Substituting fish meal with poultry by-product meal in diets for black Sea turbot *Psetta maeotica*

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Abstract

A 60 days feeding experiment was carried out with Black Sea turbot *Psetta maeotica* to determine the amount of poultry by-product meal (PBM) that could replace fish meal (FM) in formulated diets without reducing growth performance. Juvenile Black Sea turbot (initial average weight, 30 g) were fed five isoenergetic (gross energy, 20.5 ± 0.21 kJ g^{-1} diet) and isonitrogenous diets (protein content, 550 ± 0.35 g kg^{-1}). The control diet used white FM as the sole protein source, the other four diets were prepared to replace FM protein at levels of 25%, 50%, 75% and 100% with PBM. The fish readily accepted all experimental diets and no mortality were recorded during the trial. There were no significant differences in growth performance of turbot ($P < 0.05$) fed the diets with 25% and 50% replacement levels compared with fish offered the control diet (100% FM), however, final body weight and specific growth rate values in the 50% replacement diet were about 8% lower than those of the control. Total nitrogen excretion in fish fed 50% replacement diet were about 10% higher than the control group, even though these parameters were not found to be statistically different. At the levels of 750 and 1000 g kg^{-1} of the protein, PBM inclusion caused a severe decrease in growth performance, feed utilization, protein efficiency ratio and per cent nitrogen retention. The results in the present study indicate that up to 25% of FM protein can be replaced by PBM protein without causing reduction in growth performance, nutrient utilization and nitrogen retention.

KEY WORDS: Black Sea turbot, growth performance, nitrogen retention, poultry by-product meal, white fish meal

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Introduction

Production of cost-effective nutritionally balanced diets for fish is the main factor affecting intensive aquaculture because of its influence on growth, health and production cost. Fish meal (FM) is an important ingredient in aquaculture diets because of its high protein quality. However, as FM is the most expensive of all diet ingredients, the use of less expensive animal or plant protein sources as partial or total replacements for FM is an important research interest in aquaculture nutrition.

Fish meal still remains the main protein source in fish diets for the present feed industry. The global aquaculture demand for FM was 32% of the world supply level in 1999 (New & Wijkström 2002), 37% in 2000 (Chamberlain 2000) and may reach nearly 65% by 2010 (Chamberlain 2000) and nearly 70% by 2015 (New & Wijkström 2002). The sustainability of the growing aquaculture industry depends on the progressive reduction of wild fish catch as protein source for aquafeeds (Naylor *et al.* 2000).

The turbot is reported to have a high protein requirement (500–600 g kg^{-1} of the diet) (Bromley 1980; Cacerez-Martinez *et al.* 1984; Guillaume *et al.* 1991). It is therefore of interest to replace some of the FM with protein-rich ingredients. Previous studies with turbot have focused on some plant protein sources such as corn gluten meal, lupin or soybean meal (Regost *et al.* 1999; Burel *et al.* 2000; Day & Plascencia González 2000; Fournier *et al.* 2004). Poultry by-product meals (PBMs) have been tested in diets for rainbow trout (Alexis *et al.* 1985; Steffens 1994; Pfeffer *et al.* 1995; Bureau *et al.* 1999), European eel (Gallagher & Degani 1988), chinook salmon (Fowler 1991), channel catfish (Lochmann & Phillips 1995), gilthead sea bream (Nengas

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et al. 1999), Pacific white shrimp (Davis & Arnold 2000; Samocha *et al*. 2004), red sea bream (Goto *et al*. 2001) and freshwater shrimp *Macrobrachium nipponense* (Yang *et al*. 2004). To our knowledge, so far, little information is available on the use of PBM in turbot diets (Turker *et al*. 2005). The objective of this study was to evaluate the possibility of replacing a large proportion of FM in the diet of Black Sea turbot using practical ingredients readily available to the aquaculture-feed industry and to determine the effects of FM substitution on growth performance, nutrient utilization and nitrogen balance of Black Sea turbot.

