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Partial Replacement of Fishmeal with Hazelnut Meal in Diets for Juvenile Gilthead Sea bream (*Sparus aurata*)

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Abstract

Hazelnut meal was evaluated as an alternative protein source to fishmeal in diets of gilthead sea bream with an initial weight of 35.08±0.29 g. Incorporation levels of hazelnut meal were 0% (control), 10%, 20%, 30%, and 40%. Each diet was tested in triplicate for ten weeks. There were no significant differences between the experimental diets and the control in final body weight, weight gain, specific growth rate, or feed and protein efficiency ratios. In addition, the level of hazelnut meal did not significantly affect the whole body composition of the sea bream. In conclusion, up to 40% hazelnut meal could be included in diets for gilthead sea bream juveniles without adverse effects on growth rate or body composition.

Introduction

Feed cost is the largest operational cost in aquaculture and protein is the most expensive component in feeds. Fishmeal is the main protein source in most fish feeds, particularly for carnivorous species. Annual world fishmeal production averages about 7.05 million tons, 31.5% of which is used in fish feeds (Hardy, 2006). This percentage is expected to increase to 65% in 2010. Moreover, El-Niño events occurring every five to seven years on average reduces global fishmeal supplies. As a result, availability and prices of fishmeal has become problematic (Hardy, 2006). Reliance of fish diets on fishmeal is dangerous and the search for alternative sources is extremely important to ensure the sustainability of the industry (Glencross et al., 2007).

Hazelnut meal is obtained after oil is extracted from hazelnuts. Turkey is the top producer, followed by Italy, Spain, and the USA (Kilic and Alkan, 2006). Even though hazelnuts are used mainly in the manufacture of chocolate, its oil fraction has drawn considerable attention due to health-related fea-

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tures. The current amount of hazelnut meal production is unknown, but considering the interest in hazelnut oil, it is expected that hazelnut meal will become increasingly available. Hazelnut meal is a valuable protein source in diets for layers (Ozen and Erener, 1992) and quail (Erener et al., 2003). It is also promising as a partial replacement of fishmeal in diets for European sea bass (*Dicentrarchus labrax;* Emre et al., 2008), common carp (*Cyprinus carpio*; Buyucapar and Kamalak, 2007), and Black Sea turbot (*Scophthalmus maeoticus*; Ergun et al., 2008).

The current study was undertaken to determine the effects of partial replacement of fishmeal with hazelnut meal on growth and body composition of juvenile gilthead sea bream (*Sparus aurata*).

Materials and Methods

Experimental fish and rearing system. The study was conducted at the Beymelek Unit of the Mediterranean Fisheries Research Production and Training Institute. Fish were obtained from a base population of juveniles grown in the institute's hatchery. Before commencement of the experiment, the fish were acclimatized to experimental conditions for two weeks. At the start of the trial, fish weighing 35.08±0.29 g were randomly distributed into fifteen 200-l cylindrical-conical tanks at 30 fish per tank. Five diets were fed to triplicate groups of fish for ten weeks. Fish were fed by hand twice a day (09:00 and 16:00) at 3% of their body weight; the feed quantity was adjusted biweekly after weighing all animals. Throughout the study, an 8-l/min flow of sand-filtered sea water was supplied to each tank to ensure suitable water conditions. Water parameters were pH 7.96±0.01, salinity 35.98±0.13 ppt, temperature 26.50±2.03°C, and dissolved oxygen 5.50±0.57 ppm.

At stocking, twenty fish from the base population were analyzed for initial whole body composition. At the end of the trial, five fish from each tank were analyzed for final whole body composition. Samples were kept frozen at -20°C until analysis.

Diet preparation. Five isonitrogenous and isocaloric diets were prepared (Table 1). Fishmeal was the only protein source in the control diet whereas the experimental diets contained 10%, 20%, 30%, or 40% hazelnut meal by weight. Hazelnut meal was supplied by the Ordu Oil Company (Ordu, Turkey). Fair Average Quality of South African fishmeal, fish oil, and wheat middlings were purchased from a local feed plant (Kortkutelim Feed and Food Company, Antalya, Turkey). Other feed components were obtained from the local market. Crude nutrient composition and percentage of essential amino acids in the fishmeal and hazelnut meal are given Table 2. The ingredients were ground with a hammer mill, weighed at predetermined levels, and mixed through an experimental type horizontal mixer for 5 min. The diets were pelleted with a pelleting machine (2-mm pellet diameter) without steam, packed in plastic bags, and stored at 4°C until use.

