



Effects of Partial Replacement of Fish Meal by Soybean Meal on Growth of Juvenile Saddled Bream (*Sparidae*)

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Abstract

The objective of this study was to evaluate the effects of soybean meal (SBM) inclusion and treatment on growth of juvenile saddled bream. The saddled bream (1.1 ± 0.5 g body weight) were fed with four practical diets containing 44% of crude protein (CP). Diet 1 was a control diet containing 100% fish meal (FM) as a protein source. Diets 2-4 contained 29% 34% and 49% soybean meal with FM (ratio of fish protein (FP) to soybean meal protein (SP) were 3:1, 2:1 and 1:1, respectively). Fish fed the diet containing FP/SP ratio 1:1 had significantly ($P < 0.05$) lower weight gain than fish fed with the other diets. There was no significant difference in body weight of fish fed with the other three diets. When compared to the control (Diet 1), fish fed with the diets 2 and 3 did not show any significant difference in body protein content. Body fat content was significantly higher in fish fed with the control diet than the fish fed with diets containing FP/SP ratios 2:1 and 1:1. Whole body ash content was significant higher in fish fed with diet 4. The present study indicates that SBM may be included in the diet up to 34% as a substitute for FM, replacing about 28% of FP.

Keywords: Saddled bream; Fish meal replacement; Soybean meal, Growth performance.

Introduction

No studies have yet examined the effects of treatment of different protein level of defatted SBM on growth performance of saddled bream. In one of our previously experiments, the protein requirement of juvenile *S. bream* was determined to be 49% of the diet (Antolović *et al.*, 2011).

Fish meal supplies the largest portion of dietary protein in fish diets (Biswas *et al.*, 2007). Available data show that about 30% to 50% of fish meal (FM) can be successfully replaced in fish feeds by plant protein sources (Francis *et al.*, 2001). Soybean meal (SBM), having high protein content and favorable amino acid profile that closely meets the requirements of fish, is consistently available and reported to be palatable to most species of fish (Lim and Akiyama 1992). Studies have shown considerable success in partial or total replacement of FM with SBM in diets for many fish species (Reinitz, 1980; Mohsen and Lovell, 1990; Vivyakarn *et al.*, 1992; Webster *et al.*, 1992a, 1992b, 1995; Olli *et al.*, 1995; Boonyaratpalin *et al.*, 1998; Quartararo *et al.*, 1998; Arndt *et al.*, 1999; Thompson *et al.*, 2006; Biswas *et al.*, 2007; Hernandez *et al.*, 2007; Abdel-Tawwab *et al.*, 2010). Many studies shown that approximately 20 to 40%

FM protein can be replaced in diets for carnivorous fish species (Chou *et al.*, 2004; Lim *et al.*, 2004; Hernandez *et al.*, 2007; Pham *et al.*, 2007; Lim and Lee, 2008). The discrepancy among researchers regarding the use of SBM as a protein source for fish may be related to the quality and processing of SBM, variation in diet formulation, and differences in fish species, size and culture systems (Elangovan and Shim, 2000). Many studies indicated palatability problems for the feeds when SBM was included in the diets (Fowler, 1980; Hajen *et al.*, 1993). The main limitations in the use of SBM are attributed to the low level of methionine and the presence of anti-nutritional factors (Wilson and Poe, 1985; Olli *et al.*, 1994). Heat treatment of soybeans improved growth performance and feed utilization in trout (Sandholm *et al.*, 1976), common carp (Nour *et al.*, 1989), and coho salmon (Arndt *et al.*, 1999). Heating SBM at 105°C for 30–90 min destroyed most of the protease inhibitors present (Viola *et al.*, 1983). However, heating may cause loss of essential amino acids (Plakas *et al.*, 1985). Soybeans contain many antinutritional factors, such as, protease inhibitors, non-digestible carbohydrates, lectins, saponins, phytates, and possibly allergenic storage proteins (Liu, 1997).

The objective of this study was to examine the growth performance, feed utilization efficiency, and body composition on saddled bream fingerlings fed on diets containing differently protein level of defatted SBM.