Materials and methods

Diets

Five practical diets were formulated with commercially available ingredients and were produced at the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey. All diets were formulated to be iso-nitrogenous and iso-caloric on a crude protein (550 g kg^{-1}) and gross energy (20.5 kJ g^{-1} diet) basis. White FM (high quality whiting meal, 710 g kg^{-1} 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 white FM protein for PBM protein at levels of 25%, 50%, 75% and 100% replacement on a crude protein basis. Ingredient and chemical composition of the diets are presented in Table 1. The amino acid profiles of the diets were estimated according to Kaushik (1998) and reported in Table 1. Total n-3 Highly unsaturated fatty acid (HUFA) contents were calculated according to the equation given below and ranged from 0.6 g kg^{-1} for the diet containing no FM to 36.2 g kg^{-1} for the diet containing only FM (Table 1). The nutrient composition and amino acid profiles of protein sources and turbot are given in Table 2.

$$\text{Total n-3 HUFA in diet, g kg}^{-1} = (\text{total fish oil in diet, g kg}^{-1}) \times (\%\ \text{n-3 HUFA in fish oil used})$$

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 through a meat grinder with a 3 mm holes die. After pelleting, the diets were dried to a moisture content of 80–100 g kg^{-1} and stored in a cool location (−20 °C) until use.

Poultry by-product meal used in this experiment was obtained from the manufacturer 'Ay-pi Tavukculuk Gida Hayvancilik San. ve Tic. A.S., Bafra-Samsun, Turkey', where PBM is produced under high pressure steam processing of clean parts of the poultry carcass, excluding the feathers, using modern processing methods and strict hygienic controls according to the standards of the Turkish Standards Institution (TSE).

Fish and experimental rearing conditions

Hatchery reared turbot (*Psetta maeotica*) of 30.1 ± 0.1 g mean weight obtained from the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey, were transported to the facilities of the Faculty of Fisheries, Ondokuz Mayis University in Sinop, Turkey. Prior to the experiment, the fish were fed a commercial FM-based diet [550 g kg^{-1} crude protein, 160 g kg^{-1} crude lipid, 90 g kg^{-1} nitrogen-free extract (NFE), 21 kJ gross energy g^{-1} feed and 26.2 mg protein kJ^{-1} energy] to satiation once a day for 1 month for acclimatization to the new environment. Experimental fish were then randomly distributed into 15 identical 60-L rectangular polypropylene tanks filled with 45 L water (12 fish per tank with three replicate tanks per treatment). In an indoor flow-through system, seawater (17 g L^{-1} salinity) was supplied to the tanks at a flow rate of 1.5 L min^{-1}. Continuous aeration was provided by air-stones. Fish were exposed to natural light regime (35°09′E 42°01′N). The experimental tanks were cleaned daily to remove uneaten feed and faecal material. Water quality was checked periodically; pH ranged from 7.5 to 8, total ammonia nitrogen determined by the Nessler method using a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Padova, Italy) varied 0.24–0.28 mg L^{-1}. Water temperature was seawater ambient, ranging between 14 and 24 °C during the course of the 60-day trial carried out from May to June 2003. Fish were hand fed twice daily at 9:00 and 18:00 hours. Feeding activity was monitored carefully to ensure an even distribution of the feed offered among all experimental fish in each tank.

All experimental fish were individually weighed at the start of the experiment, after 15, 30, 45 days of trial and at the end of the experiment (day 60). Before weighing, fish were deprived of feed for 1 day.

Samplings and analytical methods

At the beginning of the experiment, 15 fish from an initial pool of fish were killed by lowering the body temperature in a freezer, stored in polyethylene bags and frozen (−20 °C) for analysis of muscle composition. At the end of

