The effect of dietary soybean meal on growth, nutrient utilization, body composition and some serum biochemistry variables of two banded seabream , *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817)

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Abstract

This study was performed to determine the optimum level of soybean meal diets for two banded sea bream for growth performance, nutrient utilization, body composition and serum biochemistry. Two banded seabream were fed five experimental diets which were formulated replace fish meal by soybean meal at 0, 20, 30, 40 and 50%. Up to 40% of dietary fish meal was successfully replaced with no growth depression. Whole body composition of two banded seabream was not affected by soybean meal inclusion level. Total protein, triglyceride and total cholesterol of fish fed the SM50 diets were significantly lower compared to fish fed the soybean free diet. On the other hand, serum glucose level significantly increased as dietary soybean meal inclusion increased. Results showed that 40% fish meal can be replaced in diets for the two banded seabream by defatted soybean meal. Further studies to determine the inclusion level of soybean meal more than 40% with amino acid or enzyme supplementation are needed.

Keywords: *Diplodus vulgaris*, serum biochemistry, body composition, growth performance, soybean meal

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Introduction

Fish farming has a great development in recent years. Turkish aquaculture sector is based on the production of few fish species, such as gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*).

Two banded seabream (*Diplodus vulgaris*) should be taken under consideration by researchers due to its market price as a new species for aquaculture. It has a tolerance to salinity (Horta et al., 2004) and can mature easily under culture system without any hormone treatment (Jug-Dujakovic and Glamuzina, 1988), and this makes it an excellent candidate species for aquaculture (Ozório et al., 2008).

Feedings costs constitute a large part of the total expenses of an aquaculture facility. Aquaculture researchers are looking for protein source alternatives to fish meal, due to the expensive cost and availability issue of this ingredient. Fish with omnivorous fish, such as two banded seabream, could make a more efficient use of dietary vegetable protein. Researchers are focused on replacing fish meal with plant-based protein ingredients (Kissil et al., 2000; Pereira and Oliva-Teles, 2002, 2003; Gomez-Requeni et al., 2004; Hernandez et al., 2007; Yiğit et al., 2012). Data show that plant proteins can be successfully used in fish feed instead of fish meal for about 30 to 50% (Francis et al., 2001). In rainbow trout diets, a total replacement is possible (Kaushik et al., 1995).

Soybean is one of the plant that can be used as an alternative protein source. Soybean meals have a high level of available protein with well-balanced amino acid profile, low price and wide availability. However, its methionine level is low and contains several anti-nutritional factors that may disturb the fish's digestive system (Olli et al., 1994; Storebakken et al., 2000). Nevertheless, according to Hernández et al. (2007) sharpsnout seabream (*Diplodus puntazzo*) tolerates 60% of soybean meal in its diet without any negative effects. Moreover, Piccolo et al. (2011) studied the effects of mannanoligosaccharide (MOS) and fructooligosaccharide (FOS) on sharpsnout seabream in the context partial fishmeal replacement by soybean meal, concluded that partial replacement of fish meal is possible with 40% of soybean meal without negative effect on growth performance of fish.

The aim of this study was investigate the effects of the partial replacement of fish meal protein by soybean meal protein in two banded seabream diet and show effects on growth performance, nutritional use of diet and biochemical composition of plasma.

Material and methods

A total of 225 two banded seabream with about 11.5±2.8 g initial body weight were captured in Dardanos (Çanakkale, Turkey) and kept at the Aquaculture of Marine Fish and Research Center of Fisheries Faculty in Çanakkale Onsekiz Mart University (Dardanos, Çanakkale, Turkey). Fish were held in two circular tanks for 15 days to adapt to the new rearing conditions (750 l. max volume) and during the adaptation stage, fish were fed to apperent satiation two times a day, 7 days a week with commercial gilthead seabream diet

(%49 crude protein; Bioaqua, Turkey). Then, 15 fish were weighed and distributed into 15 aquariums of 84 L (35 cm x 45 cm x 60 cm deep) in a recirculation seawater system equiped with aeration and filtration systems. Fish were kept under a constant photoperiod (12 h light/ 12 h dark). Three replicates per treatment were performed.

