{b}
Body length/total length	0.79	0.78	0.78
Body height/total length	0.56	0.56	0.55
Muscle ratio (%)	42.6±1.8	40.0±5.2	40.9±1.2
Hepatosomatic index (%)	1.57±0.35	1.55±0.45	1.13±0.24
Total diet given (g)	325	325	325
Feed efficiency (%)	141.0^{a}	100.0^{b}	37.2^{c}
Protein efficiency ratio	2.99	1.96	0.74
Muscle composition			
Moisture (%)	80.7±0.4^{a}	79.6±0.6^{b}	80.1±0.6^{ab}
Ash (%)	1.1 ± 0.0^{a}	1.2±0.0^{b}	1.2±0.0^{b}
Crude protein (%)	15.8±0.2^{a}	16.8±0.5^{b}	16.5±0.3^{b}
Lipid (%)	3.4±0.2^{a}	3.4±0.6^{a}	2.9±0.2^{b}
Glycogen (mg/g)	2.91±0.35^{a}	0.81±0.15^{b}	0.47±0.09^{c}
Liver composition			
Lipid (%)	9.3±0.9^{a}	7.8±0.4^{b}	4.9±0.7^{c}
Glycogen (mg/g)	102.8±8.4^{a}	62.1±4.80^{b}	45.8±11.0^{c}
Muscle composition after starvation			
Moisture (%)	79.4±0.3^{a}	79.3±0.1^{a}	80.9±0.4^{b}
Ash (%)	1.2 ± 0.0^{a}	1.3±0.0^{b}	1.3±0.0^{b}
Crude protein (%)	16.9±0.2^{a}	17.5±0.1^{b}	16.4±0.4^{a}
Lipid (%)	3.4±0.4^{a}	2.7±0.3^{b}	2.3±0.2^{b}
Glycogen (mg/g)	1.04±0.37^{a}	0.29±0.25^{b}	0.12±0.00^{b}
Liver composition after starvation			
Lipid (%)	7.4±0.2^{a}	3.9±0.4^{b}	2.7±0.3^{c}
Glycogen (mg/g)	2.66±1.22^{a}	1.02±0.42^{b}	1.40±0.44^{b}

Different superscripts in a row indicate significant differences ($p<0.05$).
[1] Initial body weight = 8.2±1.3 g.
[2] CV (coefficient of variation) of body weight = 100(SD of body weight/body weight).
[3] Initial body length = 65.8±3.2 mm.

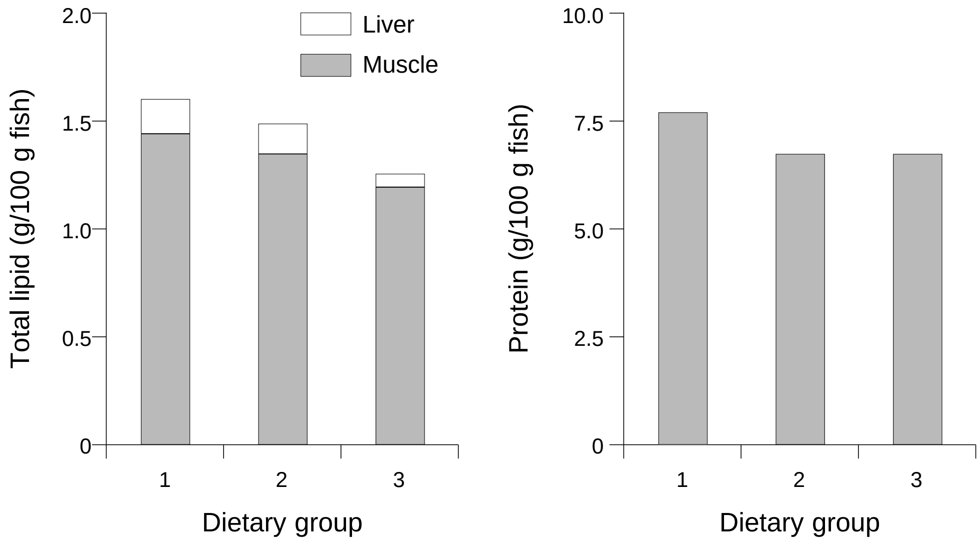

Fig. 1. Total lipid and protein deposited in Black Sea turbot fed diets containing imported fishmeal (group 1), local anchovy meal (group 2), or anchovy plus soybean meals (group 3).

was highest but loss of muscle ratio was lowest in group 2, and muscle protein was lost only in group 3 (Fig. 2).

The main fatty acids in the muscle and liver were 16:0, 18:1 n-9, 20:5 n-3 (eicosapentaenoic acid; EPA), and 22:6 n-3 (docosahexaenoic acid; DHA), the same as in the diets (Table 3). The proportions of saturated fatty acids in the muscle and liver were lower than in the diets, and the values of n-3 and n-6 fatty acids were higher. In general, there were no marked differences between dietary groups.

Discussion

Based on our findings, imported fishmeal is a superior protein source, but the price is higher than that of local anchovy fishmeal. An adequate dietary protein level in Japanese flounder was 45-50% (Kikuchi et al., 2000; Lee et al., 2000; Kim et al., 2003). In turbot, Cacerez-Martinez et al. (1984) found that 50-55% is an adequate dietary protein level. Commercial diets for grow-out stage turbot have high protein contents (52-53%) and low crude lipid contents. In the current experiment, the anchovy fishmeal produced low growth performance and feed efficiency. As oxidized lipids cause Sekoke disease and decrease fish growth performance, solvent-extraction might improve the quality of the fishmeal.