Table 1 Formulation and nutrient composition of diets used in the experiment

Ingredients	Diet (g kg^{-1})					
	FM100	PBM25	PBM50	PBM75	PBM100	Turbot requirements
Fish meal	773	580	380	190	0	
Poultry by-product meal	0	212	432	640	850	
Fish oil	79	60	40	21	2	
b-Corn starch	90	90	90	91	90	
Vit.-Min. Premix[1]	35	35	35	35	35	
Attractant[2]	3	3	3	3	3	
Guar-Gum	20	20	20	20	20	
Proximate composition (g kg^{-1} air dry basis)						
Moisture	80	88	71	84	101	
Crude lipid	153	144	139	131	121	
Crude ash	131	124	111	98	84	
Crude protein	549	548	554	554	547	
Nitrogen-free extracts[3]	87	96	125	133	147	
GE (kJ g^{-1} diet)[4]	20.5	20.3	20.7	20.6	20.2	
P : E (mg kJ^{-1})	26.8	27.0	26.7	26.9	27.0	
Crude fat (g kg^{-1})	55	55	55	55	55	
Fat from white fish meal	43	32	21	11	0	
Total fish oil in diet	122	92	61	32	2	
n-3 HUFA in fish oil[5]	298	298	298	298	298	
Total n-3 HUFA in diet	36	27	18	9	0.6	8.0[6]-6.0[7]
Amino acid content (g kg^{-1} DM)[8]						
Arg	34.1	34.1	34.2	34.2	34.3	35.9
Lys	38.3	35.3	32.2	29.3	26.4	34.2
His	11.4	10.8	10.3	9.7	9.2	14.7
Ile	23.0	22.7	22.3	21.9	21.6	18.4
Leu	36.9	36.8	36.7	36.5	36.4	32.5
Val	25.6	25.7	25.8	25.9	26.0	21.1
Met + Cys	20.6	19.9	19.2	18.6	17.9	22.7
Phe + Tyr	34.8	32.4	30.0	27.6	25.3	39.0
Thr	21.8	20.8	19.7	18.7	17.7	21.2
Trp	5.6	5.3	4.9	4.6	4.3	N/A

[1] Kadai, Riken Vitamin, Tokyo, Japan.
[2] Glutamic acid, 0.9; Inosine, 0.1 (Sigma-Aldrich ChemieGmth, Steinheim, Germany).
[3] Calculated by difference.
[4] Calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} nitrogen-free extract.
[5] According to Güner *et al.* (1998).
[6] According to Gatesoupe *et al.* (1977).
[7] According to Léger *et al.* (1979).
[8] Essential amino acid contents calculated from data in Table 2.

the trial, the same protocol of sampling was followed for each tank. Three fish per tank were randomly withdrawn for comparative analysis of fish muscle (dry matter, protein, lipid, ash) and calculation of nutrient retention rates.

Prior to analyses, conducted in triplicate, samples were prepared for analyses by homogenizing the muscle tissue of fish in a blender (Beko BKK-2160 Hotmix Hand Blender, Beko Co., Istanbul, Turkey). Muscle tissues sampled between the lateral line and the dorsal fin from both sides of the fish were used for the analyses. Chemical analyses of diets and fish muscle tissue were done according to AOAC (1984) guidelines as follows: dry matter after drying in an oven at 105 °C for 24 h until constant weight, protein (N × 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, while NFE was calculated by difference.

Calculations

Calculation of the specific growth rate (SGR), feed conversion rate (FCR), protein efficiency rate (PER), total nitrogen excretion and retention rates were performed as described by Watanabe *et al.* (1987a,b), Burel *et al.* (2000) and Yigit *et al.* (2002).

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

	Turbot	White fish meal	Poultry by-product meal
Proximate analyses (g kg^{-1})			
Moisture		80	50
Protein		714	640
Lipid		55	140
Ash		120	130
Essential amino acid (g kg^{-1})[1]			
Arg	65.3	44.1	40.3
Lys	62.2	49.6	31.0
His	26.7	14.7	10.8
Ile	33.5	29.8	25.4
Leu	59.1	47.8	42.8
Val	38.4	33.1	30.6
Met	N/A	18.4	11.3
Cys	N/A	8.2	9.8
Met + Cys	41.2	26.6	21.1
Phe	N/A	25.0	19.7
Tyr	N/A	2.0	10.1
Phe + Tyr	70.9	4.5	29.8
Thr	38.6	2.8	20.8
Trp	N/A	0.7	5.0

N/A, not available.
[1]Data on the amino acid contents of white fish meal and poultry by-product meal are from Halver (1991) and of turbot from Kaushik (1998).

