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Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, body composition and activity of digestive enzymes of juvenile yellowfin sea bream (*Acanthopagrus latus*)

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Abstract

A feeding trial was conducted to evaluate the effect of replacing fish meal protein with fermented soybean meal (FSM) on the growth performance, feed utilization, body composition and digestive enzymes activity of yellowfin sea bream (*Acanthopagrus latus*) juvenile. Five isonitrogenic and isolipidic diets were prepared with levels of 0 (control), 100, 150, 250 and 300 g kg⁻¹ FSM. Triplicate groups (20 fish per tank) of yellowfin sea bream with initial weight of 2.51±0.01 g were hand-fed to visual satiation at three meals per day for 56 days. The fish fed diets containing different levels of FSM had no significant differences regarding Final weight, Weight gain, SGR, FCR and Survival with control group. Whole body proximate compositions of fish were not affected by dietary FSM level. The activity of digestive enzymes in the intestine was not affected by dietary FSM level. This study showed that up to 30% fish meal in the diets of juvenile yellowfin (*Acanthopagrus latus*) could be replaced by fermented soybean meal.

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Introduction

Fish meal has been a major ingredient in fish diets

because of its good protein quality and palatability (Lovell, 1984). However, increasing demand, high cost and unstable supply of fish meal have resulted in nutritionists studying alternative sources, especially plant proteins to replace fish meal protein in the diet for freshwater and marine fish species (Pongmaneerat and Watanabe, 1993; Robinson and Li, 1994). Among plant proteins, soybean meal is the good candidate for partial or total replacement of fish meal protein in diets because of higher protein content, balanced essential amino acids, availability of sources and low cost, but its full nutritional value is obtained only after inactivation of soy anti-nutritional factors (ANFs) such as protease inhibitor, anti-vitamin and lectin which decrease the nutritional value of food through the mineral bioavailability and digestibility of nutrients. In salmonids, soy non starch carbohydrates or heat-stable anti-nutritional factors have been reported as important factors responsible for decreased growth performance (Harpez *et al.*, 2006). It has been reported that fermentation is a suitable technique for drying wet products with minimal nutrient loss (Yamamoto *et al.*, 2004). Fermentation is a process allows microorganisms such as *Bacillus subtilis* to degrade protein macromolecules to a large extent water-soluble low molecular weight compounds (Kiers *et al.*, 2000). It was found that fermentation of soybean meal induced removal or inactivation of anti-nutritional factors (Reddy and Pierson, 1994; Lim *et al.*, 2010), improvement of the nutritional quality (Canella *et al.*, 1984), improvement of digestibility (Kiers *et al.*, 2000) and shelf life of the processed food (Skrede and Nes, 1988). Fermented fish silage and fermented soybean meal (FSM) were reported as suitable protein sources in the diets of catfish, *Clarias gariepinus* (Burchell) and Nile tilapia *Oreochromis niloticus* L. (Fagbenro *et al.*, 1994). It was shown that FSM induced higher growth and feed efficiency compared to non-fermented soybean meal in diets of yellowtail *Seriola quinqueradiata* (Shimeno *et al.*, 1993a).

Most farmers are using commercially manufactured feeds, which mostly contain high levels of fish meal for cultivation of carnivorous fish such as yellowfin

sea bream *Acanthopagrus latus*. Several researchers reported different types of processed soybean meals as alternative protein sources in diet for gilthead sea bream, but limited information is available on using FSM in diet of yellow sea bream. Also based on our knowledge, less attention has been paid to the relationship between dietary plant protein and fish physiological status (Olsen *et al.*, 2007). Therefore, this study was conducted to determine the effect of partial substitution of dietary fish meal with FSM on growth performance, feed utilization and some biochemical parameters of juvenile yellowfin sea bream in an attempt to understand the mechanisms and appropriated level of FSM inclusion in diet of yellowfin sea bream.

Materials and methods

Diet preparation

The soybean meal was fermented using *B. subtilis* by a process modified to method as mentioned in the Lim *et al* (2010). Briefly, soybean meal was steam cooked in an autoclave at 100°C for 20 min (pH 5-6). After cooling, the steamed soybean meal was inoculated by evenly spraying spore suspension of *B. subtilis*. Then the inoculated soybean meal substrate was incubated at 37°C for 24 h (pH 8.35). The products of fermentation were dried in a vacuum drying oven at below 60°C for 15 h (pH 7-8). Finally, FSM was ground to be below 400 μ m mesh size.

