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The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869)

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ABSTRACT

The purpose of this study was to evaluate the replacement of fish meal (FM) with soybean meal (SBM) in diets for juvenile spotted rose snapper with the overall goal of identifying practical diet formulations for commercial production of the spotted rose snapper. The response of spotted rose snapper to diets contained graded levels of FM was evaluated by measuring weight gain, feed efficiency, body composition and blood chemistry during a 12-week period. Four diets were formulated (43.7% crude protein, 14% crude lipid and 19.6 kJ g^{-1} gross energy) with 0, 20, 40 or 60% SMB protein replacing FM in diets. Diets were fed to juvenile spotted rose snapper (mean initial weight 17.75 ± 0.03 g) for 12 weeks, and weight gain, feed efficiency, body composition and blood chemistry were assessed at the end of the feeding trial. Compared to the FM diet, there were no significant differences in weight gain, individual feed intake, specific growth rate or protein efficiency ratio (PER) of fish when 20% of the FM was replaced compared to fish fed the FM control diet. However, fish performance was reduced at higher levels of FM replacement, significantly so at the 60% replacement level. Hematological parameters were similar among the treatments. Fish fed the 60% SBM diet had significantly lower lipid levels than fish fed the other diets. There was no significant difference in survival of fish fed the different diets. A second order polynomial regression revealed maximum growth of the spotted rose snapper fed up to 19.4% SBM inclusion. The results of this study show that SBM is an acceptable ingredient to supply 20% of protein in spotted rose snapper diets, but that higher dietary levels reduce fish performance.

1. Introduction

The spotted rose snapper *Lutjanus guttatus* is an economically important marine carnivorous fish in Mexico and Latin America that is distributed along the Pacific coast of the American continent (Rojas-Herrera, 2001). This fish is considered to have a high degree of farming potential because of its commercial demand, its adaptive capacity and its controlled reproduction in captivity (Ibarra-Castro and Alvarez-Lajonchere, 2011).

The spotted rose snapper primarily feeds on demersal organisms, such as crustaceans and fish (Allen, 1995), and requires high levels of protein in its diet. From an economic standpoint, dietary protein is the most expensive component in fish feed formulations (Pérez et al., 1997; Thoman et al., 1999). Therefore, optimizing the source and inclusion level of various high-protein ingredients is critical to developing economical practical diets. Soy products are among the most promising of the ingredients that have been investigated as alternatives for FM (Lim et al., 1998; Storebakken et al., 2000; Swick, 2002). In 2005, the global production of soybeans was estimated at 218 mmt, and soybean accounted for 56% of the total world production of oilseeds. The price of soybean meal (SBM) varies and ranged from $360 to $525 mt between March 2011 and July 2012 (Feedstuffs, 2012); however, it is less expensive per kg protein than FM. From a nutritional, economic and market availability standpoint, products derived from soy, such as full-fat SBM, dehulled SBM and soy protein concentrates are likely to be key ingredients in future aquaculture diets (Gatlin et al., 2007).

Soybean meal is regarded as a nutritious feedstuff with high crude protein content and a reasonably balanced amino acid profile (Carter and Hauler, 2000; NRC, 2011) compared to other plant proteins

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(Gatlin et al., 2007). When used as the main protein source, the essential amino acid profile of SBM is adequate to meet the known requirements of marine fish, except for methionine (NRC, 2011). This nutritional imbalance leads to reduced growth in marine or carnivorous fish species fed diets with a high dietary SBM inclusion unless supplemental methionine is added to the diet (Kaushik et al., 1995; Martínez-Llorens et al., 2009; Tomás et al., 2005; Urán et al., 2009; Wang et al., 2006). Poor performance in marine fish fed high SBM diets is also associated with reduced feed intake assumed to be caused by reduced feed intake of the diet when FM and other marine protein sources are removed (Davis et al., 2005). However, reduced feed intake varies with fish species and feed formulation.