Statistical analyses of data

The Kolmogorov–Smirnov and Levene statistic tests were applied to test normality and homogenity of variance, respectively. Analysis of variance and the Duncan multiple-range test were used to detect significant differences ($P < 0.05$) in final body weight, SGR, feed intake, FCR, PER, nitrogen balance and proximate muscle composition. All statistical analyses were performed using the SPSS Statistical Analysis Software Program for Windows, Version 10.0.1, 1999 (SPSS Inc., Chicago, USA).

Results

Growth performance, feed and protein efficiency

At the end of the 60 days growth trial, survival was 100% in all treatments, indicating that partial or total replacement of FM protein by PBM protein had no effect on survival rates. Average final body weights and feed utilization data are shown in Table 3. Fish fed the PBM25 and PBM50 diets did not show any significant difference in final mean body weight compared with fish fed the control diet (FM100). However, even though no significance was found, the final body weight in fish fed the 500 g kg^{-1} inclusion diet was about 7% lower than the control group. Diet PBM75 and PBM100 gave mean body weight values significantly lower than diets PBM25, PBM50 and the control group. The relationship between relative growth rate and per cent replacement levels of FM protein by PBM is shown in Fig. 1.

Specific growth rate and FCR followed the same trends, being best for the FM100 treatment group and poorest for PBM100 treatment group. Although there was no significant difference between the control and 500 g kg^{-1} inclusion diet, SGR value in the latter was found about 9% lower than that of the control. PER was significantly lower in fish fed diets with increasing PBM replacement levels at 750 and 1000 g kg^{-1}, when compared with the replacement levels of 250 and 500 g kg^{-1} (Table 3).

Ammonia excretion

Total nitrogen excretion per net wet gain was significantly ($P < 0.05$) lower in fish fed diets FM100, PBM25 and PBM50 compared with the values obtained for diets PBM75 and PBM100. Similarly, total nitrogen excretion as a per cent of the nitrogen consumption increased significantly as the

Table 3 Growth performance and feed utilization of Black Sea turbot fed the experimental diets for 60 days

	Experimental diet				
	FM100	PBM25	PBM50	PBM75	PBM100
IBW (g)	30.22 ± 0.07^{a}	30.19 ± 0.11^{a}	30.12 ± 0.13^{a}	30.10 ± 0.09^{a}	30.10 ± 0.08^{a}
FBW (g)	71.98 ± 3.44^{c}	70.17 ± 3.60^{c}	66.69 ± 3.49^{c}	50.88 ± 1.62^{b}	45.98 ± 1.63^{a}
SGR (% day^{-1})	1.45 ± 0.08^{c}	1.40 ± 0.08^{c}	1.32 ± 0.08^{c}	0.87 ± 0.05^{b}	0.71 ± 0.06^{a}
FI (% day^{-1})	1.07 ± 0.01^{b}	1.06 ± 0.00^{b}	1.04 ± 0.02^{ab}	0.99 ± 0.05^{a}	0.98 ± 0.05^{a}
FCR	0.86 ± 0.03^{a}	0.88 ± 0.04^{a}	0.89 ± 0.03^{a}	1.27 ± 0.08^{b}	1.58 ± 0.15^{c}
PER	2.31 ± 0.09^{c}	2.28 ± 0.11^{c}	2.17 ± 0.08^{c}	1.56 ± 0.09^{b}	1.29 ± 0.11^{a}

Values (mean ± standard deviation of data for triplicate groups) with different superscripts in the same row are significantly different ($P < 0.05$).
IBW, initial body weight; FBW, final body weight; SGR, specific growth rate (per cent increase in body weight per day) = [(ln final wet weight − ln initial wet weight)/days] × 100; FI, feed intake (per cent body weight per day) = (total feed distributed/average live weight/2/days) × 100; FCR, feed conversion ratio = feed/weight gain; PER, protein efficiency ratio = wet weight gain/protein intake.

