Lat. Am. J. Aquat. Res., 42(1): 111-120, 2014
DOI: 103856/vol42-issue1-fulltext-8

Research Article

Replacement of fish meal by poultry by-product meal, food grade, in diets for juvenile spotted rose snapper (*Lutjanus guttatus*)

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ABSTRACT. The feasibility of replacing fish meal protein at different levels with poultry by product meal food grade (PBM-FG) was assessed in diets for spotted rose snapper *Lutjanus guttatus*. Four diets were formulated, the control diet fish meal was used as the main protein source (FM); the other three diets had increasing levels of PBM-FG replacing 25, 50 or 75% of the fish meal protein respectively. The diets were fed close to apparent satiation, three times a day to quadruplicate groups of juvenile snapper (average body weight 11.0 ± 0.04 g). The fish were randomly distributed into groups of 15 fish in a 120 L seawater tank. The response of snapper to diets containing graded levels of fish meal was evaluated by measuring weight gain, feed efficiency, body composition, hematological parameters and apparent nutrient digestibility during a 12-week period. The replacing of the 25% of fish meal protein by PBM-FG did show a similar trend for feed efficiency and growth performance than control diet. Feed efficiency and growth performance was reduced at 75% level of fish meal protein replacement by PBM-FG, due to deficiencies of lysine and methionine. The final whole-body proximate composition did not differ among treatments. The hematological characteristics were similar among the treatments control, 25 and 50%, but the fish fed PBM-FG 75% showed the lowest levels for total protein and glucose parameters. The dietary dry matter and protein apparent digestibility coefficients (ADCs) decreased with increasing dietary PBM-FG. High values for lipid ADCs were observed in all diets.

Keywords: artificial diets, hematological characteristics, amino acids, digestibility, aquaculture.

Reemplazo de la harina de pescado por harina de subproducto de ave, grado alimento, en dietas para juveniles de pargo lunarejo (*Lutjanus guttatus*)

RESUMEN. La factibilidad de la sustitución de la proteína de harina de pescado por proteína de ave, grado alimento (PBM-FG), fue evaluada en dietas para pargo lunarejo *Lutjanus guttatus.* Se formularon cuatro dietas, en la dieta control la harina de pescado (FM) se utilizó como principal fuente de proteína. Las otras tres dietas tenían crecientes niveles de PBM-FG en reemplazo del 25, 50 y 75% de la proteína de FM respectivamente. Las dietas fueron ofrecidas a saciedad aparente, tres veces al día a grupos de cuatro réplicas de juveniles (peso corporal promedio 11,0 ± 0,04 g). Los peces fueron distribuidos aleatoriamente en grupos de 15 peces en estanques de 120 L de agua de mar. La respuesta del pargo a las dietas, contenidas con niveles graduales de harina de pescado, fue evaluada midiendo la ganancia en peso, eficiencia del alimento, composición corporal, parámetros hematológicos y digestibilidad aparente de nutrientes durante un periodo de 12 semanas. El reemplazo del 25% de la proteína de FM por PBM-FG mostró una tendencia similar para la eficiencia del alimento y el desempeño productivo que la dieta control. La eficiencia del alimento y el rendimiento productivo fueron reducidos a un nivel de 75% de reemplazo de la proteína de HP por la PBM-FG, debido a las deficiencias de lisina y metionina. La composición proximal del cuerpo completo no difirió entre los tratamientos. Las características hematológicas fueron similares entre los tratamientos control, PBM-FG25%, y PBM-FG 50%, pero los peces alimentados con PBM-FG 75% mostraron niveles más bajos para los parámetros de proteína total y glucosa. Los coeficientes de digestibilidad aparente (ADCs) de materia seca y de proteína en la dieta disminuyeron con el incremento de PBM-FG en la dieta. Se observaron valores altos para ADCs de lípidos en todas las dietas.

Palabras clave: dietas artificiales, características hematológicas, aminoácidos, digestibilidad, acuicultura.