Less expensive feeds containing sustainable protein sources, such as SBM, are needed to expand production of spotted rose snappers. The primary objective of the present research was to evaluate the iso-nitrogenous replacement of FM with solvent-extracted SBM to determine appropriate SBM inclusion levels in practical diets for juvenile spotted rose snapper by measuring weight gain, feed efficiency, body composition and the blood chemistry of fish.

2. Materials and methods

2.1. Feed formulations and manufacture

Four isonitrogenous and isoenergetic experimental diets were formulated in which 0, 20, 40 and 60% of protein from FM were replaced by that from SBM. The inclusion levels of squid meal, krill meal, wheat gluten, carotenoids, antioxidants, soy lecithin, premixtures of minerals and vitamins remained constant in the four diets, whereas fish oil levels varied slightly. Sodium alginate was used as binder and dextrin was used to adjust the total to 100%. The third and fourth diets were supplemented with analytical reagent grade calcium phosphate dibasic ($CaHPO_4 2H_2O$) and DL-methionine to adjust dietary levels as FM decreased and SBM increased in the diet (Table 1). The dry ingredients were ground in a hammer mill to a particle size of 250 μm. The macro ingredients were mixed in a Hobart mixer (model AT-200) and the micro ingredients were added thereafter. Fish oil and then water were added until a homogeneous mixture was obtained. The resulting mash was passed through a meat grinder (Tor-rey® Model 22) to produce pellets. The pellets were dried with forced air at 38 °C for 12 h. Subsequently, the pellets were manually reduced to a size of approximately 0.5 mm using sieves to remove fine particles. The pellets were stored in labeled, sealed containers and were held at 4 °C until use.

Table 1
Ingredient and proximate composition of the experimental diets for the spotted rose snapper *L. guttatus*.

Ingredients (g kg^{-1} wet wt.)	Diet			
	D-0%	D-20%	D-40%	D-60%
Fish meal (sardine)[a]	52.60	46.10	39.59	33.08
Soybean meal[b]	0.00	10.52	21.04	31.56
Squid meal[b]	6.00	6.00	6.00	6.00
Krill meal[c]	7.59	7.59	7.59	7.59
Fish oil[d]	8.78	9.28	9.78	10.27
Dextrin[d]	17.47	12.95	8.12	3.59
Alginate[d]	3.00	3.00	3.00	3.00
Wheat gluten[e]	2.00	2.00	2.00	2.00
CaP dibasic[e]	0.00	0.00	0.05	0.10
DL-methionine[e]	0.00	0.00	0.25	0.25
Vitamin premix[f]	0.60	0.60	0.60	0.60
Minerals premix[f]	0.23	0.23	0.23	0.23
Carotenoids[g]	0.08	0.08	0.08	0.08
Soy bean lecithin (70%)[g]	1.50	1.50	1.50	1.50
Vitamin C[g]	0.10	0.10	0.10	0.10
Antioxidants[g]	0.05	0.05	0.05	0.05
Composition (% DM)[h]				
Crude protein	46.34 ± 0.02	47.38 ± 0.06	47.51 ± 0.08	48.13 ± 0.04
Crude lipid	14.92 ± 0.17	15.37 ± 0.10	15.69 ± 0.10	15.18 ± 0.14
Ash	15.08 ± 0.01	14.33 ± 0.06	13.30 ± 0.60	12.99 ± 0.09
NFE[i]	23.65 ± 0.17	22.90 ± 0.10	23.48 ± 0.42	23.68 ± 0.02
Gross energy (kJ g^{-1})[j]	19.3	19.5	19.8	19.8