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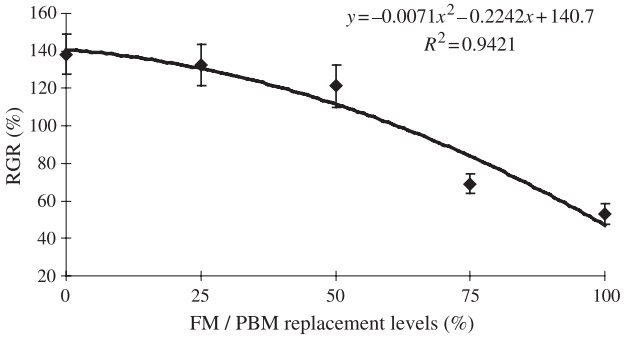


Figure 1 Relationship between relative growth rate (RGR) and replacement rate of fish meal (FM) with poultry by-product meal (PBM). The values represent means plus standard deviations at all replacement levels.

FM replacement levels rose to 750 g kg^{-1} and 1000 g kg^{-1} replacement (Table 4). However, total nitrogen excretion in fish fed 500 g kg^{-1} PBM was 10% higher than the control group, even though no significant difference was found among these groups.

Muscle composition

The muscle tissue moisture of fish fed diets PBM75 and PBM100 increased significantly ($P < 0.05$) when compared with fish fed the FM100 diet. The other groups showed no statistical difference in muscle tissue moisture when compared with fish fed the reference diet. Conversely to muscle tissue moisture, crude lipid of fish muscle tissue tended to decrease as dietary PBM increased, but no significant difference was observed ($P > 0.05$). The muscle tissue protein of fish fed diets PBM75 and PBM100 decreased significantly ($P < 0.05$) when compared with fish fed the PBM100 diet. The other groups showed no statistical difference in muscle tissue protein when compared with fish fed the reference diet. Higher levels of ash were recorded in fish fed diets with PBM substitution compared with fish in the reference group. Ash in muscle tissue of fish fed diets PBM75 and PBM100 were significantly ($P < 0.05$) higher than those of fish in the other groups (Table 5).

Discussion

Poultry by-product meal has been widely studied as an alternative protein source for FM in fish diets and seemed to be a promising protein source. Alexis *et al.* (1985) reported that FM can be partially replaced without any depression in growth performance in diets for rainbow trout by poultry by-products. Fowler (1991) reported that addition of 200 g kg^{-1} of PBM could replace half of the FM in a diet for chinook salmon without any negative effects, but that growth was reduced when fish were fed a diet with 300 g kg^{-1} PBM. Gallagher & LaDouceur (1995) stated that juvenile palmetto bass fed a diet containing 120 g kg^{-1} FM and 360 g kg^{-1} low-ash PBM had weight gains similar to those observed in fish fed a diet containing 470 g kg^{-1} FM (control diet). Steffens (1987) noted that a diet containing 530 g kg^{-1} PBM as the sole animal protein source did not provide significant differences in growth and feed efficiency when compared with an iso-nitrogenous control FM based diet. Similar results were also reported by Fowler (1991), Steffens (1994) and Yang *et al.* (2004), showing that PBM generally could efficiently substitute up to half of the FM protein in aquatic diets. Turker *et al.* (2005) reported that replacing FM protein by PBM up to 250 g kg^{-1} in Black Sea turbot diets did not affect fish growth in winter conditions at temperatures ranging 6–8 °C. Our findings are in agreement with those reported in other studies, in terms of the possible use of PBM as a partial substitute for FM in Black Sea turbot diets.

Table 4 Nitrogen (N) balances of Black Sea turbot fed to satiation the experimental diets during 60 days

	Experimental diet				
	FM100	PBM25	PBM50	PBM75	PBM100
N balance (mg g^{-1} production)					
N intake	69.3 ± 2.7^{a}	70.3 ± 3.4^{a}	73.6 ± 2.7^{a}	103.1 ± 6.2^{b}	124.5 ± 11.4^{c}
N retention	28.6 ± 1.7^{a}	28.4 ± 2.4^{a}	28.4 ± 2.3^{a}	24.4 ± 1.2^{ab}	23.9 ± 2.8^{b}
N retention (%NI)	41.2 ± 1.0^{a}	40.4 ± 1.4^{a}	38.5 ± 1.8^{a}	23.7 ± 1.3^{b}	19.2 ± 1.4^{c}
N total excretion	40.7 ± 1.1^{a}	41.9 ± 1.0^{a}	45.3 ± 0.8^{a}	78.6 ± 5.7^{b}	100.6 ± 9.3^{c}
N total excretion (%NI)	58.8 ± 1.0^{a}	59.6 ± 1.4^{a}	61.5 ± 1.8^{a}	76.3 ± 1.3^{b}	80.8 ± 1.4^{c}

