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POTENTIAL OF POULTRY BY-PRODUCT MEAL AS A SUBSTITUTE FOR FISHMEAL IN DIETS FOR BLACK SEA TURBOT SCOPHTHALMUS MAEOTICUS: GROWTH AND NUTRIENT UTILIZATION IN WINTER

Ali Turker¹, Murat Yigit^{2*}, Sebahattin Ergun², Burcu Karaali¹ and Adnan Erteken³

¹ Department of Aquaculture, Faculty of Fisheries, Ondokuz Mayis University, 57000 Sinop, Turkey

² Department of Aquaculture, Faculty of Fisheries, Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey

³Central Fisheries Research Institute, PO Box 129, 6001 Trabzon, Turkey

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Key words: Black Sea turbot, feed utilization, growth performance, poultry by-product meal, white fishmeal, winter growth

Abstract

The use of poultry by-product meal as an alternate dietary protein for Black Sea turbot $Scophthalmus\ maeoticus$ (initial avg wt 18 g) in winter was evaluated. Triplicate groups of 15 fish were fed one of five isoenergetic (gross energy 20.5 \pm 0.21 kJ/g) and isonitrogenous (protein content 55 \pm 0.35%) diets with 25%, 50%, 75%, or 100% of the fishmeal protein replaced by poultry by-product protein. White fishmeal was the sole protein source in the control diet. There was no significant (p<0.05) reduction in growth performance of the turbot fed the 25% replacement diet compared to the control diet (100% fishmeal). At the replacement levels of 50%, 75%, and 100%, however, there was a severe decrease in feed intake, growth performance, feed utilization, protein efficiency ratio, and apparent net protein utilization. Results indicate that up to 25% of the fishmeal protein can be replaced by poultry by-product meal with no negative effects in fish performance at temperatures ranging 6-8°C.

Introduction

The Atlantic turbot, *Scophthalmus maximus*, is of great economic importance for the European mariculture industry (Person-Le Ruyet, 1993), and its production is gradually increasing. The high market demand and

interest in this species has caused many workers to study its biology, especially its nutritional requirements (Burel et al., 1996, 2000; Dosdat et al., 1996; Regost et al., 1999; Day and Plascencia González, 2000;

^{*} Corresponding author. Fax: +90-286-2180543, muratyigit@comu.edu.tr

Pichavant et al., 2000; Person-Le Ruyet et al., 2002; Fournier et al., 2003, 2004). However little information is available on the Black Sea turbot (*kalkan*; *Scophthalmus maeoticus* Pallas), an endemic subspecies and new candidate for aquaculture in Turkey (Moteki et al., 2001; Sahin, 2001; Erteken and Nezaki, 2002; Yigit et al., 2003).

Production of cost-effective nutritionally balanced diets for fish is the main factor affecting intensive aquaculture due to the influence of feed on growth, health, and production costs. One way to reduce feed costs is to partially or totally substitute less expensive animal or plant proteins for more expensive animal proteins. Fishmeal is an important ingredient in aquaculture diets because of its high quality protein but, of all diet ingredients, fishmeal is the most expensive. Hence, the use of less expensive protein sources as partial or total replacements for fishmeal is of important research interest. Fishmeal remains the main protein source in today's aquafeed industry.

The global aquaculture demand for fishmeal was 32% of the world supply in 1999 (New and Wijkstöm, 2002), 37% in 2000 (Chamberlain, 2000), and may reach nearly 65% by 2010 (Chamberlain, 2000) or 70% by 2015 (New and Wijkstöm, 2002). Sustainability of the growing aquaculture industry depends on the progressive reduction of wild fish catch as a protein source for aquafeeds (Naylor et al., 2000).

Poultry by-product meals are valuable alternate protein sources for carnivorous fish. However, compared to fishmeal, poultry byproducts are deficient in one or more essential amino acids (Davies et al., 1991). Poultry byproduct meals have been tested in diets for rainbow trout (Alexis et al., 1985; Steffens, 1994; Pfeffer et al., 1995; Bureau et al., 1999), coho salmon (Higgs et al., 1979), coho salmon and rainbow trout (Sugiura et al., 1998), chinook salmon (Fowler, 1991), European eel (Gallagher and Degani, 1988), channel catfish (Lochmann and Phillips, 1995), gilthead sea bream (Nengas et al., 1999), red sea bream (Goto et al., 2001), Pacific white shrimp (Davis and Arnold, 2000; Samocha et al., 2004), and freshwater shrimp *Macrobrachium nipponense* (Yang et al., 2004).