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INTRODUCTION

The snapper family is an important fisheries resource in tropical and subtropical areas, and most snapper fisheries are being harvested at or beyond their maximum sustainable yield (Stickney, 2000). The spotted rose snapper *Lutjanus guttatus* is found along the Pacific coast from the Gulf of California to Peru, including the Galapagos Islands (Allen, 1995). It is an economically important artisanal fishery along the northwest coast of Mexico. According to official statistics, snapper landings totaled 10,478 ton in 2011 and reached a price of US$ 8-10 kg^{-1} (CONAPESCA, 2011). The market demand for snapper is high. In recent years, there has been increasing interest in the commercial grow-out of snapper in marine cages. Cultured fish are fed with farm-made feeds consisting primarily of fish and shrimp processing by-products, because there is no commercial feed available for this species that are suitable for the grow-out of wild-sourced juveniles.

Protocols for reproduction, larval rearing and juvenile culture have been developed and tested for the snapper in captivity (Ibarra-Castro & Alvarez-Lajonchere, 2011). Several studies on the biology fo the snapper and other Lutjanidae have shown that the members of this family are carnivorous, feeding primarily on fish and benthic crustaceans (Allen, 1995; Rojas, 1997; Thomson *et al*., 2000).

L. guttatus requires protein and lipid values ranging from 45 to 50% and 9 to 15%, respectively, and its diet is mainly based on fish meal as the dietary protein source (Abdo de la Parra *et al*., 2010a). The limited fish meal supply, coupled with the increasing demand for fish meal, has greatly inflated the cost of this commodity (Tacon & Metian, 2008). Therefore, it is a priority to design growth-promoting diets that combine cost-effectiveness with low dependence on fish meal as the primary protein source.

Poultry by-product meals (PBMs) are rendered byproducts from the poultry processing industry. The PBMs made in the United States are generally consistent in freshness, quality and digestibility. Mexico imports PBM with two quality grades defined by ash content. Of the two available grades, low-ash PBM is more suitable for the diet of carnivorous fish species, such as the snapper, due to its palatability, high protein content, total digestible dry matter, digestible protein and similar energy content compared to fish meal (Bureau *et al*., 1999; Zhou *et al*., 2004; NRC, 2011), making this ingredient a cost-effective and valuable protein source for several marine species (El-Sayed, 1994; Quartararo *et al*., 1998; Nengas *et al*., 1999; Kureshy *et al*., 2000; Wang *et al*., 2006; Yigit *et al*., 2006; Shapawi *et al*., 2007).

In this sense, some studies suggests, that fish meal may be partially replaced by PBM, consequently providing a substantial savings in feed cost without a decrease in performance.

The primary objective of the present research was to evaluate the iso-nitrogenous replacement of fish meal with PBM-FG in practical diets for the juvenile snapper by measuring weight gain, feed efficiency, body composition, hematological parameters and apparent nutrient digestibility.

MATERIALS AND METHODS

Fertile eggs were obtained from batch laboratory breeding fish reproduction at CIAD Mazatlan. Females were induced according to the protocol proposed by Ibarra-Castro & Alvarez-Lajonchere (2011). Snapper juveniles were produced in a pilot-scale hatchery at CIAD Mazatlan, Mexico according to the protocols established by Abdo de la Parra *et al*. (2010b). The fish were randomly distributed at a stocking density of 15 fish (weight 11.0 ± 0.15 g) per tank among fifteen tanks (volume 120 L). 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 the tanks to be cleaned. Each tank had supplemental aeration and a continuous flow of sea water at a rate of 1.5 L min^{-1}. Seawater was pumped from the seashore, passed through two parallel sand filters and delivered to four 25 m^3 high-density polyethylenes (HDPE) head/sedimentation tanks (4 x 15 m). From the head tanks, the seawater was pumped through a double parallel filtration system consisting of a pressured sand filter (265 Lpm, 100 μm relative particle retention) and multiple a filter room (Alvarez-Lajonchere *et al*., 2007).

Fish rearing and feeding

The feed was supplied by hand three times a day (09:00, 13:00 and 16:00) until apparent satiation was reached. Uneaten food was collected from the bottom of the tank with a siphon 30 min after the onset of feeding and was dried in an oven at 60°C. The feed intake was calculated as the amount of feed supplied minus the amount of unconsumed feed.

The water temperature was maintained at 21 ± 2°C; the dissolved oxygen level ranged between 5 and 6 mg L^{-1}, the salinity was 34.6 ± 0.4 g L^{-1} and the pH was 8.1 ± 0.3.