[a] "Premium" grade fish meal was obtained from Selecta de Guaymas, S.A. de C.V. Guaymas, Sonora, Mexico.
[b] Proteínas marinas y Agropecuarias, S.A. of C.V., Guadalajara, Jalisco, Mexico.
[c] PROAQUA, S.A. de C.V. Mazatlán, Sinaloa, Mexico.
[d] Droguería Cosmopolita, S.A. de C.V. México, D.F., Mexico.
[e] Sigma-Aldrich Chemical, S.A. de C.V. Toluca, Mexico State, Mexico.
[f] Trouw Nutrition México S.A. de C.V. (by cortesy). *Vitamin premix composition: Vitamin A, 10,000,000 IU o mg/g; Vitamin D3, 2,000,000 IU; Vitamin E, 100,000 g; Vitamin K3, 4.00 g; Thiamine B1, 8.00 g; Riboflavin B2, 8.70 g; Pyridoxine B6, 7.30; Vitamin B12, 20.00 mg; Niacin, 50.00 g; Pantothenic acid, 22.20 g; Inositol, 153.80 g; Nicotinic Acid, 160.00 g; Folic Acid, 4.00 g;, 80 mg; Biotin, 500 mg; Vitamin C, 100.00 g; Choline 300.00 g, Excipient c.b.p. 2000.00 g. **Mineral premix composition: Manganese, 100 g; Magnesium, 45.00 g; Zinc, 160 g; Iron, 200 g; Copper, 20 g; Iodine, 5 g; Selenium,400.00 mg; Cobalt 600.00 mg. Excipient c.b.p. 1500.00 g.
[g] DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, Mexico.
[h] mean ± SD, number of determinations = 3.
[i] Nitrogen-free extract (including fiber) = 100 − (% protein + % lipid + % ash).
[j] Gross energy (kJ $g^{-1)}$ was calculated according to the physiological fuel values of protein, 20.93 kJ g^{-1}; lipids, 37.68 kJ g^{-1}; and nitrogen-free extract, 16.75 kJ g^{-1} (Shiau and Chou, 1991).

2.2. Fish rearing and feeding

Spotted rose snapper were produced at the Research Center for Food and Development (CIAD), Mazatlán Unit (Centro de Investigación en Alimentación y Desarrollo (CIAD), Unidad Mazatlán) following the established protocols for spawning and larval rearing (Abdo de la Parra et al., 2010). Because the fish were bred in captivity experimental conditions were similar to those during the larval stage, no acclimatization was necessary. A completely randomized experimental design with three replications was used. Groups of 15 fish (weight 17.75 ± 0.03 g) were randomly stocked in a system composed of twelve cylindrical black fiberglass tanks (volume 0.6 m^3). Each of the tanks had a central 50-mm drain covered with a 0.5-cm mesh net to prevent fish escape and to allow for cleaning of the tanks. Each tank had continuous flow-through sea water at a flow rate of 6.5 L min^{-1} and supplemental aeration. Seawater was pumped from the seashore, passed through two parallel sand filters and delivered to four 25 m^3 high density polyethylene (HDPE) head/sedimentation tanks (4 m × 15 m). From the head tanks, the seawater was pumped through a double parallel filtration system consisting of a pressured Jacuzzi sand filter (265 Lpm, 100 μm relative particle retention) and multiple cartridge filters (four 9.3 m2 cartridge filters, 16 μm relative particle retention) within a filter room (3 m × 5 m) (Alvarez-Lajonchere et al., 2007).

Feed was manually supplied three times a day (08:00, 12:00 and 16:00) until the fish were satiated. Uneaten food was collected from the bottom of the tank using a siphon 30 min after the onset of feeding and dried in an oven at 60 °C. Feed intake was calculated as the amount of feed supplied minus the amount of unconsumed feed. Dead fish were recorded and weighed to calculate the feed conversion ratio (FCR). Water quality parameters were measured daily using an YSI 85-10FT. The dissolved oxygen level was maintained at 6.40 ± 0.51 mg L^{-1}. The water temperature was 21.39 ± 1.5 °C, and the salinity was 32.8 ± 0.87 g L^{-1}.

The fish were weighed every two weeks to calculate their mean body weight, the biomass present in each tank. The fish were caught with scoop nets and anesthetized with 2-phenoxyethanol (Sigma®) at a concentration of 0.3 ml L^{-1}. Then fish were weighed individually on a digital scale (accurate to ± 0.01 g).