Superscript letters indicate intergroup statistical differences. Mean ± standard deviation of data for triplicate groups with no common letter are significantly different (one way ANOVA and Duncan's multiple range test, $P < 0.05$).
N intake (mg g^{-1} production) = (Protein intake/6.25)/(FBW – IBW).
N retention (mg g^{-1} production) = (total g protein retained in fish/6.25)/(FBW – IBW).
N excretion (mg g^{-1} production) = (Nitrogen intake(g) – Nitrogen retained(g))/(FBW – IBW).

Table 5 Muscle proximate composition (dry basis, except for moisture) of Black Sea turbot fed experimental diets in which fish meal protein was partially replaced by poultry by-product meal protein

Diet	Moisture wet weight	Crude protein dry basis	Crude lipid dry basis	Crude ash dry basis
Initial	806 ± 0.10^{b}	865 ± 0.39^{ab}	63 ± 0.10^{a}	62 ± 0.35^{a}
FM100	801 ± 0.04^{a}	870 ± 0.92^{a}	62 ± 0.37^{ab}	61 ± 0.26^{a}
PBM25	801 ± 0.07^{a}	867 ± 0.44^{a}	61 ± 0.16^{ab}	64 ± 0.16^{a}
PBM50	801 ± 0.08^{a}	866 ± 0.51^{a}	60 ± 0.17^{ab}	64 ± 0.14^{a}
PBM75	811 ± 0.13^{c}	854 ± 0.90^{b}	58 ± 0.20^{b}	69 ± 0.09^{b}
PBM100	811 ± 0.09^{c}	852 ± 0.41^{c}	58 ± 0.11^{b}	70 ± 0.18^{b}

Mean values within the same column with different superscript letters are significantly different ($P < 0.05$).

The replacement diets contained less n-3 HUFA than the control diet because of the lower content of anchovy oil and lipids in the FM. However, the amounts of lipid contained in diets with up to 500 g kg^{-1} replacement, met the essential fatty acid requirements of turbot, estimated to be 8 or 6 g kg^{-1} by Gatesoupe *et al.* (1977) and Léger *et al.* (1979), respectively. The estimated n-3 HUFA in our PBM75 group (9 g kg^{-1} n-3 HUFA), however, was just at the limit, while our full replacement diets (0.6 g kg^{-1} n-3 HUFA) did not meet the requirements of turbot. The insufficient fatty acid content in the experimental diets with high levels of PBM inclusion could have lead to low performance of fish, as also was reported by Nengas *et al.* (1999), who stated that low performance of some of the PBMs tested in their studies could be due to insufficient essential amino acid as well as fatty acid levels. Gropp *et al.* (1979) reported that a full value FM-free diet for rainbow trout based exclusively on poultry waste meals could be produced by supplementing lysine and methionine. In the present study, some of the amino acid levels exceeded requirements for 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, compared with the control group the PBM100 diet had 32% less lysine which is generally considered the first limiting amino acid in most fish species. Therefore, the 36% lower mean final weight in our full replacement diet could be attributed solely to the lower lysine content of the diet or to the lower n-3 HUFA, as this was apparently below the requirements for turbot.