Materials and Methods

Experimental Diets

Four isoenergetic diets were formulated to contain various percentages of SBM (defatted) as partial replacement for FM. All diets were isonitrogenous and contained 44% protein. Diet 1 is with 84% FM served as the control. Diets 2, 3 and 4 contained 29%, 34%, and 49% SBM and 59%, 53%, and 37% FM, respectively (ratio of fish meal protein (FP) to soybean meal protein (SP), 3:1, 2:1 and 1:1, respectively). The composition and proximate analysis of the experimental diets are given in Table 1. SBM was ground to powder form in a mill. All dry ingredients were mixed thoroughly for 30 min in a food mixer. Then, oil was added and the diets were mixed again for 10 min. The prepared diets were packed in separate plastic bags and stored at -30°C. Wet feed was prepared weekly. The calculated amount of dry feed for a week for each tank was mixed with an equal amount of distilled water to form a paste wet feed and stored at 4°C. Fish were fed at a rate of 6% body weight five times daily (08:00, 10:00, 12:00, 14:00, and 16:00 h). During the course of the experiment, fish in all tanks ate all the food as soon as they were fed and no food was left over. Fish (n=30) were bulk-weighed every month and to record growth and determine the daily ration for the subsequent fortnight. The feeding trial was carried out for 90

days.

Experimental Procedure

Juvenile tin foil saddled bream were collected from south Adriatic Sea and stocked in the experimental tanks (1,000 L) for 1 week before the beginning of the experimental regime, in order to condition the fish to the laboratory system and handling procedures. The photoperiod was set at 12-h light : 12-h dark. During the acclimatization period, the fish were fed a commercial powdered diet 64% protein. At the start of the growth trial, uniform-sized fish (1.1±0.5 g body weight) were randomly distributed into 12 tanks, with three replicates per diet. Hundred fish were stocked in each tank. The tanks were connected to a continuous circulatory system, in which seawater from a holding tank was circulated through biological and mechanical filters and sterilized with UV light. Continuous aeration was provided to each tank through air stones connected to a central air compressor. Water temperature and pH were measured every 3 days. The water temperature varied between 18 and 21°C and the pH ranged from 6.5 to 7.3 during the experimental period.

Sampling Procedure and Evaluation of Growth Parameters

Prior to final weighing and sampling for chemical analysis, fish were starved for 24 h. At the termination of the experiment, 15 fish from each tank were randomly collected for proximate analysis. Fish were killed by immersing in ice water. Fish carcass samples were analyzed for crude protein, crude fat, ash, and moisture according to the methods described

Table 1 Composition and proximate analysis of experimental diets (g/100 g diet)

Ingredient (g/100g)	Diet 1	Diet 2	Diet 3	Diet 4
	Control 100% FP	3 : 1	FP : SP 2 : 1	1 : 1
Fish meal	84	59	53	39
Soybean meal	/	29	34	49
Fish oil	6	2	1	1
Carboxymethyl cellulose	4	2	3	1
Starch	3	5	6	7
Mineral mix ^a	2	2	2	2
Vitamin mix ^b	1	1	1	1
Total	100	100	100	100
<i>Proximate composition analysed</i>				
Crude protein	44.21	43.45	44.12	44.02
Crude lipid	14.3	13.8	14.1	14.8
Ash	13.21	12.54	12.05	12.11
GE ^c (kJ/kg)	21.3	21.2	21.7	21.1

^aMineral mix contains (g/kg diet): CaHPO₄P₂H₂O, 18.00; CaCO₃, 14.00; MgSO₄P₇H₂O, 5.10; FeSO₄P₇H₂O, 1.00; NaHCO₃, 6.88; MnSO₄PH₂O, 0.35; KIO₃, 0.01; CoCl₂P₆H₂O, 0.002; Na₂MoO₄P₂H₂O, 0.008; NaSeO₃, 0.002; KH₂PO₄, 11.996; ZnCO₃, 0.15; CuSO₄P₅H₂O, 0.03; NaCl, 2.47; Al₂SO₄.3, 0.02.

^bVitamin mix contains (g/100 g of mix): thiamin, 0.100; pyridoxine, 0.100; folic acid, 0.025; ascorbic acid, 2.00; pantothenic acid, 0.300; myo-inositol, 2.00; biotin, 0.010; niacin, 0.750; cyanocobalamin, 0.001; riboflavin, 0.100; retinol acetate, 0.040; tocopheryl acetate, 0.200; menadione, 0.400; cholecalciferol, 0.003; dextrin, 93.971.

^cGE= gross energy.

by the Association of Official Analytical Chemists AOAC, 1990. Water content was measured by drying samples at 105°C to constant weight in an oven. Crude protein was determined using a Kjeltac autoanalyser. Crude fat was estimated using Soxhlet apparatus with petroleum ether and ash by heating at 550°C for 24 h in a Thermolyne type 6000 programmable ashing furnace (Carbolite Furnaces, model CSF 1100).

