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Incorporation of Soybean Meal and Hazelnut Meal in Diets for Black Sea Turbot (*Scophthalmus maeoticus*)

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Key words: Black Sea turbot, hazelnut meal, fishmeal, soybean meal, feed utilization

Abstract

The effects of soybean and hazelnut meals as partial replacements of fishmeal were studied in Black Sea turbot (*Scophthalmus maeoticus*). Juvenile fish (mean 26 g) were fed six isoenergetic (19.1±0.18 kJ/g diet) and isonitrogenous (511±0.48 g protein/kg diet) diets for 60 days. The control diet contained fishmeal as the sole protein source. In two of the six experimental diets, soybean meal replaced 20% and 35% of the fishmeal. The other three diets contained 65% fishmeal plus soybean and hazelnut meal at ratios of 25/10, 15/20, 5/30. There was no significant difference ($p<0.05$) in growth performance between the groups fed the diet containing 20% soybean meal and those fed the control diet. Fish fed the 10% or 20% hazelnut meal diets had similar results as fish fed the diet containing 35% soybean meal. Growth performance, feed utilization, protein efficiency, and nitrogen retention were significantly lower in the group fed the 30% hazelnut diet. Total nitrogen excretion and retention were similar in all groups except the 30% hazelnut group. Results indicate that soybean meal can replace up to 20% of the fishmeal without causing reduction in growth performance, nutrient utilization, or nitrogen retention and that hazelnut meal can be incorporated with 20%, but not 35%, soybean meal at a level of no more than 20% of the diet.

Introduction

Fishmeal is a major ingredient in fish diets due to its high protein quality and utilization. It is also among the most expensive ingredients. Due to the limited global supply and increasing demand for fishmeal, less expensive animal or plant protein sources are being examined as partial or total replacements for fishmeal. However, the lack of essential amino acids or presence of anti-nutritional factors in most plant feedstuffs prevents the total replacement of fishmeal by plant protein sources. Only a few studies have succeeded in totally replacing fishmeal in fish diets (Kaushik et al., 1995, 2004; Adelizi et al., 1998).

Earlier studies with turbot deal with plant protein sources such as corn gluten, lupin, or

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soybean meal in diets for Atlantic turbot (*Psetta maxima*; *Scophthalmus maxima*). As far as we know, the few studies on fishmeal replacement in diets for the Black Sea turbot (kalkan; *Psetta maeotica*; *Scophthalmus maeoticus*) include Yigit et al. (2003, 2005ab, 2006) and Turker et al. (2005).

The hazelnut is commercially cultivated in Turkey, the USA, Europe, China, and Australia. Turkey, the world's largest hazelnut producer, produces about 70% of the world supply. Hazelnut production in Turkey was about 584.000 tons (in shell) in 2005 (Fiskobirlik, 2007). Hazelnuts are extensively used in confections, together with chocolate in truffles and other products, and in biscuits and cakes. Excess hazelnuts are used to produce oil for human consumption. The production of hazelnut oil and meal reduces the value to one-fifth of its value for domestic sales as hazelnuts. Hazelnut meal obtained after oil extraction has high nutritive value due to its high protein and low fiber content (Akkilic et al., 1982; Sehu et al., 1996; Yalcin et al., 2005).

The present study evaluated the incorporation of hazelnut meal with soybean meal as a replacement for fishmeal in Black Sea turbot diets and its effect on growth performance, nutrient utilization, and nitrogen balance in the fish.

Materials and Methods

Experimental diets. Six experimental diets, formulated from commercially available ingredients, were produced in the Fish Nutrition Laboratory on the Dardanos Campus of the Faculty of Fisheries in Canakkale Onsekiz Mart University. The diets were isonitrogenous and isocaloric with 511 g crude protein per kg diet and 19.1 kJ gross energy per g diet (Table 1). In the control diet, brown fishmeal (anchovy meal) was the sole protein source. In two diets, 20% or 35% of the fishmeal was replaced by defatted soybean meal. In three diets, 10%, 20%, or 30% of the soybean meal was replaced by hazelnut meal. The proximate composition and amino acid profiles of the protein sources and turbot are given in Table 2; amino acid profiles of the diets are given in Table 3; total n-3 HUFA contents of the diets, calculated according to Yigit et al. (2006) as (total fish oil in g/kg diet) x (% n-3 HUFA in fish oil), are given in Table 4.