As feed intake values were significantly depressed with increasing FM substitution in the present study, the poor growth performance of fish fed over 50% replacement could be due to lower dietary available energy. Furthermore, increasing FM substitution lead to a decline in dietary fat. Thus, a potentially lower protein-sparing effect may have aggravated the poorer amino acid profiles of high PBM containing diets, with more energy being used for deamination of protein for energy. The findings concerning the significant and progressive decrease of PER values as the replacement levels increased, might suggest that the proportion of dietary protein used for catabolic processes increased with the level of FM replacement. The reduction in PER was partly due to reduced growth, as protein required for maintenance consumes a greater share of the protein intake. Consequently, the elevated protein catabolism led to extreme higher ammonia excretion rates in diets with more than 500 g kg^{-1} PBM. FCRs and PERs in the present study fell within the range reported by Day & Plascencia González (2000) and Fournier *et al.* (2003, 2004) in Atlantic turbot and Turker *et al.* (2005) in Black Sea turbot, which were fed diets where FM was substituted by soybean protein concentrate, a mixture of plant proteins or PBM, respectively. Our data were slightly lower than those reported by Person-Le Ruyet *et al.* (2002) for Atlantic turbot reared in different O_2 concentrations for 30 days.

Higher replacement levels than the PBM50 caused a significant reduction of nitrogen retention, which can be explained by a possibly limited content of some essential amino acids in replacement diets exceeding 50%. Although digestibility coefficients were not determined, the similarities in nitrogen retention suggest that the availability of protein in 50% replacement diet was close to that of the control diet and therefore, that PBM is a suitable partial substitute for FM in Black Sea turbot diets. Our findings regarding nitrogen retention are in close agreement with those reported by Burel *et al.* 2000 (28–36% of intake) and Fournier *et al.* 2004 (36–42%) in Atlantic turbot, and Turker *et al.* 2005 (38–40%) in Black Sea turbot fed diets in which protein from FM was substituted by plant protein sources and PBM.

In the present study, 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). Our values for the FM, PBM25 and PBM50 diets were slightly lower than the best-performing groups in

Turker *et al.* (2005), where groups fed a control (FM) diet and 25 g kg^{-1} PBM inclusion diets produced nitrogen excretion rates of 60% and 62%, respectively, and in Burel *et al.* (2000), where groups fed diets with 300 or 500 g kg^{-1} extruded lupin inclusion levels produced nitrogen excretion rates of 64% and 67%, respectively, and the 100 g kg^{-1} FM control produced a rate of 70%.

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

A slight decline in muscle tissue crude protein composition of fish fed diets with PBM inclusion was similar to the nitrogen retention results, i.e. significant lower nitrogen retention with higher PBM inclusion, probably because of the elevated protein catabolism which resulted also in higher ammonia excretion rates in diets with high PBM inclusion. The low lipid content in the muscle tissue of fish fed diets higher than PBM50 could be due to the low possibility of lipid accumulation in the tissues because of lower available energy in the diet, and thus forcing the protein-sparing effect of lipids to the limits. Our results do not agree with the findings of Gouveia (1992) who reported an increase of body lipid with the inclusion of poultry in the diets. However, our findings are similar to Nengas *et al.* (1999) and Turker *et al.* (2005) who reported significantly lower carcass lipid with increasing dietary PBM at replacement levels up to 750 g kg^{-1} in gilthead seabream and up to complete replacement in Black Sea turbot, respectively. The increased moisture content with the poultry by-product inclusion is in agreement with Turker *et al.* (2005), who reported an increase in body moisture with decreasing levels of dietary FM in diets for Black Sea turbot. The negative relation between ash and protein levels in the muscle tissue, might be due to the elevated protein catabolism, which also resulted in a slight decrease in muscle tissue crude protein composition when PBM inclusion increased. Our findings are in agreement with those of Fournier *et al.* (2003) and Turker *et al.* (2005). However, Fournier *et al.* (2004), reported a decrease in body ash with decreasing levels of FM in diets for Atlantic turbot.

Even though there were no significant differences in final body weights, SGRs and total nitrogen excretion rates, but still mean values varied 7–10%. This might from a commercial point of view, produce an important difference.

As a conclusion of this study, it is suggested that without amino acid supplementations, PBM could safely replace up to 250 g kg^{-1} FM protein in the diets for Black Sea turbot without loss in growth performance under the conditions of this study. Further experiments are suggested to formulate PBM diets with amino acid supplementation. This may allow higher PBM incorporation and formulation of less expensive diets for Black Sea turbot, and may reduce the diet costs for producers, thereby increasing profitability and assuring a continuous expansion of the Black Sea turbot industry.

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