Calculations. Growth and feed utilization parameters were calculated as follows: weight gain = W_t - W_0, where W_t = fish body weight in g at day t, W_0 = fish body weight in g at day 0, and t = day, specific growth rate (SGR %/day) = 100(ln W_t - ln W_0)/t, feed conversion ratio (FCR) = feed consumed (g dry weight)/ weight gain (g wet weight), protein retention efficiency (PER) = weight gain/protein fed, and condition factor (CF) = W_t/L^3, where L = total fish length in cm.

Analytical procedures. Proximate analyses of feedstuffs, diets, and fish were performed according to AOAC (1990) methods: dry matter after drying in an oven at 104ºC until constant weight, ash content by incineration in a muffle furnace at 600ºC for 2 h, crude protein (N x 6.25) by the Kjeldhal method after acid digestion, and lipid by petroleum ether extraction in a Soxhlet extractor. All analyses were conducted in duplicate. Amino acid compositions of fishmeal and hazelnut meal were determined in triplicate with a Varian gas chromatograph (model CP-3800, Varian Inc., Walnut Creek, CA) using a Phenomenex EZ Faast GC-FID kit (EZ: Faast Phenomenex, Torrance, CA). Gross energy was calculated using conversion factors of 39.5, 23.7, and 17.2 MJ/kg for fat, protein, and carbohydrate, respectively (Young et al., 2005).

Table 1. Formulation and nutrient composition of experimental diets.

	Hazelnut meal (%)					
	0 (control)	*10*	*20*	*30*	*40*	*Sea bream requirements (% of dietary protein)*[1]
Ingredient (%)						
Fishmeal	63.26	56.84	50.41	43.99	37.57	
Hazelnut meal	0	10	20	30	40	
Dextrin[2]	17.91	13.72	9.53	5.33	1.14	
Fish oil	7.33	7.94	8.56	9.18	9.79	
Wheat middling	10.00	10.00	10.00	10.00	10.00	
Vitamin mix[3]	0.50	0.50	0.50	0.50	0.50	
Mineral mix[4]	0.20	0.20	0.20	0.20	0.20	
Pellet binder[5]	0.30	0.30	0.30	0.30	0.30	
Colin chloride[6]	0.50	0.50	0.50	0.50	0.50	
Proximate composition (dry matter basis)						
Dry matter (%)	93.11	92.65	92.96	93.55	93.24	
Crude protein (%)	43.08	43.37	43.63	44.04	44.92	
Crude lipid (%)	13.77	14.23	15.11	15.85	16.67	
Crude ash (%)	11.93	11.44	11.26	10.97	10.60	
Gross energy (MJ/kg)	21.02	21.22	21.47	21.71	22.01	
Essential amino acid composition (% of dietary protein)[7]						
Arginine	6.4	6.8	7.1	7.5	7.7	5.4
Histidine	2.4	2.3	2.3	2.3	2.2	1.7
Isoleucine	5.0	4.8	4.7	4.6	4.4	2.6
Leucine	8.5	8.5	8.5	8.5	8.4	4.5
Lysine	6.7	6.4	6.1	5.8	5.4	5.0
Methionine+cysteine	5.1	4.8	4.6	4.3	4.0	4.0
Phenylalanine+tyrosine	8.7	8.7	8.8	8.9	8.9	2.9
Threonine	4.1	4.1	4.0	3.9	3.8	2.8
Valine	5.0	5.0	5.0	5.0	4.9	3.0

[1] Kaushik, 1998
[2] Sunar Misir, Adana, Turkey
[3] Vitamin mix contains (per kg): 4,000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6000 mg vitamin B2, 40,000 mg niacin, 10,000 mg Ca-pantothenate, 4000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1200 mg folic acid, 40,000 mg vitamin C, and 60,000 mg inositol (Ekol Gida, Istanbul, Turkey)
[4] Mineral mix contains (per kg): 23.75 g Mn, 75 g Zn, 5 g Cu, 2 g Co, 2.75 mg I, 0.1 g Se, 200 g Mg (Ekol Gida, Istanbul, Turkey)
[5] Calcium lignosulfonate, Korkutelim Feed and Food Company, Antalya, Turkey
[6] Ufuk Chemical Medicine Company, Istanbul, Turkey
[7] Based on Table 2. Amino acid composition of wheat middling adapted from NRC, 1993.