The dry ingredients and oil were mixed in a food mixer for 15 min. Tap water was blended into the mixture to attain a consistency suitable for passing through a meat grinder with a 3-mm hole die. After pelleting, the diets were dried to a moisture content of 80-100 g/kg and stored in at -20°C until use.

Fish and experimental rearing conditions. Hatchery reared turbot, *Scophthalmus maeoticus* (26.06±0.11 g mean), were obtained from the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey, and transported to the facilities of the Faculty of Fisheries of Ondokuz Mayis University in Sinop, Turkey. The fish were acclimatized for one month during which they were fed a commercial fishmeal-based diet (550 g/kg crude protein, 160 g/kg crude lipid, 90 g/kg nitrogen-free extract, 21 kJ/g feed gross energy, and 26.19 mg protein/kJ energy) to satiation once a day.

After acclimation, fish were randomly stocked into 18 identical 60-l rectangular polypropylene tanks filled with 45 l water at 10 fish per tank with three replicate tanks per treatment. The system was an indoor flow-through system. Sea water (17 g/l salinity) was supplied to the tanks at a flow rate of 1.5 l/min. Continuous aeration was provided by air-stones. Fish were exposed to the natural light regime (35º09'E, 42º01'N). The tanks were cleaned daily to remove uneaten feed and fecal material.

Water quality was controlled periodically: pH ranged 7.5-8, total ammonia nitrogen, determined by the Nessler method using a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Italy), ranged 0.23-0.28 mg/l; ambient water temperature ranged 8-16°C. Fish were hand fed twice daily at 09:00 and 18:00 for 60 days, from April to June 2004. Feeding activity was monitored carefully to ensure even distribution of the feed to all fish in each tank. Fish were individually weighed at the start of the experiment, and on days 15, 30, 45, and 60. Fish were deprived of feed for one day prior to weighing.

Table 1. Ingredients and nutrient composition of experimental diets.

	Diet					
	100% Fishmeal (control)	*20% Soybean meal*	*35% Soybean meal*	*10% Hazelnut meal*	*20% Hazelnut meal*	*30% Hazelnut meal*
Ingredient (%)						
Fishmeal	79.3	64.8	54.0	54.0	54.0	54.0
Soybean meal	0.0	20.0	35.0	25.75	16.5	7.3
Hazelnut meal	0.0	0.0	0.0	10.0	20.0	30.0
Fish oil	4.4	5.2	5.8	5.6	5.4	5.2
Corn starch	13.3	7.0	2.2	1.6	1.0	0.35
Vitamin-mineral premix[1]	2.8	2.8	2.8	2.8	2.8	2.8
Lysine	0.0	0.0	0.0	0.05	0.1	0.15
Binder (guar gum)	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition (% on air dry basis)						
Dry matter	91.87	91.44	90.26	91.21	91.35	90.10
Crude lipid	10.14	9.80	9.45	9.12	9.65	11.66
Crude ash	13.27	12.48	11.12	11.10	11.65	11.21
Crude protein	51.25	51.42	50.10	51.22	51.15	51.21
NFE[2]	17.21	17.74	19.59	19.77	18.90	16.02
GE (kJ/g feed)[3]	19.08	19.07	18.94	19.11	19.15	19.46
P:E (mg/kJ)	26.87	26.96	26.45	26.81	26.71	26.31

[1] Kadai, Riken Vitamin, Tokyo, Japan
[2] Nitrogen free extracts = matter - (crude lipid + crude ash + crude protein)
[3] Gross energy, calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, 17 kJ/g NFE

Before the start 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 at -20°C for subsequent analysis of whole body composition. At the end of the feeding trial, the same protocol was followed. Three fish from each tank were randomly withdrawn for comparative whole body analysis (dry matter, protein, lipid, ash) and calculation of nutrient retention rates. The three samples from each tank were individually prepared for analysis by homogenizing the body in a blender (Beko BKK-2160 Hotmix Hand Blender, Beko Co., Istanbul). Diets and fish were chemically analyzed according to AOAC (1984) guidelines as follows: dry matter, by drying in an oven at 105°C for 24 h until a constant weight was obtained; protein (N x 6.25), by the Kjeldahl method after acid digestion; lipids, by ethyl ether extraction in a Soxhlet System; ash, by incineration in a muffle furnace at 550°C for 12 h; nitrogen free extracts, as the difference between total dry matter and (crude lipid + crude ash + crude protein).