Experimental diets and feeding

Five isonitrogenous and isoenergetic diets were formulated using commercial ingredients (Table 1). Soybean meal was added in the diets at levels of 0, 20, 30, 40 and 50% of fish meal replacement. The control diet was prepared using fish meal as the major source of protein. The dry weighed ingredients were carefully mixed using a laboratory food mixer. The mixtures were primed with water to yield a suitable pulp. Wet diets were made into 2 mm pellet size and dried at 40 °C in a drying cabinet. Crude protein content was about %40 and lipid content was %17 in all experimental diets (Ozório et al., 2009). Fish were fed by hand twice a day (1000 – 1700 h) until satiety for 45 days.

Table 1: Ingredients and composition of experimental diets in which fishmeal was replaced by defatted soybean meal on two banded seabream fed experimental diets (g kg $^{-1}$)

Ingredients g kg $^{-1}$	Groups				
	SM0	**SM20**	**SM30**	**SM40**	**SM50**
Fish meal	603	461	400	341	274
Soybean meal	0	232	368	425	505
Wheat meal	245.4	142.4	62.4	61.4	41.4
Fish oil	114	127	132	135	142
Vitamin mix[1]	20	20	20	20	20
Mineral mix[2]	12	12	12	12	12
Choline chloride	5	5	5	5	5
Vitamin C	0.6	0.6	0.6	0.6	0.6
Chemical composition (% Dry Basis)					
Protein	40.64±0.94	40.16±0.50	40.55±0.35	40.38±0.29	40.93±1.47
Lipid	18.56±0.48	18.02±0.46	18.59±0.17	18.43±1.03	18.85±1.41
Ash	14.65±0.52	14.96±0.21	14.85±0.18	14.56±0.24	14.37±0.13

[1]Vitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU ; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d–pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg.

[2]Mineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg.

Data on weight gain were collected at days 15, 30 and 45 after starting the experiment. At the end of the treatment 15 fish per group were taken for whole body composition analyses and stored at -20°C. Growth performance of two banded seabream fed with different soy level diets was considered by calculating weight gain (WG), specific growth rate (SGR) and feed conversion rate (FCR). Blood samples were taken the caudal vein with heparinized syringes. The fish anesthetized with clove oil which is a natural product are commonly used (Mylonas et al., 2005). Blood samples were centrifuged at 4000 × 10 min to separate serum.

WG (%) =[(final weight (g) – initial weight (g)) / initial weight (g)] x 100

SGR (% day $^{-1}$) = [(l_n final weight (g) – l_n initial weight (g)) / days] x 100

FCR = feed intake (g) / weight gain (g)

Chemical analysis

Feedstuffs and fish samples were analyzed for proximate composition according to AOAC (1998). All the samples were frozen at -20 °C until analyzed. Dry matter was detected after drying at 105 °C until a constant weight was achieved. Ash content was measured in a muffle furnace at 525 °C for 12 h. The amount of crude protein was analyzed by the Kjeldahl method. Lipid extractions were determined by the SOXTEC system.

Result were expressed as mean ± SD and group mean difference were compared using Student's t-test. A significant level of $P < 0.05$ was employed at all cases.

Results

All fish were acclimated to experimental conditions and trial diets before the 6-week feeding trials. Result on fish growth performance, feed conversion ratio (FCR), specific growth rate (SGR) are shown in Table 2. The survival was 100% in all experimental groups. Weight gain and SGR decreased while FCR significantly increased as the ratio of soybean meal in the diet increased. However, SM20 and SM30, SM40 treatment groups showed similar weight gain, SGR and FCR levels with the control group. Growth performance parameters like weight gain (P =0.009), SGR (P =0.013) and FCR (P =0.001) were decreased when soybean was included at a level of 50% in diet compared with control diet.