The fish were weighed every two weeks to calculate their mean body weight and the biomass in

each tank. The fish were caught with scoop nets and anesthetized with 2-phenoxyethanol (Sigma®, St. Louis, MO, USA) at a concentration of 0.3 mL L^{-1}. The fish were weighed individually on a digital scale (accurate to ± 0.01 g).

The growth and feed efficiency of the fish were assessed by calculating the weight gain (WG), feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR), survival (SR), protein efficiency ratio (PER), apparent nitrogen utilization (ANU), and the economic performance of the diets was calculated from the method of Vincke (1969) as PI = Profit index as follows:

WG = final mean weight (g) - initial weight (g)

FI = $\sum_i n$[(total feed consumption (g)) / (number of fish)] / number of days

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

SGR = [(Ln final weight- Ln initial weight) / number of days] x 100

SR = (final number / initial number) x 100

PER = weight gain / protein intake

ANU = [(final body protein - initial body protein) / protein intake] x 100

PI = value of fish (kg) / cost of feed (US$)

Experimental design

Four isonitrogenous (50% crude protein) and isolipidic (15% crude lipid) diets were formulated to replace 0% (PBM-FG 0), 25% (PBM-FG25), 50% (PBM-FG50) or 75% (PBM-FG75) of the protein contributed by fish meal with PBM-FG.

All diets had constant inclusion levels of squid meal, krill meal, wheat gluten, carotenoids, antioxidants, soy lecithin, pre-mixtures of minerals and vitamins. Sodium alginate was used as binder, and corn dextrin was used to adjust the total to 100%. The diets were supplemented with chromic oxide (0.5%) as an indigestible marker to obtain the apparent digestibility coefficient (ADC) (Table 1).

Chemical analysis

Ten randomly chosen fish were sampled from the initial population to determine the initial 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. The fish samples, meals and diets were homogenized and dried at 105°C for 24 h prior to the chemical analyses. The moisture, protein, lipid and ash levels in the test ingredients, diets, carcasses and fecal samples were determined using standard methods (AOAC, 1995). The samples were homogenized and dried at 105°C for 24 h prior to the chemical analyses. The level of crude protein was determined by the Dumas combustion method (Ebling, 1968) using a Leco FP-528 nitrogen analyzer (LECO Instrument Corporation, St. Joseph, MI, USA). The lipid content was analyzed using a micro Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden) after extraction with petroleum ether. The ash content was determined by calcination of the samples in a muffle furnace at 550°C (Fisher Scientific International, Inc. Pittsburgh, PA, USA). The gross energy content was measured by combustion in a Parr bomb calorimeter 1241 (Parr, Instrument Company, Moline, IL, USA) using benzoic acid as the standard. The amino acid composition of the experimental diets was quantified following Vázquez-Ortiz *et al.* (1995) with high performance liquid chromatography (HPLC, Varian 9012, Walnut Creek, CA, USA) with a Microsorb guard column (4.5 x 30 mm) packed with octadecylsilane and a C18 Microsorb Short 3-m column (4.6 x 100 mm). Standards were used for the amino acids, and α-aminobutyric acid was added as an internal standard. Given the contents of essential amino acids (EAA), the chemical score of each diet: PBM-FG 0, PBM-FG25, PBM-FG50 and PBM-FG75 were computed as in Vidotti *et al.* (2003), considering essential amino acids in the body composition. In brief, the chemical score was calculated using the following relation:

$$\text{Chemical score} = \frac{\text{EAA in diet protein } (\text{g } 100\ \text{g}^{-1})}{\text{EAA in body composition protein } (\text{g } 100\ \text{g}^{-1})}$$

Collection of blood samples

At the end of the feeding experiment, the fish were carefully handled to minimize stress. The fish were anesthetized with 0.3 mL L^{-1} of 2-phenoxyethanol and in less than 3 min, blood samples were collected from the caudal vein using 1 mL non-anticoagulant insulin syringes (Terumo Mexico DF, Mexico). Ten fish were selected randomly from each experimental diet. A volume of 400 µL of blood was extracted. The blood sample was divided into 200 µL samples and placed into two Eppendorf tubes. The first tube, with no anticoagulant, was immediately centrifuged for 10 min, and the serum was stored in a -20°C freezer for further analysis of the total protein concentration, glucose and triglyceride levels. The second tube included K_2 EDTA (BD Microtainer, Franklin Lakes, NJ, USA) to prevent coagulation. This tube was used to determine the hematocrit and hemoglobin concentrations.