Growth and feed efficiency of the fish were monitored in terms of weight gain (WG), feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR), survival (S), protein efficiency ratio (PER) and apparent nitrogen utilization (ANU).

The biological indicators were calculated as follows:

$$WG = \text{final mean weight (g)} - \text{initial weight (g)}$$

$$FI = \sum_i n[(\text{total feed consumption (g)})/(\text{No. of fish})]/\text{number of days}$$

$$FCR = \text{feed intake (g)}/\text{weight gain (g)}$$

$$SGR = [\text{Ln}(\text{final weight} - \text{initial weight})/\text{number of days}] \times 100$$

$$S = (\text{Final number}/\text{initial number}) \times 100$$

$$PER = \text{weight gain}/\text{protein intake}$$

$$ANU = [(\text{final body protein} - \text{initial body protein})/\text{protein intake}] \times 100.$$

2.3. Chemical analysis

Ten randomly chosen fish were sampled from the initial population to determine the starting carcass composition. To analyze the final composition, two fish were selected at random from each tank for a total sample size of six fish per treatment group. The fish samples, meals and diets were homogenized and dried at 105 °C for 24 h prior to chemical analysis. Moisture, protein, fat and ash levels in the meals, diets and carcasses were determined according to standard methods (AOAC, 1984). The protein level was evaluated with the Dumas combustion method (Ebling, 1968) using a Leco FP-528. The crude fat content was analyzed using a micro Soxhlet Foss Soxtec Avanti. Moisture was determined using a Craft stove, and the ash by calcination of the samples in a muffle furnace (Felissa®).

The amino acid contents of the diets were determined using samples (1.0 mg) that had been hydrolyzed with 6 N HCl for 6 h. Sodium thioglycolate was added to the samples to prevent oxidation. The samples of the ingredients and diets were suspended in sodium citrate buffer (pH 2.2), derivatized with o-phthaldialdehyde (OPA), and injected (10 μL) into a Varian 9012 HPLC apparatus equipped with a fluorescent detector with a 340–380 nm excitation filter and 460 nm emission filter. Amino acid standards were used and α-aminobutyric acid was added as an internal standard. The solvent flow rate was 1.5 mL/min at 25–29 °C. The amino acids were all eluted within 20 min, and the column was equilibrated for 10 min with the starting solvent (Vázquez-Ortiz et al., 1995).

2.4. Hematological analysis

At the end of the experiment, blood samples were taken by cardiac puncture for hematology analysis from anesthetized fish. Ten fish were selected randomly from each experimental diet. A volume of 400 μL of blood was extracted from the fish using a 1-mL TERUMO tuberculin syringe via tail vein puncture. The blood sample was divided into 200-μL samples and placed into two tubes. The first tube contained an anticoagulant, and the blood from this tube was used to determine hematocrit and hemoglobin concentration. The second tube, which did not contain an anticoagulant, was centrifuged to obtain serum and determine glucose, total protein and triglyceride levels. The parameters were determined using commercial kits supplied by Randox y Biosystem Co. The mean corpuscular hemoglobin concentration (MCHC) was determined with a standard formula using the values of hemoglobin concentration and the percentage of microhematocrit (Del Rio-Zaragoza et al., 2008).

2.5. Data analysis

Data for each parameter was tested for normality and homoscedasticity. One-way analysis of variance (ANOVA) was performed with diet as the independent variable. Duncan's New Multiple Range Test was used as a post-how test to determine significant differences among dietary treatment groups with a significance level of 5% (Zar, 1984). Given the dose–response design of the experiment, the optimal level of SBM according to the best weight gain was determined using a second-order polynomial regression analysis. All of the statistical procedures were performed using the Sigma Stat ver. 3 software package.

3. Results

All experimental diets were close to isonitrogenous (46.3–48.1% CP) and isoenergetic (19.3–19.8 kJ g^{-1}) (Table 1). The essential amino acid (EAA) profiles of the diets with high amounts of SBM revealed lower levels of methionine, lysine and arginine compared to levels in the basal diet (Table 2).