Table 2: Weight gain, feed conversion ratio (FCR), specific growth rate (SGR) of the two banded seabream fed experimental diets in which fish meal was replaced by defatted soybean meal

	SM0	SM20	SM30	SM40	SM50
Initial weight (g)	11.49±2.95^{z}	11.03±2.34^{z}	11.74±2.73^{z}	11.13±2.95^{z}	11.84±2.70^{z}
Final weight (g)	17.97±3.11^{z}	17.90±2.23^{z}	17.67±2.63^{z}	16.74±2.49^{z}	17.11±2.62^{z}
Weight gain (%)	55.93±3.06^{z}	62.26±1.64^{z}	50.50±3.85^{z}	51.92±4.70^{z}	44.50±1.36^{y}
SGR	0.98±0.04^{z}	1.07±0.02^{z}	0.90±0.05^{z}	0.88±0.07^{z}	0.81±0.02^{y}
FCR	1.25±0.04^{y}	1.22±0.02^{y}	1.31±0.04^{y}	1.36±0.12^{y}	1.56±0.04^{z}

Values are means±SD (n=3). Different letters in same line indicate significant differences within groups (*P* < 0.05).

The proximate body compositions of fish fed using the different diets are presented in Table 3. On a dry weight basis, no significant differences were found in ash and crude lipid contents among the treatment groups. However, fish fed diet SM50 showed a whole body protein content higher than control group (*P* =0.02).

The results of the biochemical variables of fish serum used in the experiments are presented in Table 4. Total protein (TPROT), glucose (GLU), triglyceride (TRI) and cholesterol (CHOL) levels were significantly affected by the soybean meal replacement. Specifically glucose levels were increased, while total protein, cholesterol and triglyceride levels were decreased with the rising of the proportion of soybean meal replacement in the diet increased. Serum GLU levels in fish that were fed to SM30 (*P* =0.021), SM40 (*P* =0.009) and SM50 (*P* =0.020) were significantly higher than the control values. Relative to the value for the control group, serum TRI and CHOL were significantly lower in two banded seabream that were fed the SM30 (*P* =0.039 and *P* =0.011), SM40 (*P* =0.007 and *P* =0.010) and SM50 (*P* =0.003 and *P* =0.011) groups.

Table 3: Whole body composition (dry weight basis) of two-banded seabream fed experimental diets in which fish meal was partially replaced by soybean meal

	SM0	**SM20**	**SM30**	**SM40**	**SM50**
Crude protein (%)	41.83±2.1[a]	42.16±1.46[a]	42.31±2.66[a]	43.57±1.67[a]	45.38±2.2[b]
Crude lipid (%)	35.69±1.6[a]	35.24±2.35[a]	35.18±2.16[a]	35.01±2.20[a]	35.15±1.8[a]
Ash (%)	15.25±3.3[a]	14.74±1.10[a]	14.08±1.63[a]	14.14±0.90[a]	14.15±0.9[a]

Values are means±SD (n=3). Different letters in same line indicate significant differences within groups (*P* < 0.05).

Table 4: Concentration of plasma constituents of two-banded seabream fed experimental diets in which fish meal was partially replaced by soybean meal

	GLU (mg/lt)	**TPROT (g/dl)**	**TRIG (mg/dl)**	**CHOL (mg/dl)**
SM0	32.33±6.31[a]	9.44±0.52[b]	97.16±10.50[b]	84.00±13.26[b]
SM20	44.91±7.26[a]	10.22±0.67[b]	77.30±14.17[b]	64.80±20.50[b]
SM30	48.90±4.54[b]	6.02±1.63[a]	68.79±12.45[a]	47.20±5.00[a]
SM40	50.90±2.61[b]	7.44±0.74[b]	60.28±6.50[a]	47.20±3.67[a]
SM50	51.21±5.99[b]	5.41±1.00[a]	55.32±4.26[a]	48.67±2.72[a]

Values are means±SD (n=3). Different letters in same line indicate significant differences within groups

Discussion

Two banded seabream is a potential marine fish species to be used as an alternative commercial species in aquaculture industry. Nevertheless nutritional information about this species is scare. In this study, crude protein (40% DM) and crude lipid (18% DM) of experimental diets were formulated as suggested by Ozório (2009). There are not studies published about effects of soy products in two banded seabream diets. The growth rate, FCR and SGR were comparable to that observed in other sparid fish studies (Biswas et al., 2007;Hernández et al., 2007; Tomás et al., 2009; Piccolo et al., 2011).