Five isonitrogenous and isolipidic diets were formulated to contain 0, 100, 150, 250 and 300 g kg⁻¹ FSM produced by *Bacillus subtilis* designated as control, FSM10, FSM15, FSM25 and FSM30. Ingredients and nutrient contents of the experimental diets are presented in Table 1. Kilka fish meal was used as the primary protein source and fish oil and soybean oil were used as lipid sources. All ingredients were thoroughly mixed with 300 g kg⁻¹ distilled water, and pellets were prepared using a moist pelleting machine. The pellets were dried at room temperature for 24 h and ground into desirable particle sizes. All diets were stored at -20°C until used.

Growth experiment

Juvenile yellowfin sea bream were obtained from a local farm (Bushehr, Iran). The fish were acclimated to laboratory condition for 2 weeks before starting the feeding trial. Juvenile fish were allocated randomly into 150 L circular plastic tanks with 20 fish per each tank for the feeding trial after being collectively weighed. Three replicate groups of fish were hand-fed to apparent satiation three times a day (9:00, 13:00 and 17:00) for 56 days. Filtrated sea water was supplied at a flow rate of 5 L min⁻¹ in each tank and the mean water temperature was 23.8±0.74°C. The photoperiod was left under natural conditions during the feeding trail. At the initiation and termination of experiment, juvenile sea bream in each tank were collectively weighed and counted for calculations of growth performance and survival.

Diets and whole body chemical analysis

Five fish from each tank at the end of experiment were randomly sampled and stored at -20°C in freezer for proximate composition. Proximate analysis of diets and fish were determined according to the method of AOAC (1995). Crude protein content was determined using the Kjeldahl method using an Auto Kjeldahl System. Crude lipid was analyzed by ether extraction, moisture content by a dry oven drying at 105°C for 6 h and ash by a furnace muffler (550°C for 4 h).

Digestive enzymes activity

At the end of the feeding trial (after one day starvation) five fish from each tank were washed in cold distilled water and dissected on a glass maintained on ice. Samples of intestine were homogenized immediately in 50 mM Tris-HCl containing 20 mM CaCl₂ and 50 mM KCl (pH 7.5) by a homogenizer, followed by centrifugation (15000 g for 40 min at 4°C). We used 100 mg tissue mL⁻¹ buffer for homogenization and then supernatants were kept frozen in -80°C to determine biochemical analysis. All the assay techniques were based on photometric procedures in which disappearance of the substrate for product formation was measured.

The activity of α -amylase, lipase and trypsin was assayed according to the methods described below.

Enzyme activity expressed as a specific activity of U mg⁻¹ protein. The specific activity of α -amylase, lipase and trypsin was performed by the enzymatic photometric method using amylase kit (Pars Azmon, Tehran, Iran). Also protein content in the supernatants was measured using the Bradford (1976) method.

Statistical analysis

In outline, this study was planned and executed entirely by accident. All data are collected normal distribution using the Shapiro-Wilk test was performed, and significant differences between treatments at different levels ($p \leq 0.05$) using ANOVA (One-way ANOVA) and post- Duncan test was examined. Analysis of all the data and the operations were performed by SPSS 16.0 software.

Results

The results of growth performance and feed efficiency were shown in Table 2. Final body weight, weight gain (WG), weight gain percent (WG %), specific growth rate (SGR) and survival were not affected significantly by dietary FSM level compared with control group ($P > 0.05$). Also, FCR of fish fed different levels of fermented soybean meal had not significantly different compared with control group ($p > 0.05$).

Body composition of fish fed diets containing different levels of FSM was shown in Table 3. The Crude protein, lipid and ash contents of whole body of fish fed diet containing different levels of FSM was not significantly difference compared with control group ($P > 0.05$).

The activity of digestive enzymes was presented in Table 4. The activity of α -amylase, lipase and trypsin in the intestine of fish were not affected by dietary FSM level compared with control group ($P > 0.05$).