Fish survival was greater than 95% and was not affected by dietary treatment. Polynomial regression analysis relating the increase in weight with respect to the degree of substitution of FM by SBM indicated that 96.76% (R^2) of the original uncertainty was explained by the model: $Y = -0.024X^2 + 0.7384X + 185.545$. The replacement level that provides the greatest percentage of weight gain was within the range of 10.5 to 19.4% (Fig. 1). The treatment that produced the best results for weight gain (WG), feed intake (FI) and specific growth rate (SGR) was the diet with an SBM inclusion level of 20% (D-20%), but this result was not significantly different from that obtained with the control diet (D-0%) ($P<0.05$, Table 3). The lowest feed conversion ratio (FCR) was achieved in the fish fed the D-20% diet. However, this treatment was not significantly different from the diets with 0 or 40% inclusion of SBM (D-0% and D-40%) ($P<0.05$). The highest protein efficiency ratio (PER) was obtained with the D-20% diet but this was not significantly different from PER values from the D-0% and D-40% treatments ($P<0.05$). There were no significant differences in apparent utilization of nitrogen (ANU) values ($P<0.05$). The highest body protein content was obtained using the diets that contained SBM at the level of 60 and 40% (D-60% and D-40%), while the control diet (D-0%) and the D-20% diet resulted in the lowest result ($P<0.05$, Table 4). Fish fed the 60% SBM diet (D-60%) had the lowest body fat content and highest moisture content, although it was not significantly different from fish fed the D-0% or D-20% diets. The lowest ash composition was found in the diet with an SBM inclusion level of 20% (D-20%), which showed no significant differences ($P<0.05$) compared

Table 2
Concentrations of essential amino acids (g AA per 100 of protein) in *L. guttatus* diets containing different levels of SBM.

Amino acid	D-0%	D-20%	D-40%	D-60%
Alanine	6.469 ± 0.571	5.756 ± 0.460	6.890 ± 0.412	6.474 ± 0.234
Arginine	8.140 ± 0.471	8.576 ± 0.256	7.656 ± 0.842	6.460 ± 0.520
Aspartic acid	9.575 ± 0.145	9.894 ± 0.162	9.902 ± 0.180	10.539 ± 0.188
Glutamic acid	15.294 ± 1.027	15.965 ± 0.163	17.636 ± 1.175	18.104 ± 1.148
Glycine	6.966 ± 0.118	5.872 ± 0.120	7.310 ± 0.493	6.069 ± 0.403
Histidine	2.159 ± 0.117	2.390 ± 0.051	2.535 ± 0.305	2.537 ± 0.092
Isoleucine	4.936 ± 0.324	4.413 ± 0.384	5.193 ± 0.004	5.845 ± 0.122
Leucine	7.317 ± 0.200	7.677 ± 0.676	7.878 ± 0.257	8.629 ± 0.702
Lysine	6.276 ± 0.237	6.940 ± 0.311	6.760 ± 0.237	6.524 ± 0.614
Methionine	2.699 ± 0.208	2.780 ± 0.246	2.635 ± 0.087	2.456 ± 0.037
Phenylalanine	4.421 ± 0.238	4.755 ± 0.487	4.664 ± 0.049	5.237 ± 0.519
Serine	3.273 ± 0.255	4.012 ± 0.138	3.295 ± 0.265	3.294 ± 0.281
Threonine	3.363 ± 0.135	3.770 ± 0.136	3.536 ± 0.130	4.879 ± 0.136
Tyrosine	6.340 ± 0.281	7.640 ± 0.612	6.793 ± 0.498	6.806 ± 0.484
Valine	5.245 ± 0.298	4.706 ± 0.307	5.305 ± 0.047	5.953 ± 0.316

Tryptophan was not determined by the analytical method used.