Partial replacement of fishmeal by plant protein has been examined in turbot (Regost et al., 1999; Burel et al., 2000; Day and Gonzalez , 2000). In the current feeding trial, dietary soybean meal reduced growth. In tests of soybean protein in Japanese flounder, growth was not influenced at a replacement level of 20% when supplemented with an amino acid mixture (Kikuchi et al., 1994). Soybean is known to contain polysaccharides and anti-nutrient factors such as trypsin inhibitor, tannin, saponin, and phytic acid (Linenar, 1994; Francis et al., 2001). Extrusion and solvent-extraction were not carried out on the anchovy fishmeal and soybean meal in this experiment

In the current experiment, the anchovy fishmeal did not influence muscle lipid content but caused lower glycogen accumulation in

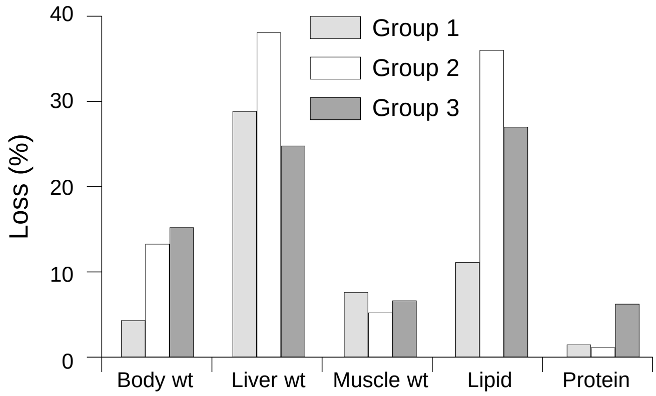

Fig. 2. Changes in biometric parameters after starvation for 14 days.

Table 3. Fatty acid composition of muscle and liver of Black Sea turbot juveniles fed diets containing imported fishmeal (diet 1), local anchovy meal (diet 2), or anchovy plus soybean meals (diet 3).

	Muscle			*Liver*		
Fatty acid	*1*	*2*	*3*	*1*	*2*	*3*
14:0	4.4	5.3	5.0	5.1	6.1	6.6
16:0	16.4	17.2	16.8	14.7	14.2	15.6
18:0	2.7	2.7	2.8	1.9	3.9	2.3
Total saturated	23.5	25.2	24.6	21.7	24.2	24.5
18:1 n-7	4.3	3.2	3.1	5.9	4.4	3.7
16:1 n-9	6.8	7.5	6.8	7.9	8.6	9.7
18:1 n-9	15.0	15.7	13.9	15.5	23.3	15.6
22:1 n-9	0.3	0.3	0.4	0.4	0.5	0.3
20:1 n-11	2.1	2.4	2.9	1.2	1.2	0.8
22:1 n-11	1.5	1.6	2.1	0.7	0.4	0.2
Total monoenes	30.0	30.7	29.2	31.6	38.4	30.3
18:3 n-3	0.9	1.2	1.2	0.7	1.0	1.1
18:4 n-3	1.3	1.7	1.7	0.8	1.0	1.4
20:4 n-3	0.6	0.6	0.7	1.0	1.0	0.8
20:5 n-3	10.7	8.2	7.8	9.5	5.8	7.7
22:5 n-3	2.5	1.9	2.0	2.8	2.1	1.8
22:6 n-3	17.8	15.3	16.4	16.6	12.2	16.0
Total n-3	33.8	28.9	29.8	31.4	23.1	28.8
18:2 n-6	4.2	6.3	8.2	2.2	2.9	4.3
20:2 n-6	0.3	0.4	0.4	0.5	0.2	0.5
20:4 n-6	1.1	0.7	0.8	1.4	0.7	1.6
22:4 n-6	0.2	0.1	0.2	1.0	0.3	0.8
22:5 n-6	0.4	0.3	0.4	0.4	0.4	0.6
Total n-6	6.2	7.8	10.0	5.5	4.5	7.8
Total unsaturated	70.0	67.9	69.0	68.5	66.0	66.9

both muscle and liver, indicating that there was no influence of lipogenesis under our conditions. Kikuchi et al. (1992), working with Japanese flounder, and Oliva-Teles et al. (1999), working with Atlantic turbot, found that the dietary protein level did not affect the proximate composition of muscle. However, Kim et al. (2002, 2003) found that higher dietary protein induced higher muscle protein and lower lipid contents in Japanese flounder. Dietary lipid influenced liver weight in Japanese flounder (Kikuchi et al., 2000) and turbot (Regost et al., 2001). High dietary lipid led to high lipid deposition

The soybean meal used in the current experiment negatively influenced lipid and glycogen accumulation, supporting the theory that low energy intake caused by low digestibility and/or anti-nutritive substances in soybean meal may affect growth. Anti-digestive substances in both the anchovy fishmeal and the soybean meal might have caused the lower growth performance. Since oxidized lipids in the anchovy fishmeal may have reduced dietary effects, solvent extraction might improve growth performance. The quality of anchovy and soybean meal as protein sources in Black Sea turbot culture could be improved through heating and solvent extraction as digestibility of plant proteins for Atlantic turbot is improved by thermal treatment and solvent-extraction (Burel et al., 2000).

The starvation treatment revealed different results. Fish fed the anchovy fishmeal had a greater loss of body weight than fish fed the imported fishmeal. Groups 2 and 3 had greater lipid losses, while the loss of muscle protein was suppressed. Under normal physiological conditions, lipid reserves are preferentially mobilized into energy. During starvation, lipid and glycogen in the muscle and liver were also consumed as energy in groups 2 and 3. The changes in biochemical composition caused by starvation do not imply any serious metabolic disorder such as lipolysis and glycolysis in groups 2 and 3. Low energy accumulation in these groups might have induced the higher loss of body weight after starvation.

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