The objective of this study was to evaluate the effects of replacing fishmeal with poultry by-product meal on growth performance, feed utilization, nitrogen balance, and body composition of Black Sea turbot reared during the winter.

Materials and Methods

Experimental diets. Five practical diets were formulated from commercially available ingredients and produced at the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey (Table 1). The diets were isonitrogenous and isocaloric on a crude protein (55%) and gross energy (20.5 kJ/g diet) basis. White fishmeal (high quality Black Sea whiting meal, 71% crude protein) was the sole protein source in the control diet, as suggested by Yigit et al. (2003) for Black Sea turbot diets. The test diets were formulated by substituting poultry by-product meal protein for the white fishmeal protein at levels of 25%, 50%, 75%, or 100%. The protein, lipid, ash, and moisture contents of the diets were determined by methods of AOAC (1984). The amino acid profiles of the diets met or exceeded the requirements for turbot estimated by Kaushik (1998). Total n-3 HUFA contents ranged 0.06% for the diet containing no fishmeal to 3.62% for the diet containing only fishmeal. The nutrient composition and amino acid profiles of the protein sources and turbot are given in Table 2.

The dry ingredients and oil were mixed in a food mixer for 15 min. Tap water was then blended into the mixture to attain a consistency appropriate for passing the mixture through a meat grinder with a 3 mm die. After pelleting, the diets were dried to a moisture content of 8-10% and cool-stored until use.

Experimental fish and rearing conditions. Hatchery reared turbot (S. maeoticus) of 18.1±0.06 g mean weight were obtained from the Japan International Cooperation Agency (JICA) and the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey, and transported to the Faculty of Fisheries, Ondokuz Mayis University, in Sinop, Turkey. After

arrival, the fish were acclimatized to the new conditions for one month during which they were fed once a day to satiation with a commercial fishmeal based diet (55% crude protein, 16% crude lipid, 9% nitrogen free extracts, 21 kJ gross energy/g feed, 26.19 mg

protein/kJ). After one month acclimation, fish were randomly distributed among 15 identical 60-l rectangular polypropylene tanks filled with 45 l water (15 fish per tank with three replicates per treatment). Tanks were part of an indoor flow-through system in which sea

Table 1. Ingredients and nutrient composition of diets used in the experiment.

	Replacement level (%)					
	0	25	50	75	100	Turbot requirements
Ingredient (g/100 g feed)						
Fishmeal	77.3	58.0	38.0	19.0	-	
Poultry by-product meal	-	21.2	43.2	64.0	85.0	
Anchovy oil (Black Sea)	7.9	6.0	4.0	2.1	0.2	
Corn starch	9.0	9.0	9.0	9.1	9.0	
Vitamin-mineral premix	3.5	3.5	3.5	3.5	3.5	
Attractant	0.3	0.3	0.3	0.3	0.3	
Binder (guar gum)	2.0	2.0	2.0	2.0	2.0	
Proximate composition (g/100	g air dry ba	sis)				
Moisture	8.0	8.8	7.1	8.4	10.1	
Crude lipid	15.3	14.4	13.9	13.1	12.1	
Crude ash	13.1	12.4	11.1	9.8	8.4	
Crude protein	54.9	54.8	55.4	55.4	54.7	
Nitrogen free extracts a	8.7	9.6	12.5	13.3	14.7	
Gross energy (kJ/g diet) b	20.5	20.3	20.7	20.5	20.2	
Protein:energy (mg/kJ)	26.8	27.0	26.7	26.9	27.0	
Protein energy:gross energy	0.63	0.64	0.63	0.64	0.64	
Crude fat (%)	5.5	5.5	5.5	5.5	5.5	
Fat from white fishmeal (%)	4.25	3.19	2.09	1.05	-	
Total fish oil in diet (%)	12.15	9.19	6.09	3.15	0.20	
n-3 HUFA in fish oil (%) c	29.76	29.76	29.76	29.76	29.76	
Total n-3 HUFA (%)	3.62	2.73	1.81	0.94	0.06	0.8d - 0.6e