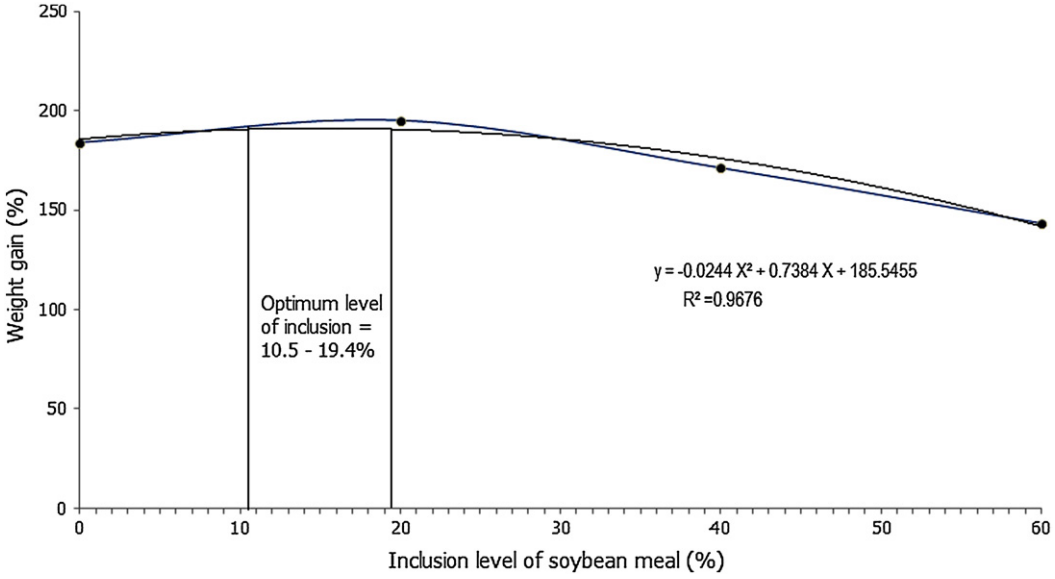

Fig. 1. Quadratic polynomial regression analysis relating the increase in growth by weight to the level of FM replaced by SBM.

to treatment with diets containing an SBM inclusion level of 0 or 40% (D-0 and D-40%).

Hematocrit, hemoglobin, mean corpuscular hemoglobin concentration (MCHC), total protein and glucose were not significantly different among the treatments. The maximum triglyceride level occurred in the fish fed the diet with 60% SBM, significantly different from the other treatments (Table 5).

4. Discussion

Achieving the substitution up to 20% of FM protein with SBM protein in practical diets for spotted rose snapper was an important step for this high value species because true sustainability in fish farming requires the replacement of most of the fish meal and fish oil in fish feeds. The substitution of more than 40% of FM protein with SBM protein in practical diets for this species reduced fish performance, similar to results reported for other marine species, such as Japanese flounder, Korean rockfish and olive flounder (Choi et al., 2004; Kikuchi, 1999; Lim et al., 2004). However, some marine species, such as Asian seabass, silver seabream, hybrid striped bass and cobia, have been successfully reared using low FM diets with various combinations of soy protein concentrate and SBM (El-Sayed, 1994; Gallagher, 1994; Boonyaratpalin et al., 1998; Salze et al., 2010). Red drums have been reared using 5% FM diets (McGoogan and Gatlin, 1997). Spotted rose snapper have a much lower tolerance for the use of SBM as a substitute for FM than do red drum (McGoogan and Gatlin, 1997), but their tolerance is similar to that of the above-mentioned marine fish species. In the present study, the diets were formulated to contain 43.7% protein and 14% lipid because this combination was found to be suitable for spotted rose snapper growth (Abdo de la Parra et al., 2010). The SGR observed (1.27%) was similar to SGR values recorded with the spotted rose snapper in previous works (Hernández et al., 2010a, 2010b), indicating that the experimental diets fed were supported normal weight gains.