Hematological analysis

To calculate the blood hematocrit, the tube was placed for 10 min in a microhematocrit centrifuge (SOL-BAT

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

Ingredients*	Diet (g kg^{-1} wet weight)			
	PBM-FG0	PBM-FG25	PBM-FG50	PBM-FG75
Fish meal[a]	52.60	39.45	29.71	18.27
Poultry by-product meal[b]	0.00	15.11	30.63	45.94
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	7.91	6.47	5.31
Dextrin[d]	17.47	15.88	11.54	8.83
Alginate[d]	3.00	3.00	3.00	3.00
Wheat gluten[e]	2.00	2.00	2.00	2.00
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
Chromic oxide	5.00	5.00	5.00	5.00
Proximate analysis (g kg^{-1} dry weight)[h]				
Crude protein	51.3	51.5	51.7	52.0
Crude lipid	15.7	16.1	15.7	15.4
Ash	13.4	12.9	12.5	12.3
NFE[i]	19.6	19.5	20.1	20.2
Gross energy (kJ g^{-1})[j]	21.6	21.7	21.7	21.7
P/E	23.7	23.6	23.7	23.9

[a]Premium" grade fish meal was obtained from Selecta de Guaymas, S.A. de C.V. Guaymas, Sonora, México. [b]Proteínas marinas y Agropecuarias, S.A. of C.V., Guadalajara, Jalisco, México. [c]PROAQUA, S.A. de C.V. Mazatlán, Sinaloa, México. [d]Droguería Cosmopolita, S.A. de C.V. México D.F., México. [e]Sigma-Aldrich Chemical, S.A. de C.V. Toluca, Mexico. [f]Trouw Nutrition México S.A. de C.V. (by courtesy). *Vitamins 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, 80 mg; Biotin, 500 mg; Vitamin C, 100.00 g; Choline 300.00 g, Excipient c.b.p. 2,000.00 g. **Mineral premix composition: Manganese, 100.00 g; Magnesium, 45.00 g; Zinc, 160.00 g; Iron, 200.00 g; Copper, 20.00 g; Iodine, 5 g; Selenium,400.00 mg; Cobalt 600.00 mg. Excipient c.b.p. 1,500.00 g. [g]DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, México. [h]mean ± SD, number of determinations = 3. [i]Nitrogen-free extract = 100 - (% protein + % lipid + % ash).

P600, Mexico DF, Mexico). The packed cells were measured using a hematocrit reader and reported as a percentage (Del Rio-Zaragoza *et al*., 2008). The hemoglobin concentration in the erythrocytes was determined using the cyanmethemoglobin method (HemogloWiener reactive, Wiener Lab) following the manufacturer's instructions. The total protein concentration, glucose and triglyceride levels were determined using commercial kits supplied by Randox and Biosystem Laboratories LTD (Admore, Diamond Road, Crumlin, Co. Antrim, United Kingdom). The mean corpuscular hemoglobin concentration (MCHC) was determined with a standard formula using the values for the hemoglobin concentration and the microhematocrit percentage (Del Rio-Zaragoza *et al.,* 2008).

Apparent digestibility coefficient

The digestibility for each experimental diet was determined using three replicates (tanks) per treatment, with 20 fish (average body weight 70 ± 5 g) per replicate. The fish were adapted to the experimental diets for 15 days before the collection of feces. The digestibility trial conditions were similar to those used for the growth trial.

Before beginning the fecal collections, preliminary tests were conducted to determine the length of time needed for optimal fecal production. The results of the

preliminary feeding tests indicated that maximal fecal production occurred 3-5 h after feeding.

Fecal material was collected weekly for 8 weeks. To acclimate the fish to the feeding and handling practices, the fish were fed to apparent satiation on collection days at 09:00, 11:00 and 13:00 h with the assigned diets.

To prevent leaching losses, fecal material was manually stripped 3.5-4 h after feeding by applying pressure to the lower abdominal region, resulting in the expression of fecal matter onto a slightly inclined, flat sheet of labeled aluminum foil. Care was taken to exclude urine, mucus and water from the fecal samples (Rawles & Gatlin, 2000; Glencross *et al*., 2005; Rawles, 2010).