Table 1. Cont'd

		Replacement level (%)				
	0	25	50	75	100	Turbot requirements
Amino acid content (%/DM) f						
Arg	3.4	3.4	3.4	3.4	3.4	2.6
Lys	3.8	3.5	3.2	2.9	2.6	2.7
His	1.1	1.1	1.0	1.0	0.9	0.8
lle	2.3	2.3	2.2	2.2	2.2	1.4
Leu	3.7	3.7	3.7	3.6	3.6	2.5
Val	2.6	2.6	2.6	2.6	2.6	1.6
Met+Cys	2.1	2.0	1.9	1.9	1.8	1.5
Phe+Tyr	3.5	3.2	3.0	2.8	2.5	2.9
Thr	2.2	2.1	2.0	1.9	1.8	1.6
Trp	0.6	0.5	0.5	0.5	0.4	0.3

a Calculated by difference

water (17 ppt salinity) was supplied at a flow rate of 1.5 l/min. Continuous aeration was provided by airstones. Fish were exposed to a natural light regime. The experimental tanks were cleaned daily to remove uneaten feed and fecal matter. Water quality was checked periodically. pH ranged 7.5-8. Total ammonia nitrogen, determined by the Nessler method with a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Italy), varied 0.23-0.28 mg/l. Water temperature ranged 6-8°C. Fish were hand fed twice daily at 9:00 and 17:00. Feeding was monitored carefully to ensure even distribution to all experimental fish in the tank. The experiment was carried out for 60 days.

Fish were individually weighed at the start of the experiment, on days 15, 30, and 45, and at the end of the experiment. Prior to weighing, fish were deprived of feed for one day. Before starting the experiment, 15 fish from the initial batch were sacrificed by lowering the body temperature in a freezer, stored in polyethylene bags, and frozen (-20°C) for subsequent analysis of body composition. At the end of the feeding trial, three fish from each tank (nine fish per treatment) were randomly sampled, sacrificed, and stored for analysis in the above manner. Prior to analyses, samples were prepared by homogenizing the whole fish body in a blender. All analyses were performed in triplicate.

 $^{^{\}rm b}$ Determined according to 23.6 kJ/g protein, 39.5 kJ/g lipid, 17 kJ/g nitrogen free extract

c According to Guner et al. (1998)

d According to Gatesoupe et al. (1977)

e According to Léger et al. (1979)

f Essential amino acid contents based on data in Table 2.

Table 2. Proximate analyses of white fishmeal, poultry by-product meal, and turbot.

	White fishmeal	Poultry by-product meal	Turbot	
Proximate analysis (%)				
Moisture	8.0	5.0		
Protein	71.4	64.0		
Lipid	5.5	14.0		
Ash	12.0	13.0		
Essential amino acids (%)*				
Arg	4.41	4.03	4.8	
Lys	4.96	3.10	5.0	
His	1.47	1.08	1.5	
lle	2.98	2.54	2.6	
Leu	4.78	4.28	4.6	
Val	3.31	3.06	2.9	
Met	1.84	1.13	N/A	
Cys	0.82	0.98	N/A	
Met+Cys	2.66	2.11	2.7	
Phe	2.50	1.97	N/A	
Tyr	2.00	1.01	N/A	
Phe+Tyr	4.50	2.98	5.3	
Thr	2.82	2.08	2.9	
Trp	0.73	0.50	0.6	

N/A = not available

Calculations. Relative growth rate, specific growth rate, feed intake as a percent of body weight, daily feed intake in g, daily protein intake, daily energy intake, feed conversion rate, feed efficiency, protein efficiency rate, apparent net protein utilization, total nitrogen intake, and nitrogen excretion and retention rates were calculated as described by Watanabe et al. (1987a,b) and Yigit et al. (2002).

Statistical analysis. Results were analyzed by analysis of variance (ANOVA) using SPSS for Windows, Version 10.0, for significant dif-

ferences among treatment means. Duncan's multiple range test (Duncan, 1955) was used to compare differences among individual means. Probability values less than 0.05 were considered significant. Results are presented as means±SD.

Results

At the end of the 60-day trial, survival was 100% in all treatments (Table 3). Fish fed diets where 50% or more of the fishmeal was replaced gained significantly less weight than those fed the control or 25% replacement

^{*} Data on amino acid contents of white fishmeal and poultry by-product mean are from Halver (1991) and of turbot from Kaushik (1998).