Low growth and feed intake of fish fed high SBM diets may also be due to the presence of anti-nutritional factors (Peres et al., 2003; Refstie et al., 1998), low protein digestibility (Refstie et al., 1998) or a deficiency of the essential amino acids (Chong et al., 2003; Tantikitti et al., 2005). The SBM used in this experiment is a widely-used commercial product without further treatment. Because the protein digestibility of the experimental diets was not evaluated, the effects of any anti-nutritional factors and the digestibility of the protein on the growth and feed intake of spotted rose snappers are not known. However, digestibility evaluations of other feed ingredients using spotted rose snapper gave values similar to those reported for other marine species (Davis et al., 2005; Hernández et al., 2010a, 2010b). Methionine is often the limiting amino acid in SBM (Hertrampf and Piedad-Pascual, 2000), and methionine deficiencies in soy products have been observed in other studies (Chong et al., 2003; Chou et al., 2004). In this study, supplementing the diets containing 40 or 60% SBM with methionine (0.25%) did not prevent lower weight gain compared to fish fed the 0% or 20% SBM diet, suggesting that factors other than methionine level in the diet were responsible.

Blood parameter values of fish fed the different diets did not indicate any adverse effect on the spotted rose snapper's health. Blood parameter value levels were similar to those reported for clinically healthy snappers of the same species (Del Rio-Zaragoza et al., 2011). Similar to this study, studies in juvenile Japanese flounder

Table 3
Growth and feed efficiency of juvenile spotted rose snapper *L. guttatus* fed experimental diets for 85 days.

Diet	WI (g)	FW (g)	WG (g)	FI (g fish^{-1})	FCR	SGR (%day^{-1})	S (%)	PER	ANU (%)
D-0%	17.7 ± 0.04^{a}	50.42 ± 1.30^{ab}	32.67 ± 1.34^{ab}	41.69 ± 2.48^{ab}	1.28 ± 0.05^{ab}	1.23 ± 0.03^{ab}	97.78 ± 3.85^{a}	1.82 ± 0.07^{ab}	61.12 ± 6.85^{a}
D-20%	17.77 ± 0.02^{a}	52.45 ± 3.13^{a}	34.68 ± 3.11^{a}	42.31 ± 2.72^{a}	1.22 ± 0.04^{b}	1.27 ± 0.07^{a}	95.56 ± 7.70^{a}	1.90 ± 0.06^{a}	64.53 ± 2.50^{a}
D-40%	17.74 ± 0.05^{a}	48.12 ± 0.25^{b}	30.38 ± 0.20^{b}	37.71 ± 1.77^{b}	1.24 ± 0.05^{ab}	1.17 ± 0.00^{b}	97.78 ± 3.85^{a}	1.81 ± 0.08^{ab}	64.11 ± 3.15^{a}
D-60%	17.76 ± 0.02^{a}	43.22 ± 1.36^{c}	25.45 ± 1.34^{c}	33.46 ± 1.65^{c}	1.31 ± 0.01^{a}	1.05 ± 0.04^{c}	93.33 ± 11.55^{a}	1.73 ± 0.02^{b}	62.92 ± 3.50^{a}

Values in the same column with the same superscript are not significantly different ($P > 0.05$). a > b.
FW = Final weight; WG = Weight gain; FI = Feed intake; FCR = Factor conversion ratio; SGR = Specific growth rate; S = Survival; PER = Protein efficiency ratio; ANU = Apparent nitrogen utilization.

Table 4
Whole body composition of juvenile spotted rose snapper *L. guttatus* fed experimental diets for 85 days.

Diet	Moisture	Lipid	Ash	Protein
Initial	71.74 ± 0.15	5.79 ± 0.05	5.27 ± 0.03	16.00 ± 0.13
D-0%	68.21 ± 2.24^{ab}	9.53 ± 1.56^{a}	4.86 ± 0.15^{ab}	16.63 ± 0.73^{b}
D-20%	68.64 ± 0.20^{ab}	9.29 ± 0.24^{a}	4.63 ± 0.20^{b}	16.81 ± 0.12^{b}
D-40%	67.26 ± 0.72^{b}	9.26 ± 0.79^{a}	4.97 ± 0.15^{ab}	17.66 ± 0.08^{a}
D-60%	69.45 ± 0.10^{a}	7.22 ± 1.17^{b}	5.13 ± 0.50^{a}	17.63 ± 0.54^{a}

The values (mean ± SD, n = 3) with different superscripts denote significant differences between the treatments ($P<0.05$) according to the Duncan test.