Discussion

The result of this experiment showed that up to 30% fish meal in the diets of juvenile yellowfin sea bream could be replaced by fermented soybean meal with attention to growth performance and digestive

enzyme activity. Soybean meal is considered to be the best widely available plant protein source. So, many researchers carried out experiments on the use of soybean meal in diets of freshwater fish such as tilapia *Oreochromis niloticus* L. (Wee and Shu, 1989), grass carp *Ctenopharyngodon idella* (Steindachner) (Dabrowski and Kosak, 1979) and blue catfish *Ictalurus furcatus* (Lesueur) (Webster *et al.*, 1995). Also several studies were carried out about soybean meal utilization in marine fish (Lim and Lee, 2008). It was found that fish meal protein could be replaced up to 200 g kg⁻¹ with soybean meal in diet of yellowtail (Shimeno *et al.*, 1993b). Day and Plascencia (2000) reported that fish meal protein could be replaced up to 250 g kg⁻¹ with soybean protein concentration in diet of turbot. It was shown that 330 g kg⁻¹ of fish meal protein could be replaced with soybean meal in diet of Pacific salmon *Oncorhynchus nerka* (Suckley) (Carter and Hauler, 2000). Kikuchi (1999) found that about 350 g kg⁻¹ of fish meal protein could be replaced by defatted soybean meal in combination with other protein sources as corn gluten meal and blood meal in the diet of juvenile

olive founder. Also it was reported significant reductions in growth performance of gilthead sea bream *Sparus aurata* at 300 g kg⁻¹ substitution of fish meal protein with soybean meal (Negas *et al.*, 2008). Generally, poor growth performance is observed in fish fed the diets containing plant protein sources such as soybean meal due to low palatability, deficiency of some essential amino acid, less availability of phosphorus, disorder in lipid metabolism and high content of anti-nutritional factors (ANFs) (Gomes *et al.*, 1995; Ye *et al.*, 2011). ANFs may reduce the potential for using conventional soybean meal in feed formulations, and much effort has been expended in devising processing techniques for improving the nutritional value of soybean meal. Therefore, several practical ways have been suggested for improving the nutritional value of soybeans blending (Jackson *et al.*, 1982), feeding stimulants (Deng *et al.*, 2006) and fermentation (Kader *et al.*, 2011; Lee *et al.*, 2010). Modern processing techniques may employ a range of chemical, enzymatic and physical treatments (Phillips, 1989; Anderson and Wolf, 1995).

Table 1. Ingredient and proximate composition of experimental diets.

Ingredients (g kg ⁻¹)	Diets				
	CON	FSM10	FSM15	FSM25	FSM30
Kilka fish meal	642.6	564.2	525.0	447.4	408.7
Fermented soybean meal	0	100	150	250	300
Wheat flour	187.3	165.7	154.9	129.0	115.7
Corn gluten meal	50	50	50	50	50
potato-starch	50	50	50	50	50
Kilka fish oil	30	30	30	30	30
Soybean oil	30	30	30	30	30
Vitamin premix ¹	20	20	20	20	20
Mineral premix ²	20	20	20	20	20
Proximate analysis (g kg ⁻¹ dry matter basis)					
Crude protein	50	50	50	50	50
Crude lipid	10.77	10.31	10.08	10.00	10.00
DE(kcal kg ⁻¹)	4245	4181	4195	4200	4200

¹ Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): DL- α -tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid (98%), 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

² Mineral premix contained the following ingredients (g/kg mix): MgSO₄·7H₂O, 80.0; NaH₂PO₄·2H₂O, 370.0; KCl, 130.0; Ferric citrate, 40.0; ZnSO₄·7H₂O, 20.0; Ca-lactate, 356.5; CuCl, 0.2; AlCl₃·6H₂O, 0.15; KI, 0.15; Na₂Se₂O₃, 0.01; MnSO₄·H₂O, 2.0; CoCl₂·6H₂O, 1.0.

Table 2. Growth performance of juvenile yellowfin sea bream fed the experimental diets for 8 weeks.

	Diets				
	Con	FSM10	FSM15	FSM25	FSM30
Initial average weight (g fish ⁻¹)	2.51±0.03 ^{ns}	2.53±0.05	2.50±0.05	2.52±0.04	2.52±0.03
Final average weight (g fish ⁻¹)	6.88±0.12 ^{ns}	6.87±0.15	6.84±0.20	6.85±0.85	6.82±0.82
¹ Weight gain (g fish ⁻¹)	4.40±0.10 ^{ns}	4.36±0.12	4.36±0.16	4.33±0.13	4.30±0.20
² Weight gain percent	173.73±2.6 ^{ns}	172.40±2.2	173.70±4.2	171.16±1.77	170.63±5.1
³ Specific growth rate ¹ (%)	1.79±0.01 ^{ns}	1.79±0.01	1.79±0.02	1.75±0.01	1.77±0.03
⁴ Food conversion ratio	1.50±0.17 ^{ns}	1.36±0.12	1.26±0.12	1.56±0.03	1.43±0.06
⁵ Survival	95±2.8 ^{ns}	95±5	96.66±1.6	93.33±4.4	93.33±1.66

Values (means ± SE of three replication) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

ns= not significant ($P > 0.05$).