Table 3. Results of 60-day feed trial for Black Sea turbot juveniles (means±SD for triplicate groups).

		1	Replacement level (%)	(%)	
	0	25	50	75	100
Initial wt (g)	18.09±0.04a	18.03±0.03ª	18.18±0.02ª	18.16±0.04a	18.09±0.06a
Final wt (g)	30.09±0.90d	29.38±0.77d	25.43±0.62°	22.98±0.22b	20.25±0.10a
Relative growth rate (%)	66.36±4.67d	62.96±4.39d	39.86±3.26	26.59±1.43b	11.97±0.45a
Specific growth rate (%)	0.85±0.05d	0.81±0.04d	0.56±0.04c	0.39±0.02b	0.19±0.01a
Survival (%)	100±0.0a	100±0.0a	100±0.0a	100±0.0a	100±0.0a
Total feed intake (%/day)	0.67±0.01d	0.66±0.02cd	0.63±0.02∘	0.55±0.03b	0.43±0.03a
Daily feed intake (g)	0.16 ± 0.005^{d}	0.16 ± 0.006^{d}	0.14±0.003≎	0.11±0.006b	0.08±0.006a
Daily protein intake (g)	0.09±0.003d	0.09±0.003d	0.08±0.002∘	0.06±0.003b	0.05±0.003a
Daily energy intake (kJ)	3.32±0.11d	3.17±0.11d	2.83±0.06c	2.32±0.12b	1.67±0.11a
FCR	0.88±0.04a	0.91±0.03a	1.22±0.10b	1.53±0.03≎	2.54±0.10d
PER	2.25±0.09d	2.21±0.09d	1.60±0.14c	1.29±0.03b	0.80±0.03a
Total nitrogen intake (mg/g)	71.27±3.01a	72.44±2.76a	100.53±8.29b	124.19±2.50c	200.07±8.06d
Nitrogen in diet (%)	8.79	8.77	8.87	8.87	8.75
Nitrogen in fish (%)	2.70±0.02∘	2.67±0.06bc	2.63±0.06ab	2.58±0.01a	2.56±0.001a
Total nitrogen excretion (mg/g)	42.87±1.55a	44.88±0.71a	73.51±7.09b	99.05±2.10∘	178.50±8.47d
Total nitrogen excretion (% NI)	60.19±1.93a	62.03±3.17a	73.07±1.09b	79.76±1.02c	89.20±0.70d
Total nitrogen retention (mg/g)	28.40±2.24b	27.56±3.27b	27.02±1.21b	25.14±1.48ab	21.57±0.77a
Total nitrogen retention (% NI)	39.81±1.93d	37.97±3.17d	26.93±1.09°	20.24±1.02b	10.80±0.70a

Values in a row with different superscripts significantly differ at a 5% level.

Relative growth rate = % increase in wt = [(final wet wt - initial wet wt)/initial wet wt] x 100

Specific growth rate = % increase in body wt per day = [(In final wet wt - In initial wet wt)/days] x 100

Feed intake = (total food distributed/average live wt/2/days) x 100

Daily feed intake = (air dry feed intake/number of fish)/days

Daily protein intake = (feed intake x crude protein in diet/100)/days

Daily energy intake = (feed intake x energy in diet/100)/days

FCR, Feed conversion ratio = feed/wt gain

PER, Protein efficiency ratio = wet wt gain/protein intake

Total nitrogen intake = total protein intake/6.25/total wet wt gain

Total nitrogen excretion = (total nitrogen intake - total nitrogen retained)/net wet wt gain

Fotal nitrogen retention = total protein retained in fish/6.25/total wet wt gain

diets. The relationship between relative growth rate and replacement level is shown in Fig. 1. Feed intake negatively correlated with replacement level and was about 35% lower in fish fed the diet with no fishmeal than in the control fish. A similar trend was noted in daily protein and energy consumption, food conversion ration, protein efficiency ratio, and nitrogen retention. No differences were observed between the control and the diet in which only 25% of the fishmeal protein was replaced.

Whole body moisture and ash were significantly higher and fat content was significantly lower in fish fed the 50-100% replacement diets while there was no difference between fish fed the control and the 25% replacement diet (Table 4).