(Kikuchi, 1999) and mangrove red snappers (*Lutjanus argentimaculatus*) (Abbas and Siddiqui, 2009) detected no marked differences in hematological values when the fish were fed diets containing 0%, 20 or 40% SBM, suggesting that these parameters are not useful indicators of diet quality as it relates to SBM level.

Feed intake of fish fed the D-40% and D-60% diets was reduced compared to that of fish fed the D-0% or diets presumably because of changes in palatability (Davis et al., 1995; Meilahn et al., 1996; Reigh and Ellis, 1992) as a consequence of the replacement of FM by SBM. The diet formulations included krill meal, which is often used to improve palatability and feed intake of fish fed low FM diets containing plant proteins (Kubitza and Lovshin, 1997; Moura et al., 2000). However, krill supplementation did not restore feed intake to the level of the FM diet-fed fish in this study, thereby reducing weight gain. Many studies show that feed intake is reduced when fish such as red drum or Asian seabass are fed diets in which most or all FM is replaced with SBM (Boonyaratpalin et al., 1998; Chong et al., 2003; Davis et al., 1995; Reigh and Ellis, 1992; Tantikitti et al., 2005). In this experiment, spotted rose snappers showed acceptance of diets containing up to 20% inclusion of SBM, consistent with the conclusion that taste is an important factor in the consumption of diets based on SBM (Chou et al., 2004; Robaina et al., 1995; Wang et al., 2006).

Spotted rose snappers fed the 60% SBM diet (D-60%) had lower fat content. A similar result was observed in cyprinid fish *Barbodes altus* (Elangovan and Shim, 2000). The low content of fat, the high moisture and ash content in the final carcass of fish fed high SBM-content diets are likely the result of reduced feed intake and weight gain of these fish.

In summary, the results showed that commercial SBM appeared to be a good feed ingredient for spotted rose snapper *L. guttatus* in terms of growth performance, nutrient composition, and blood parameter value levels as long as dietary levels remain 20% or less. These results may serve to aid in the formulation of cost-effective diets for spotted rose snapper and as a basis to explore ways to improve the palatability of diets containing SBM.

Table 5
Hematological parameters of spotted rose snapper *L. guttatus* fed experimental diets for 85 days.

Parameter	Diet			
	D-0%	D-20%	D-40%	D-60%
Hematocrit (%)	51.3 ± 5.2^{a} (9)	54.2 ± 3.34^{a} (9)	51.8 ± 5.1^{a} (9)	50.7 ± 5.8^{a} (9)
Hemoglobin (g dL^{-1})	15.2 ± 1.9^{a} (10)	15.1 ± 1.3^{a} (10)	14.8 ± 2.5^{a} (10)	15.4 ± 1.7^{a} (10)
CHCM (g dL^{-1})[a]	29.6 ± 5.3^{a} (9)	28.1 ± 2.4^{a} (9)	28.7 ± 5.7^{a} (9)	30.1 ± 3.5^{a} (9)
Total protein (g L^{-1})	58.3 ± 3.9^{a} (10)	61.8 ± 17.5^{a} (10)	59.6 ± 21.4^{a} (10)	54.7 ± 3.2^{a} (10)
Triglycerides (mg dL^{-1})	283.4 ± 120.8^{a} (10)	314.1 ± 98.4^{a} (10)	370.1 ± 90.1^{a} (5)	455.9 ± 55.3^{b} (7)
Glucose (mg dL^{-1})	79.6 ± 12.1^{a} (9)	74.9 ± 11.9^{a} (9)	76.7 ± 10.8^{a} (5)	73.7 ± 58.9^{a} (6)

The values (mean ± SD with *n* in parentheses) with different superscripts denote significant differences between the treatments ($P<0.05$) using evidence from the Duncan test.

[a] MCHC = [(Concentration of hemoglobin × 100)/(% microhematocrit)].

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