Calculations. Specific growth rate, feed conversion rate, protein efficiency rate, total nitrogen excretion, and retention rates were calculated as described by Watanabe et al. (1987ab), Burel et al. (2000), and Yigit et al. (2002, 2006).

Statistical analysis. Results were analyzed by analysis of variance (ANOVA) using the

Table 2. Proximate analyses of fishmeal, soybean meal, hazelnut meal, and turbot.

	Turbot	*Fishmeal*	*Soybean meal*	*Hazelnut meal*
Proximate analysis (%)				
Moisture	-	8.0	11.0	10.0
Protein	-	65.9	47.7	43.6
Lipid	-	7.7	1.6	3.7
Ash	-	18.4	6.4	6.0
*Essential amino acids (%)**				
Arg	4.80	4.11	3.41	2.15
Cys	NA	0.66	0.63	NA
His	1.50	1.76	1.26	0.38
Ile	2.60	3.38	2.92	0.52
Leu	4.60	5.43	4.02	1.03
Lys	5.00	5.49	3.10	0.47
Met	NA	2.16	0.72	0.15
Met+Cys	2.70	2.82	1.35	NA
Phe	NA	3.03	2.45	0.68
Phe+Tyr	5.30	5.47	4.17	1.11
Thr	2.90	3.00	1.92	0.50
Trp	0.60	0.82	0.68	NA
Tyr	NA	2.44	1.72	0.44
Val	2.90	3.81	2.53	0.63

NA = not available
* Data on amino acid contents of turbot from Kaushik (1998); of fishmeal and soybean meal from Halver (1991); of hazelnut meal from Koksal et al. (2006).

SPSS Statistical Analysis Software Program for Windows, Version 10.0.1, 1999, for significant differences among treatment means. Duncan multiple-range test was used to detect significant differences (p<0.05) in final body weight, specific growth rate, feed intake, feed conversion rate, protein efficiency rate, nitrogen balance, and proximate body composition.

Results

Survival was 100% in all treatments, indicating that partial replacement of fishmeal by soybean meal with or without hazelnut meal had no effect on survival rates. Average final body weights and feed utilization are given in Table 5. Fish fed the 20% soybean diet did not significantly differ in final mean body weight or specific growth rate from the control. All other diets resulted in significantly lower weight gains.

The lowest nitrogen excretion was obtained in fish fed the control (Table 6). In general, body composition did not significantly differ among treatment groups, except for the 30% hazelnut meal diet (Table 7).

Discussion

Plant feed stuffs have been widely studied as alternative protein sources for fishmeal in fish diets. However, studies on the use of plant protein sources in turbot diets are rare. Regost et al. (1999) reported that protein from corn gluten meal can replace one third of fishmeal protein in Atlantic turbot diets. Burel et al. (2000) found that extruded lupin can be

Table 3. Essential amino acid contents of the experimental diets.

Amino acids (% DM)[1]	*Diet*						
	100% Fishmeal (control)	*20% Soybean meal*	*35% Soybean meal*	*10% Hazelnut meal*	*20% Hazelnut meal*	*30% Hazelnut meal*	*Turbot requirements*
Arg	3.26	2.66	2.22	2.43	2.65	2.86	2.45
His	1.40	1.14	0.95	0.99	1.03	1.06	0.77
Ile	2.68	2.19	1.83	1.88	1.93	1.98	1.33
Leu	4.31	3.52	2.93	3.04	3.14	3.24	2.35
Lys	4.35	3.56	2.96	3.01	3.06	3.11	2.55
Met+Cys[2]	2.24	1.83	1.52	1.54	1.55	1.57	1.38
Phe+Tyr	4.34	3.54	2.95	3.07	3.18	3.29	2.70
Thr	2.38	1.94	1.62	1.67	1.72	1.77	1.48
Trp	NA	NA	NA	NA	NA	NA	0.31
Val	3.02	2.47	2.06	2.12	2.18	2.25	1.48

NA = not available
[1] calculated from data in Table 2
[2] There is no cystine in hazelnut meal.