Discussion

Poultry by-product meal has been widely studied as an alternate protein source for fishmeal in fish diets and seems to be a promising protein source. Alexis et al. (1985) reported that fishmeal can be partially replaced by poultry by-products without any decrease in growth performance in diets for rainbow trout. Fowler (1991) observed that addition of 20% poultry by-product meal could replace 50% of the fishmeal in a diet for chinook salmon without any negative effects, but that growth was reduced when fish were fed a diet with 30% poultry byproducts. Gallagher and LaDouceur (1995) showed that juvenile palmetto bass fed a diet containing 12% fishmeal and 36% low-ash poultry by-products had weight gains similar to fish fed a diet containing 47% fishmeal (control diet). Steffens (1987) noted that a diet containing 53% poultry by-products as the sole animal protein source did not cause significant differences in growth and feed efficiency when compared to an isonitrogenous control fishmeal based diet. Similar results were reported by Steffens (1994) and Yang et al. (2004), showing that poultry by-products generally efficiently substitute up to 50% fishmeal protein in aquatic diets. Our findings in Black Sea turbot agree with these reports.

Due to the lower content of anchovy oil and lipids contained in the fishmeal, the replacement diets contained less n-3 HUFA

than the control diet. However, the amounts of lipid contained in diets with up to 75% replacement meet the essential fatty acid requirements of turbot, estimated at 0.8% by Gatesoupe et al. (1977) or 0.6% by Léger et al. (1979). The estimated n-3 HUFA in the 100% replacement diet was below the requirement for turbot.

Nengas et al. (1999) stated that low performance of some of the poultry by-product meals tested in their studies could be due to an insufficient essential amino acid or fatty acid content. Gropp et al. (1979) reported that a full value fishmeal-free diet for rainbow trout based exclusively on poultry waste meals could be produced by supplementing lysine and methionine. In the present study, the amino acid requirements of turbot were provided by the experimental diets, even though they were not fortified with essential amino acids. The estimated n-3 HUFA (except in the 100% replacement diet) and amino acid contents suggest that the poorer growth of fish fed over 25% replacement diets could not be attributed to a lack of HUFA or amino acids, but rather to processing conditions, quality of the ingredients, poor digestibility, poor palatability, or a combination of these factors.

Poultry by-product meals contain differing amounts of bone, meat, blood, etc., and therefore have different nutrient compositions, processing methods, and digestibility (Nengas et al. 1999; Webster et al., 2000). When high quality poultry by-product meals are used, many species tolerate up to 100% replacement (Steffens, 1994; Nengas et al., 1999; Kureshy et al., 2000; Webster et al., 2000).

The protein efficiency rates (PER) significantly and progressively decreased as the replacement levels increased, suggesting that the proportion of dietary protein used for catabolic processes (energy production) increased with the level of fishmeal replacement. The reduction in PER is partly due to reduced growth, as protein required for maintenance consumes a greater share of the protein intake. As expected, higher protein catabolism led to higher ammonia excretion rates in diets with more than 25% replacement levels.

Feed conversion and protein efficiency rates in the present study fell within the range

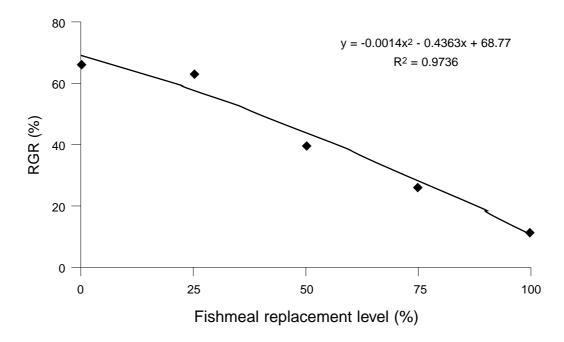


Fig. 1. Relationship between relative growth rate (RGR) and replacement rate of fishmeal protein with poultry by-product meal.

Table 4. Whole body chemical composition of fish and gross energy of Black Sea turbot fed the experimental diets for 60 days.