Table 2. Proximate composition (dry matter basis) and amino acid contents (% of protein) of fishmeal and hazelnut meal.

	Fishmeal	*Hazelnut meal*
Nutrient		
Dry matter	93.53	91.62
Crude protein[1]	64.63	46.81
Crude lipid[1]	10.89	4.29
Crude ash[1]	18.97	7.72
Essential amino acids		
Arginine	6.60[2]	10.57[3]
Histidine	2.40	1.93
Isoleucine	5.06	3.62
Leucine	8.70	8.35
Lysine	6.95	3.57
Methionine (Cystine)	2.35 (4.2)[2]	0.95 (1.54)[3]
Phenylalanine (Tyrosine)	5.14 (3.82)	5.49 (3.79)
Threonine	4.22	3.43
Valine	5.08	4.82
Non-essential amino acids		
Alanine	6.56	5.05
Aspartic	10.40	14.62
Glutamic	10.31	14.60
Glycine	6.27	5.05
Proline	4.20	4.01
Serine	3.15	3.86
Total amino acids	95.43	95.25

[1] of dry matter
[2] De Koning, 2005
[3] Ozen and Erener, 1992

Statistical analysis. The experimental design was a completely random scheme for five treatments and three replicates. Normality and homogeneity were checked by Shapiro-Wilk W. test and Bartlett's test, respectively. All percentage values were arcsine transformed before analysis of variance. One-way analysis of variance (ANOVA) was used to reveal the effects of treatments on the selected criteria. Differences were considered significant when $p<0.05$.

Results

No mortality or external clinical signs were related to any diet. All experimental animals appeared healthy throughout the study. The animals almost tripled their initial weight during the trial, with no significant differences among treatments (Table 3). Final whole body protein and lipid levels were higher than initial values whereas moisture and ash were lower, though differences were not significant (Table 4).

Discussion

Based on crude nutrient composition, hazelnut meal is similar to solvent extracted soybean meal (Karsli et al., 2006). Hazelnut meal totally replaced soybean meal in diets for rainbow trout (Bilgin et al., 2007) and 40% of the soybean meal in diets for common carp using hazelnut meal with a protein level of 39% (Buyucapar and Kamalak, 2007), well below that used in the present study. Differences in results may be due to the use of different hazelnut varieties in meal production as nutrient levels vary among hazelnut cultivars in Turkey (Ozdemir and Akinci, 2004; Koksal et al., 2006).

Hazelnut meal contains lower protein, lipid, and ash levels than fishmeal. In particular, the methionine+cysteine and lysine levels in hazelnut meal are lower than in fishmeal. The diet containing 40% hazelnut meal included only 4.0% methionine+cysteine and 5.4% lysine, just meeting the requirement levels for gilthead sea bream (Kaushik, 1998). Nevertheless, incorporation of up to 40% hazelnut meal in the diets did not reduce final weight, weight gain, or SGR compared to the fishmeal control. These findings comply with our previous results in European sea bass where a diet containing 30% hazelnut meal performed as well as the control (Emre et al., 2008). Moreover, our unpublished results from rainbow trout showed that inclusion of 30% hazelnut meal produced better growth than a control containing no hazelnut meal. When 35% of the fishmeal was replaced by 28% hazelnut meal in common carp diets, there were no significant reductions in growth or feed utilization (Buyucapar and Kamalak, 2007). While these findings indicate that hazelnut meal is a valuable protein source for fish, growth was impaired in Black Sea turbot fed diets containing up to 30% hazelnut meal in place of fishmeal (Ergun et al., 2008). Such a discrepancy might result from differences among the fish species or in the hazelnut meal and fishmeal sources. While the essential amino acid levels of the experimental diets, particularly of methionine+cysteine, hint that growth performance would be negatively affected at the 40% hazelnut meal level, this was not the case.