Table 4. Estimated n-3 HUFA contents in the experimental diets.*

	Diet					
	100% Fishmeal (control)	*20% Soybean meal*	*35% Soybean meal*	*10% Hazelnut meal*	*20% Hazelnut meal*	*30% Hazelnut meal*
Fishmeal in diet (%)[1]	79.3	64.8	54.0	54.0	54.0	54.0
Crude fat (%)	7.7	7.7	7.7	7.7	7.7	7.7
Fat from fishmeal (%)	6.11	4.99	4.16	4.16	4.16	4.16
Fish oil in the diet (%)[2]	4.4	5.2	5.8	5.6	5.4	5.2
Total fish oil in diet (%)	10.51	10.19	9.96	9.76	9.56	9.36
n-3 HUFA in fish oil (%)[3]	29.76	29.76	29.76	29.76	29.76	29.76
Total n-3 HUFA in diet (%)	3.13	3.03	2.96	2.90	2.84	2.78
n-3 HUFA requirements of turbot range from 0.8%[4] to 0.6%[5].						

[1] Anchovy meal (Black Sea)
[2] Anchovy oil (Black Sea)
[3] According to Guner et al. (1998)
[4] According to Gatesoupe et al. (1977)
[5] According to Leger et al. (1979)

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

	Diet					
	100% Fishmeal (control)	*20% Soybean meal*	*35% Soybean meal*	*10% Hazelnut meal*	*20% Hazelnut meal*	*30% Hazelnut meal*
Initial body wt (g)	25.93±0.32	26.03±0.71	25.90±0.35	26.27±0.83	26.33±0.21	25.90±0.46
Final body wt (g)	57.17±0.61^{c}	55.03±1.90^{bc}	53.83±1.88^{b}	53.87±1.12^{b}	53.83±1.83^{b}	48.10±1.38^{a}
SGR (%)[1]	1.32±0.03^{c}	1.25±0.06^{bc}	1.22±0.08^{bc}	1.20±0.06^{b}	1.19±0.07^{b}	1.03±0.02^{a}
FI (%/day)[2]	0.98±0.06	1.00±0.14	1.01±0.04	1.01±0.07	1.06±0.06	1.08±0.07
FCR[3]	0.86±0.06^{a}	0.91±0.09^{a}	0.96±0.06^{a}	0.96±0.12^{a}	1.02±0.10^{a}	1.20±0.09^{b}
PER[4]	2.49±0.17^{b}	2.35±0.23^{b}	2.30±0.15^{b}	2.24±0.26^{b}	2.11±0.22^{ab}	1.82±0.14^{a}

Values in a row with different superscripts are significantly different at 5% level.
[1] Specific growth rate = [(ln final wet wt - ln initial wet wt)/days] x 100
[2] Feed intake = (total food distributed/average live wt/2/days) x 100
[3] Feed conversion ratio = feed/wt gain
[4] Protein efficiency ratio = wet wt gain/protein intake

Table 6. Nitrogen (N) balances of Black Sea turbot fed six different diets for 60 days.

	Diet					
	100% Fishmeal (control)	*20% Soybean meal*	*35% Soybean meal*	*10% Hazelnut meal*	*20% Hazelnut meal*	*30% Hazelnut meal*
N in diet (%)	8.20	8.23	8.02	8.20	8.18	8.19
TNI (mg/g)[1]	64.44±4.34^{a}	68.52±6.94^{a}	69.74±4.47^{a}	72.01±8.74^{a}	76.33±7.38^{a}	88.35±6.73^{b}
N in fish (%)	2.74±0.03^{b}	2.73±0.03^{b}	2.75±0.02^{b}	2.73±0.02^{b}	2.75±0.03^{b}	2.44±0.02^{a}
TNE (mg/g)[2]	36.39±3.69^{a}	40.47±8.87^{a}	41.34±3.35^{a}	43.61±7.16^{a}	47.50±5.85^{a}	66.60±5.93^{b}
TNE (% NI)	56.39±2.02^{a}	58.61±6.96^{a}	59.26±2.14^{a}	60.36±2.46^{a}	62.14±2.20^{a}	75.34±1.52^{b}
TNR (mg/g)[3]	28.05±0.75^{b}	28.05±2.00^{b}	28.40±2.05^{b}	28.40±1.58^{b}	28.83±2.13^{b}	21.75±1.44^{a}
TNR (% NI)	43.61±2.02^{b}	41.39±6.96^{b}	40.74±2.14^{b}	39.64±2.46^{b}	37.86±2.20^{b}	24.66±1.52^{a}