In this study with two banded seabream, when soybean meal was used to replace 50% of the fish meal, growth was significantly reduced. Growth reduction could have been due to higher FCR and lower SGR (Table 2). Higher FCR or/and lower SGR have also been previously reported in juvenile tin foil barb (Elangovan and Shim, 2000) and sharpsnout seabream (Hernández et al., 2007) when soybean meal was included at high levels in their diets. On the other hand, use of high level soybean meal in fish diets means lower price with no changes in the fish texture (Hernández et al., 2007). Biswas et al. (2007), evaluated soybean meal with phytase supplementation by comparing fish meal in red seabream diets. They prepared six diets; one contained only fish meal as a protein source, one of fish meal with soybean meal and others contained respectively with fish meal and soybean meal 1000, 2000, 3000, 4000 phytase activity unit (FTU). Their result showed that diet with 2000 FTU kg^{-1} gave the best growth performance. These suggest that phytase supplementation could effectively reduce antinutritional factors of soybean meal (Liu, 1997).

Prebiotics, such as mannanoligosaccharide (MOS) are commonly use in fish diets for increasing growth performance (Staykov et al., 2007). Piccolo et al. (2011) used soybean meal up to 40% replacement by fish meal in sharpsnout seabream diets with MOS and FOS supplementation. Their results showed similarities with our study, when fish meal were replaced by soybean meal sharpsnout seabream diets at 40% no negative effects were found in growth performance and supplementation of MOS and FOS did not affect growth performance. This explained oligosaccharide naturally occurring in many plants.

Some serum biochemical variables may be useful tools to evaluate the health condition of the fishes (Yılmaz and Ergün, 2012; Çelik et al., 2012). The present study demonstrated that glucose level increased with an increasing dietary level of soybean meal. It is known that glucose is a carbohydrate used as a source of energy by the fish body (Pozo et al., 2012). Nutritional status is a factor that can have an effect in the glucose response. The intake of diets with different lipid and protein content resulted in different responses of blood glucose of the fish (Martínez-Porchas et al., 2009). Soybean meal contains approximately 30% carbohydrates, with 10% oligosaccharides, 1% starch, and 20% non-starch polysaccharides (Liu, 1997). Thus, high carbohydrate levels in the soybean meal diet than in the fish meal diet presumably resulted in more blood glucose.

In this study, serum cholesterol and triglycerides were lower in two banded seabream fed diets containing 30–50% soybean meal than in fish fed the fish meal diet. Similarly, Romarheim et al. (2006) reported that the plasma cholesterol and triglyceride levels of rainbow trout were reduced significantly when 50% of the fish meal was replaced by soybean meal. This can be demonstrated a drain of bile acids. Because, several compounds in soybean may bind bile acids and prevent them from being reabsorbed in the distal intestine (Romarheim et al., 2006). Soybean and other plants proteins have been investigated to have cholesterol-lowering effects in fish (Dias et al., 2005; Lim and Lee, 2009). Lim et al. (2011) reported that total cholesterol levels were lower in tiger puffer fed soybean meal diets compared to fish meal diets. In the present study similar result were obtained, cholesterol levels in plasma were lower with the rising the inclusion of soybean meal.

The serum total protein resulted in our study agreed with those found by Ye et al. (2011), Lin et al. (2013): the total protein content in serum decreased with the inclusion of soybean meal as a substitute for fish meal in diets. Moreover, the results of our study underline the relationship between protein quality and metabolism since the triglyceride content decreased as the total protein rate when soybean meal level increased in the diets (Lim et al., 2011).

Moreover in our study the whole body composition of two banded seabream resulted to be not affected by soybean meal inclusion level and this result is in agreement with several studies on other fish species (Hernández et al., 2007; Bonaldo et al., 2008; Lin et al., 2011; Mamamuag et al., 2011). Whole body protein level slightly increased with increasing soybean meal in diets. There was a negative correlation to whole body lipid and ash content a slight decrease was observed when soybean meal inclusion increased. Similar results were also reported by Lin et al. (2011) and Bonaldo et al. (2008).

In conclusion, the results of our study reported that in two banded seabream's diets can be used up to 40% soybean meal instead of fish meal with no serious effects on growth performance, feed conversion rate, specific growth rate and serum biochemical variables. These results assume high relevance because this is the only study present in the literature about the effects of soy products in two banded seabream diets. Nevertheless further studies to determine the inclusion level of soybean meal more than 40% with amino acid or enzyme supplementation are needed.

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