The data obtained were analyzed for feed conversion efficiency (FCR), protein efficiency ratio (PER), specific growth rate (SGR), using following formula:

$$\text{FCR} = \frac{\text{Dry feed consumed (g)}}{\text{Wet weight gain (g)}}$$

$$\text{PER} = \frac{\text{Wet weight gain (g)}}{\text{Protein consumed (g)}}$$

$$\text{SGR} = \frac{[\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}]}{\text{Time (days)}} \times 100$$

Data were analyzed by one-way analysis of variance ANOVA using Statistica 7 ANOVA procedure. Duncan's multiple-range test was used to compare differences among means. The level of significance was chosen at $P < 0.05$ and the results are presented as means \pm standard error of the mean (SEM).

Results

After 90 days, the weight gain response and feed performance data of saddled bream fed diets

containing different percentages of SBM and FM are shown in Table 2. Among the treatments, the weight gain of fish fed with Diet 4 was significantly different from diet 1, 2 and 3. FCR ranged from 7.7 to 16.9 and PER varied from 0.12 to 0.26 among the treatments. PER and FCR were significantly lower and higher, respectively, in fish fed with Diet 4 than that of fish fed with the other three diets. However, FCR and PER were not significantly different among fish fed with Diets 2 and 3. The general health and appearance of all test fish were good and the fish in all treatments were very active. The whole body composition of fish after 90 days of growth trial is given in Table 3. Whole-body moisture content significantly increased as the amount of SBM in the diets increased. Conversely, the whole-body lipid significantly decreased. However, ash content was significantly higher in fish fed with Diet 4, containing 49% SBM than in those fed with the other three diets, but whole-body ash content was not significantly different among the fish fed with Diets 1–3. Crude protein content was highest in fish fed with Diet 3 and it was not significantly different from that of fish fed with Diets 1 and 2. However, crude protein of the fish fed with Diet 4, containing FP and SP in the ratio 1:1, was significantly lower than fish fed the other three diets.

Discussion

The result of the present study shows that inclusion of SBM up to 34% in the diet, did not affect the growth rate when compared with the control diet containing 84% FM (100% FP). This is in agreement with results obtained with several fish species. Watanabe *et al.* (1992) demonstrated that SBM could be included as a protein source up to 30% in place of

Table 2. Mean initial body weight, weight gain, FCR, SGR, PER, of saddled bream fed test diets containing different ratios of FP and SP for 90 days¹

	Diet			
	1 (100% FP)	2 (3 FP : 1 SP)	3 (2 FP : 1 SP)	4 (1 FP : 1 SP)
Initial weight (g/fish)	1.09±0.4	1.09±0.7	1.10±0.6	1.10±0.5
Weight gain (g/fish)	6.6±0.18 ^a	6.5±0.21 ^a	6.4±0.12 ^a	4.3±0.08 ^b
SGR	3.1±0.08 ^a	3.17±0.09 ^a	3.12±0.04 ^a	2.56±0.05 ^b
FCR	7.7±0.14 ^a	10.23±0.18 ^b	11.14±0.25 ^b	16.95±0.32 ^c
PER	0.26±0.02 ^a	0.17±0.01 ^b	0.17±0.01 ^b	0.12±0.01 ^c

¹Data are mean values of three replicates. Values are mean \pm SEM. Means within a row having different superscripts were significantly different ($P < 0.05$).

Table 3. Whole body composition (% wet weight) of saddled bream after 90 days of feeding test diets containing different ratios of FP and SP¹

Diet	As percentage of wet weight			
	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)
1 (100% FP)	74.12±0.14 ^a	20.99±0.11 ^a	1.71±0.09 ^a	2.21±0.18 ^a
2 (3 FP : 1 SP)	74.61±0.05 ^a	21.28±0.08 ^a	1.56±0.21 ^b	2.24±0.15 ^a
3 (2 FP : 1 SP)	75.32±0.06 ^a	21.38±0.15 ^a	1.41±0.18 ^b	2.23±0.11 ^a
4 (1 FP : 1 SP)	76.70±0.08 ^b	20.25±0.18 ^b	0.20±0.04 ^c	2.78±0.13 ^b

¹Data are mean values of three replicates. Values are mean \pm SEM. Means within a column having different superscripts were significantly different ($P < 0.05$).