Values in a row with different superscripts statistically differ at $p<0.05$.
[1] Total nitrogen intake = (DPI x days/6.25)/(final wt - initial wt)
[2] Total nitrogen excretion (nitrogen intake - nitrogen retained/(final wt - initial wt)
[3] Total nitrogen retention = (g protein retained in fish/6.25)/(final wt - initial wt)

Table 7. Proximate composition of whole body of Black Sea turbot fed diets in which fishmeal was replaced by soybean and hazelnut meals for 60 days.

	Initial	*Diet*					
		100% Fishmeal (control)	*20% Soybean meal*	*35% Soybean meal*	*10% Hazelnut meal*	*20% Hazelnut meal*	*30% Hazelnut meal*
Moisture (wet wt)	79.23 ± 0.07^{a}	79.62 ± 0.09^{b}	79.68 ± 0.10^{b}	79.33 ± 0.07^{a}	79.67 ± 0.10^{b}	79.58 ± 0.14^{b}	80.18 ± 0.13^{c}
Crude protein (dry basis)	79.66 ± 0.73^{b}	84.03 ± 1.09^{c}	83.96 ± 1.38^{c}	83.17 ± 0.68^{c}	84.08 ± 0.78^{c}	84.17 ± 0.58^{c}	76.88 ± 0.74^{a}
Crude lipid (dry basis)	8.60 ± 0.31^{c}	7.39 ± 0.39^{b}	7.33 ± 0.20^{b}	8.43 ± 0.29^{c}	8.57 ± 0.23^{c}	8.41 ± 0.12^{c}	8.21 ± 0.34^{c}
Crude ash (dry basis)	6.07 ± 0.32^{b}	5.67 ± 0.23^{ab}	5.68 ± 0.30^{ab}	5.26 ± 0.18^{a}	5.44 ± 0.13^{a}	5.94 ± 0.21^{b}	5.40 ± 0.39^{a}
Crude protein (wet basis)	16.55 ± 0.09^{b}	17.13 ± 0.17^{c}	17.06 ± 0.20^{c}	17.19 ± 0.10^{c}	17.09 ± 0.12^{c}	17.19 ± 0.16^{c}	15.24 ± 0.16^{a}

Means with different superscripts in the same row differ significantly at 5% level.

incorporated in Atlantic turbot diets up to a level of 50% without affecting growth performance or fish quality. Fournier et al. (2004) found that a sizeable amount of fishmeal in Atlantic turbot diets could be replaced by proteins from lupin, corn gluten, or wheat gluten meal, if amino acids were supplemented. Day and Plascencia Gonzalez (2000) reported that replacement of up to 25% fishmeal by soy protein concentrate did not reduce fish performance or feed efficiency in Atlantic turbot. A subsequent trial indicated that supplemental dietary methionine and lysine improved utilization of these acids, but this finding was not statistically significant. Bilgin et al. (2007) reported that hazelnut meal can be used to replace up to 30% of soybean meal in extruded diets for rainbow trout. Turker et al. (2005) and Yigit et al. (2006) reported that up to 25% of fishmeal can be replaced by poultry by-product meal without any reduction in growth performance of Black Sea turbot.

The findings in the present study agree with those reported in other studies. Growth dropped as the soybean meal or hazelnut meal content rose but the similar feed intake, FCR, and PER among groups and the fact that fish were fed *ad libitum* indicate that there was no problem of palatability of the diets. The use of a combination of protein sources is a worthy alternative (Watanabe et al., 1997). Rodehutscord et al. (1995) found that fishmeal could be completely replaced by a mixture of wheat gluten and crystalline amino acids without negative influences on growth in rainbow trout.