Palatability is important in fish feed ingredients (Glencross et al., 2007). Reduced feed intake is the major reason for depressed growth in fish fed plant protein-based diets (Gomez-Requeni et al., 2004). However, this

Table 3. Mean growth and feed utilization of gilthead sea bream fed various levels of hazelnut meal (n = 3).

	Hazelnut meal (%)					
	0 (control)	*10*	*20*	*30*	*40*	*Pooled SEM*
Initial wt (g)	34.97	35.55	34.75	34.86	35.29	0.74
Final wt (g)	96.88	108.57	95.07	96.39	93.66	4.25
Wt gain (g)	61.91	73.01	60.32	61.53	58.37	3.82
Specific growth rate (%/day)	1.45	1.59	1.43	1.45	1.40	0.05
Feed conversion rate	2.09	1.84	2.11	2.10	2.15	0.08
Protein efficiency ratio	1.12	1.26	1.09	1.08	1.04	0.05
Condition factor	2.57	2.55	2.55	2.45	2.54	0.03

There were no significant differences among experimental treatments ($p>0.05$).

Table 4. Mean initial and final whole body composition of gilthead sea bream fed various levels of hazelnut meal (n = 3).

	Initial	*Hazelnut meal (%)*					*Pooled SEM*
		0 (control)	*10*	*20*	*30*	*40*	
Moisture	68.14	62.83	62.81	62.98	63.08	63.90	0.83
Protein	16.27	17.32	16.89	17.64	17.48	16.70	0.26
Lipid	9.38	15.25	15.36	14.93	14.75	14.46	0.96
Ash	4.02	3.84	3.87	3.89	3.89	3.85	0.11

There were no significant differences among experimental treatments ($p>0.05$).

was not the case in the present study, even though a fixed ration was fed. Similarly, no feed rejection was recorded in common carp and European sea bass fed diets with hazelnut meal up to 38% and 30%, respectively (Buyucapar and Kamalak, 2007; Emre et al., 2008) and high levels of hazelnut meal in diets offered *ad libitum* to layers and Japanese quail did not affect feed intake (Ozen and Erener 1992; Erener et al., 2003). On the other hand, the reduced body weight of rainbow trout fed a diet including 30% hazelnut meal was attributed to low preference (Bilgin et al., 2007). In the present study, the hazelnut meal levels in the experimental diets did not cause feed refusal, indicating that hazelnut meal was palatable to the fish.

Final body composition did not differ between treatments. Similarly, partial replacement of fishmeal with hazelnut meal did not significantly change body nutrient levels of European sea bass (Emre et al., 2008). Japanese quail fed diets containing up to 30% hazelnut meal had a similar meat composition to those fed a soybean meal based control diet (Yalcin et al., 2005). Conversely, rainbow trout fed a diet containing 30% hazelnut meal had higher moisture and ash, and lower protein, lipid, and energy levels than those given a fishmeal and soybean meal based diet (Bilgin et al., 2007) and inclusion of more than 20% hazelnut meal in turbot diets decreased the body protein concentration but elevated the moisture and crude lipid contents (Ergun et al., 2008). It is difficult to compare the findings of the present study and those of Buyucapar and Kamalak (2007) due to the large variation of values in their study, indicating no clear trend.

Neither growth performance nor body composition of gilthead sea bream were significantly changed by the inclusion of up to 40% hazelnut meal. It has been suggested that upper inclusion levels of new ingredients in fish diets be 40-50% (Glencross et al., 2007). Since the diet containing 40% hazelnut meal did not differ from the control in terms of growth performance and feed utilization, we were unable to determine the maximum level of hazelnut meal that can be incorporated into gilthead sea bream diets instead of fishmeal. However, due to the methionine+cysteine contents and the drop in growth performance of the 40% hazelnut diet, the maximum level might be around 40%. Use of hazelnut meal at this level may lower the price of sea bream diets.

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