Prior to stripping, the area around the anus was gently dried with a towel, and the fish was anesthetized with 0.3 mL L^{-1} of 2-phenoxyethanol and allowed to recover and being returned to the culture tank. The fecal samples from individual fish were pooled by tank and stored at -20°C. The samples were freeze-dried for a minimum of 48 h prior to chemical analysis.

The ADC for each experimental diet was calculated as the ratio of nutrients and markers in the feed and feces (Maynard & Loosli, 1969):

ADC dry matter (%) = 100 - [(Cr_2O_3 in feed / %Cr_2O_3 in feces) x 100];

ADC nutrients (%) = 100 - 100[(%Cr_2O_3 in feed / %Cr_2O_3 in feces) x (% nutrient in feces / % nutrient in feed)].

Statistical analysis

The data for each parameter were tested for normality and homoscedasticity. A one-way analysis of variance was performed with diet as the independent variable. A Tukey's HSD test was used for post hoc identification of significant differences among the dietary treatment groups at a significance level of 5% (Zar, 1984). All of the statistical procedures were performed using the SigmaPlot 12.0 software package.

RESULTS

Amino acids composition of fish and experimental diets

The essential amino acid (EAA) profile of the experimental diets showed lower values compared to levels in the basal diet (Table 2). The EAA profile content progressively decreased as PBM-FG inclusion levels increased. The chemical score of diets PBM-FG25, PBM-50, PBM-FG 75 showed low chemical scores for glycine, histidine, lysine, isoleucine, methionine and phenylalanine, which therefore become limiting o deficient in these amino acids (Table 2).

Growth performance and feed utilization

Survival ranged between 97.8-100% for all diets ($P > 0.05$). Food intake exhibited significant differences between treatments ($P < 0.05$). The FCR values did not differ significantly among the diets containing PBM-FG ($P > 0.05$). The dietary treatments with a PBM-FG inclusion level of 0 and 25% (PBM-FG0 and PBM-FG25) produced the best results for weight gain (WG), feed intake, specific growth rate (SGR), protein efficiency ratio (PER) and apparent nitrogen utilization (ANU); however, the fish fed with diets higher in PBM-FG (PBM-FG50 and PBM-FG75) produced the lower growth performance than control diet ($P > 0.05$) (Table 3).

Whole-body composition

The whole-body proximate composition of the fish is shown in Table 4. There were no significant differences ($P > 0.05$) in moisture, protein, ash or lipids in the whole-body composition of the fish fed different diets.

Hematological analysis

The measured blood parameters did not differ significantly among treatments, except glucose ($P < 0.05$). Fish fed PBM-FG 75% showed the lowest levels for almost all hematological parameters. There were similar triglyceride levels in the fish fed the overall dietary treatment ($P > 0.05$, Table 5).

Apparent digestibility coefficients

The ADCs of the experimental diets are shown in Table 6. The replacement of FM by PBM-FG affected the digestibility of protein, dry matter and energy ($P < 0.05$). Significantly higher protein, energy, dry matter and lipid digestibility coefficients were observed for the PBM-FG0, PBM-FG25 and PBM-PG50 diets ($P < 0.05$). The protein, energy, dry matter and lipid coefficients for the PBM-FG75 diet were significantly lower than those observed for the other diets ($P < 0.05$).

DISCUSSION

This study provides useful information regarding the replacement of fish meal in diets for snapper and the potential utilization of PBM-FG as an alternative ingredient. Likewise, PBM-FG could potentially

Table 2. Concentrations of essential amino acids (g AA per 100 g of protein) in body composition of *L. guttatus* diets containing different levels of poultry by-products meal feed-grade (PBM-FG).