Inclusion of high levels of some plant protein sources can result in deficiencies of essential amino acids or negative effects of anti-nutritional factors. However, in the present study, some amino acids exceeded the requirements of turbot (Kaushik, 1998), even though the diets were not fortified with essential amino acids. Nevertheless, the imbalances in the amino acid composition between experimental groups were explicit. For example, the 30% hazelnut diet contained 28% less lysine than the control diet. Lysine is generally considered the first limiting amino acid in most fish species. Therefore, the 16% lower mean final weight in the 30% hazelnut group could be attributed to the lower lysine content of the diet.

All the experimental diets met the essential fatty acid requirements of turbot. However, due to the lower amount of anchovy oil and lipids provided by the lower amount of fishmeal, the experimental diets contained less n-3 HUFA than the control. The differences of n-3 HUFA content between diets could explain the differences in growth performance.

The similarity of growth performance between the all the experimental groups (except 30% hazelnut) indicate that hazelnut meal can be incorporated into diets together with soybean meal. However, it seems preferable to incorporate soybean at a level of 20% and not 35% so that the fishmeal content will remain 65%. This will improve the fatty acid level in the diet. Further studies are recommended to test incorporation of 20% soybean meal with various levels of hazelnut meal.

The 30% hazelnut diet caused a significant reduction of nitrogen retention, which may be explained by a limited amount of some essential amino acids. Although digestibility coefficients were not determined, the similarities in nitrogen retention suggest that the available protein in the experimental diets was close to that of the control diet. Our findings closely agree with similar studies that report intakes of 28-36% (Burel et al., 2000), 30-34% (Van Ham et al., 2003), 35-39% (Regost et al., 2003), and 36-42% (Fournier et al., 2004) in Atlantic turbot, and 38-40% (Turker et al., 2005) and 39-41% (Yigit et al., 2006) in Black Sea turbot for fish fed diets in which protein from fishmeal was substituted by plant protein sources and poultry by-product meal. Our results were higher than the 22-24% intake reported by Fournier et al. (2003) in Atlantic turbot. Our total nitrogen excretion rates were higher than those reported for Atlantic turbot (Burel et al., 1996; Fournier et al., 2003) and Japanese flounder (Kikuchi et al., 1992), but agree with those reported for Atlantic turbot by Burel et al. (2000) and for Black Sea turbot by Turker et al. (2005) and Yigit et al. (2006).

Feed conversion and protein efficiency rates were within the ranges reported by Day and Plascencia Gonzalez (2000) and Fournier et al. (2003, 2004) for Atlantic turbot and Turker et al. (2005) and Yigit et al. (2006) for Black Sea turbot fed diets in which the fishmeal was substituted by soybean protein concentrate, a mixture of plant proteins, or poultry by-product meal. Our data were slightly lower than those reported by Person-Le Ruyet et al. (2002) for Atlantic turbot reared in different oxygen concentrations for 30 days.

Whole body crude protein contents in all groups were similar except for the 30% hazelnut diet. Body lipid increased as the inclusion rate of soybean or hazelnut meal increased. This agrees with the findings of Gouveia (1992), who reported an increase of body lipid as the inclusion rate of poultry-by product in diets for rainbow trout increased. However, these results do not agree with those of Fournier et al. (2003, 2004), who reported higher whole body lipids in Atlantic turbot fed fishmeal-based diets than in those fed plant protein-based or combination diets.

The general decrease in the whole body ash content that accompanied the decrease in fishmeal agrees with findings of Fournier et al. (2004) in Atlantic turbot. The general increase of moisture content with the reduction of fishmeal agrees with findings of Turker et al. (2005) and Fournier et al. (2004).

In conclusion, the present study indicates that soybean meal or soybean meal together with hazelnut meal is a reasonable substitute for fishmeal. The limit is that hazelnut meal should be incorporated with 20% soybean meal content, and not 35%, and that hazelnut meal should constitute no more than 20% in the diet. Use of these substances may allow formulation of less expensive diets for Black Sea turbot, reducing overall expenses, increasing profitability, and assuring expansion of Black Sea turbot culture. Whereas juveniles were used in the present study, similar experiments need to be carried out with other size Black Sea turbot.

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