FM in soft-dry pellets for yellowtail. Replacement up to 20% of FP with SP in diets for Atlantic salmon was suitable without growth reduction (Olli and Krogdahl, 1995). SBM may be included in the diet up to 37% as a substitute for FM, replacing about 33% of FP in juvenile tin foil barb (Elangovan and Shim, 2000). Venou *et al.* (2006) reported that increasing level up to 40% of SBM had no effect in specific growth rate in gilthead sea bream. Hernandez *et al.* (2007) reported that in small size sharpsnout seabream increasing level of SBM 40% or higher percent impact the tendency for growth to decrease as the diets percentage of SBM increased. Substitution of FP with SBM supplemented with amino acids or other protein sources in the diets of fishes have also been documented. Substituting 75% of FP with SBM was possible in the diet of hybrid striped bass with methionine supplements (Gallagher, 1994). McGoogan and Gatlin (1997) grew red drum successfully with diets in which 90% of FP was replaced by SBM with additions of amino acids. Webster *et al.* (1995) suggested that a methionine supplemented diet with an all plant protein source (SBM) could totally replace FM in a diet for blue catfish, without adverse effects on weight gain or body composition, when the protein level was 35% and fish were fed to satiation. Kikuchi (1999) has shown that about 45% of FP can be replaced with defatted SBM in combination with blood meal or corn gluten meal and blue mussel meal without any amino acid supplement. In this study with juvenile saddled bream when SBM was used to replace 44% of the FP (55% SBM in Diet 4), growth was significantly reduced. Growth reduction could have been due to higher FCR and lower PER (Table 2). Higher FCR and lower PER have also been previously reported in yellowtail (Vivayakarn *et al.*, 1992) and Japanese flounder (Kikuchi, 1999) when SBM was included at high levels in the diet. The increase in FCR with increasing levels of both types of SBM is in accordance with other studies (Pongmaneerat and Watanabe, 1992; Arndt *et al.*, 1999; Fagbenro and Davies, 2001; Chou *et al.*, 2004; Venou *et al.*, 2006; Lim and Lee, 2008).

The reduced carcass fat content with increased SBM in diet is also consistent with earlier findings (Mohsen and Lovell, 1990; Reigh and Ellis, 1992; Olli *et al.*, 1995; Elangovan and Shim, 2000; Venou *et al.*, 2006; Biswas *et al.*, 2007), even though levels of lipid and gross dietary energy were equal among all diets. It's also demonstrated that alcohol-soluble components of SBM comprise antinutrients, which negatively affect fat digestibility, particularly the long chained, saturated and monosaturated fatty acids in Atlantic salmon (Olli and Krogdahl, 1995). This may also be one of the reasons for reduced carcass fat content and growth rate in fish fed with the Diet 4, containing larger proportion (49% of the diet) of SBM. SBM contains approximately 30% carbohydrates, with 10% oligosaccharides (5%

sucrose, 4% stachyose, and 1% raffinose), 1% starch, and 20% non-starch polysaccharides (Snyder and Kwon, 1987; Liu, 1997). Non-starch polysaccharides, mainly consisting of cellulose, hemicellulose, and pectins, and oligosaccharides, such as stachyose and raffinose are not digested and absorbed by monogastric animals (Lim and Akiyama, 1992). Only, sucrose and a trace of starch from SBM are available. Non-starch polysaccharides affect digestion of nutrients negatively (Refstie *et al.*, 1999) and are responsible for a delayed gastrointestinal evacuation in Atlantic salmon (Storebakken *et al.*, 1999). Therefore, it is understandable that to the fish fed with Diet 4, containing 49% SBM, most of the carbohydrates may have been completely unavailable. The relative use of dietary carbohydrate by fish varies and appears to be associated with the complexity of the carbohydrate (NRC, 1993). Moreover, it may be possible that saddled bream fed higher levels of FM might have converted a larger portion of dietary protein to lipid, if amino acid intake was in excess of requirements. Moisture and fat usually vary inversely in fish flesh, while the protein is more constant (Belal and Assem, 1995). In this study, whole-body moisture content increased and body fat decreased with increasing dietary SBM as has been reported for Atlantic salmon (Olli *et al.*, 1995), Japanese flounder (Kikuchi *et al.*, 1994) and juvenile tin foil barb (Elangovan and Shim, 2000).

The results of this experiment suggest that a diet containing 44% crude protein with 2:1 mixture of FP and SP (34% SBM in the diet) was adequate for normal growth in saddled bream.

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