		Replacement level (%)							
	Initial	0	25	50	75	100			
Moisture (% wet wt)	79.62±0.07b	79.23±0.13a	79.28±0.12a	79.47±0.11b	80.01±0.07¢	80.29±0.05d			
Crude protein (% dry basis)	80.05±0.19a	81.21±1.02a	80.44±1.80a	79.96±1.02a	80.76±0.44a	81.28±0.16 ^a			
Crude lipid (% dry basis)	13.66±0.32d	12.40±0.08¢	12.19±0.37¢	11.28±0.44b	10.75±0.20ab	10.68±0.37ª			
Crude ash (% dry basis)	5.70±0.11bc	5.51±0.23ab	5.26±0.10a	5.91±0.19°	7.37±0.07e	6.90±0.19d			
Crude protein (% wet basis)	16.32±0.09abc	16.87±0.12d	16.67±0.40 ^{cd}	16.41±0.27bc	16.14±0.05ab	16.02±0.01a			

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

reported by Day and Plascencia González (2000) and Fournier et al. (2003, 2004) for Atlantic turbot fed diets where fishmeal was substituted by soybean protein concentrate, other feed ingredients, or a mixture of plant proteins. Our data were slightly lower than reported by Person-Le Ruyet et al. (2002) for Atlantic turbot reared in different O2 concentrations for 30 days (FCR = 0.69-0.70, PER = 3.2-3.3, SGR = 1.75-2.02). Our results may have been worse than those obtained by other authors due to the fish size and low water temperature, that is known to negatively affect Atlantic turbot (Imsland et al., 2001). Or they may be the result of genetic characteristics of Black Sea turbot. Differences in RNA:DNA ratios among Atlantic turbot populations reared in Iceland, Norway, and France were noted by Imsland et al. (2001), where populations in lower latitudes had lower ratios.

Total nitrogen retention was similar in fish fed the control and 25% replacement diets. However, higher replacement levels caused a significant reduction of nitrogen retention, possibly explained by a limited content of some essential amino acids in replacement diets exceeding 25%. Although digestibility coefficients were not determined, the similarities in nitrogen retention suggest that the availability of protein in the 25% replacement diet was similar to that of the control diet and, therefore, that poultry by-product meal is a suitable partial substitute for fishmeal in Black Sea turbot diets. Our protein retention results closely agree with those reported by Burel et al. (2000; 28.3-35.7% of intake) and Fournier et al. (2004; 35.5-41.6%) for Atlantic turbot fed diets in which fishmeal protein was replaced by plant protein sources.

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). Our values for the control and 25% replacement diet were lower than the best-performing groups in Burel et al. (2000), where groups fed diets with 30% or 50% extruded lupin inclusion levels produced nitrogen excretion rates of 64.3% and 67.1%,

respectively, and the 100% fishmeal control produced a rate of 69.5%.

Higher levels of total ammonia nitrogen excretion were reported for Atlantic cod (Gadus morhus; 75%, Ramnarine et al., 1987), sea bass (Dicentrarchus labrax; 58%, Vitale-Lelong, 1989), and rainbow trout (Oncorhynchus mykiss; 60-69%, Oliva-Teles and Rodrigues, 1991; 49%, Lanari et al., 1993; 42%, Dosdat et al., 1996). These differences are probably due to different diet characteristics, feeding conditions, and fish species. Fish size may also affect nitrogenous excretion (Kikuchi et al., 1992; Dosdat et al., 1996). In most cases, comparisons are difficult since the nitrogen balance is influenced by the proportion of total energy supplied by lipids (Beamish and Thomas, 1984; Arzel et al., 1994).

Whole body crude protein contents in all groups were similar. There were significantly lower lipid contents in the groups fed diets with over 25% replacement. These findings are similar to those of Nengas et al. (1999) who reported significantly lower carcass lipid in gilthead seabream with increasing dietary poultry by-product meal replacement at 40%, 50%, and 75%. Whole body lipid contents were higher in Atlantic turbot fed fishmealbased diets than in those fed plant proteinbased diets or combined diets (Fournier et al., 2003, 2004). However, these results do not agree with Gouveia (1992) who reported an increase of body lipid as the inclusion rate of poultry by-product in diets for rainbow trout increased. Moisture content increased with the poultry by-product inclusion (i.e., as fishmeal decreased), in agreement with Fournier et al. (2004), who worked with Atlantic turbot. However, our finding that body ash content increased as the level of fishmeal decreased disagrees with their findings.

In conclusion, poultry by-product meal protein could replace up to 25% of the fish-meal protein in diets for Black Sea turbot with-out significant negative effects on growth and survival in winter conditions. This may allow formulation of less expensive diets for Black Sea turbot, thereby reducing costs, increasing profitability, and assuring expansion of the Black Sea turbot industry.

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