¹Weight gain= final weight-initial weight

²Weight gain percent= [(final weight-initial weight)/initial weight] × 100

³Specific growth rate (%) = [ln (final fish wt.) - ln (initial fish wt.)] × 100/days of feeding.

⁴Food conversion ration= weight gain/ feed intake

⁵Survival= (final fish number / initial fish number) × 100.

The process of fermentation has advantages for higher replacement of fish meal with alternative proteins through inactivation of anti-nutritional factors (Reddy and Pierson 1994), increased low molecular weight protein and higher digestibility (Kader *et al.*, 2011). The technique allows higher inclusion levels (300–400 g kg⁻¹) of oilseed meals, legumes and aquatic macrophytes compared to non-fermented raw meals (100–200 g kg⁻¹) in the diets of *Labeo rohita* Hamilton (Mukhopadhyay and Ray,

1999; Bairagi *et al.*, 2002). Recently Zhou *et al.* (2011) reported that up to 200 g kg⁻¹ fish meal protein could be replaced by FSM by *Candida utilis* for black sea bream. Kader *et al.* (2011) reported that fish meal protein could be replaced up to 360 g kg⁻¹ by FSM and squid by product as an attractant in diet of flounder. Also Wee (1991) suggested that nutrient value of plant ingredient improved during fermentation period by microbial activities.

Table 3. Proximate composition (%) of the whole body of juvenile yellowfin sea bream fed the experimental diet for 8 weeks.

	Diets				
	Con	FSM10	FSM15	FSM25	FSM30
Crude protein	15.71±0.62 ^{ns}	15.72±1.09	15.59±0.42	15.82±0.36	15.39±0.55
Crude lipid	7.88±0.22 ^{ns}	7.58±0.37	7.64±0.17	7.68±0.20	7.56±0.49
Moisture	69.82±0.74 ^{ns}	69.56±0.94	70.40±0.79	70.36±0.88	69.79±0.52
Ash	4.33±0.16 ^{ns}	4.57±0.22	4.62±0.27	4.63±0.25	4.31±0.15

Values (mean ± SE of three replication) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

ns= not significant ($P > 0.05$).

The activity of digestive enzymes in sea bream fed the different levels of FSM in the diet were poorly mirrored in the parallel decreases in protein and feed efficiency. This suggests that FSM underwent processes enabling proper inactivation of trypsin inhibitors. So, it seems that other possible causes

affecting the digestive–absorptive function and as a result feed utilization. It have been suggested Oligosaccharides and non-starch polysaccharides (NSPs) in soy preparations lead to reduced bioavailability of nutrients and energy through mechanisms involving a binding action with bile salts

combined with changes in digesta viscosity and transit rate (Francis *et al.*, 2001). Also, reduced nutrient/energy bioavailability in response to diets containing high levels of conventionally-processed soybean meal because of oligosaccharides and NSPs has been observed in almost all carnivorous fish species investigated to date (Harpez *et al.*, 2006).

On the other hand, the intake of feeds containing different protein sources and levels has been reported to be inversely related to their digestible energy content (Morales *et al.*, 1994). So, it seems that the fish compensated the negative effect of residual ANFs of FSM on reducing bioavailability of energy in diets by significant increases of feed intake and protein intake.

Table 4. Digestive enzyme activity in juvenile yellowfin sea bream fed the experimental diets for 8 weeks.

	Diets				
	Con	FSM10	FSM15	FSM25	FSM30
Amylase (U mg ⁻¹ protein)	0.8±0.09 ^{ns}	0.8±0.11	0.7±0.14	0.8±0.07	0.8±0.12
Lipase (U mg ⁻¹ protein)	1.3±0.13 ^{ns}	1.3±0.11	1.5±0.09	1.6±0.17	1.2±0.15
Trypsin (U mg ⁻¹ protein)	359±12.3 ^{ns}	333±18.1	343±14.7	363±19.6	349±11.6

Values (mean ± SE of three replication) in the same row not sharing a common superscript are significantly different ($P < 0.05$).

ns= not significant ($P > 0.05$).

We concluded that using FSM produced by *Bacillus subtilis* had beneficial effect on the nutritional quality of soybean meal. Therefore, fish meal protein could be replaced up to 300 g kg⁻¹ by FSM in diet without negative effect on the growth performance of juvenile yellowfin sea bream.

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