Amino acid	Body composition	Diet				Chemical score			
		PBM-FG0	PBM-FG25	PBM-FG50	PBM-FG75	PBM-FG0	PBM-FG25	PBM-FG50	PBM-FG75
Alanine	7.1 ± 0.22	7.4 ± 0.05	7.2 ± 0.20	6.8 ± 0.04	6.3 ± 0.27	1.04	1.01	0.96	0.89
Arginine	5.9 ± 0.09	7.9 ± 0.52	7.7 ± 0.75	6.3 ± 0.03	6.0 ± 0.11	1.34	1.31	1.07	1.02
Aspartic acid	9.3 ± 0.06	8.9 ± 0.03	7.6 ± 0.08	7.2 ± 0.47	7.0 ± 0.52	0.96	0.82	0.77	0.75
Glutamic acid	13.1 ± 0.75	15.3 ± 0.56	14.5 ± 0.27	13.1 ± 0.08	12.0 ± 0.25	1.17	1.11	1.00	0.92
Glycine	9.8 ± 0.23	8.9 ± 0.27	9.1 ± 0.35	8.1 ± 0.76	8.2 ± 0.60	0.91	0.93	0.83	0.84
Histidine	1.9 ± 0.45	2.2 ± 0.06	1.7 ± 0.06	1.5 ± 0.45	1.4 ± 0.73	1.16	0.89	0.79	0.74
Isoleucine	4.3 ± 0.12	5.0 ± 0.71	4.6 ± 0.77	4.3 ± 0.02	4.1 ± 0.42	1.16	1.07	1.00	0.95
Leucine	7.0 ± 0.05	8.6 ± 0.91	8.6 ± 0.45	8.7 ± 0.51	9.3 ± 0.04	1.23	1.23	1.24	1.33
Lysine	6.0 ± 0.26	7.7 ± 0.90	7.0 ± 0.95	5.8 ± 0.66	5.8 ± 0.82	1.28	1.17	0.97	0.97
Methionine	2.4 ± 0.43	2.4 ± 0.48	2.3 ± 0.78	2.0 ± 0.82	2.0 ± 0.55	1.00	0.96	0.83	0.83
Phenylalanine	5.4 ± 0.07	5.2 ± 0.55	4.9 ± 0.03	5.1 ± 0.57	5.5 ± 0.93	0.96	0.91	0.94	1.02
Serine	1.4 ± 0.09	3.6 ± 0.35	4.2 ± 0.42	4.5 ± 0.82	4.9 ± 0.08	2.57	3.00	3.21	3.50
Threonine	2.3 ± 0.29	4.4 ± 0.27	4.2 ± 0.56	4.1 ± 0.92	3.3 ± 0.61	1.91	1.83	1.78	1.43
Tyrosine	1.5 ± 0.56	2.6±0.48	2.5 ± 0.32	2.7 ± 0.07	2.9 ± 0.95	1.73	1.67	1.80	1.93
Valine	4.9 ± 0.89	5.6 ± 0.03	5.6 ± 0.45	5.6 ± 0.57	5.5 ± 0.61	1.14	1.14	1.14	1.12

Tryptophan was not determined by the analytical method used.

Table 3. Growth and feed efficiency of spotted rose snapper *L. guttatus* that were fed the experimental diets containing graded levels of poultry by-product feed grade meal for 83 days. Values are the mean ± SD of four replicates (n = 4 tanks per diet)[1]. IBW: initial body weight, FBW: final body weight, WG: weight gain, FI: feed intake, FCR: factor conversion ratio, SGR: specific growth rate, SR: survival rate, PER: protein efficiency ratio, PI: Profit index.

	Diet				
Variables	PBM-FG0	PBM-FG25	PBM-FG50	PBM-FG75	SEM[2]
IBW (g)	10.99 ± 0.00^{a}	11.02 ± 0.02^{a}	11.01 ± 0.02^{a}	11.00 ± 0.02^{a}	0.01
FBW (g)	36.43 ± 1.82^{a}	36.17 ± 2.52^{a}	32.740.68^{ab}	31.350.73^{b}	3.22
WG (g)	25.44 ± 1.82^{a}	25.14 ± 2.52^{a}	21.72 ± 0.68^{ab}	20.34 ± 0.73^{b}	1.46
SGR (% day^{-1})	1.44 ± 0.06^{a}	1.43 ± 0.09^{a}	1.31 ± 0.02^{ab}	1.26 ± 0.03^{b}	0.05
FI (g fish^{-1})	31.16 ± 1.03^{a}	30.07 ± 1.55^{ab}	27.08 ± 0.79^{c}	27.21 ± 0.87^{bc}	1.10
FCR	1.23 ± 0.05^{a}	1.20 ± 0.06^{a}	1.24 ± 0.04^{a}	1.33 ± 0.08^{a}	0.06
PER	1.71 ± 0.07^{a}	1.72 ± 0.09^{a}	1.55 ± 0.05^{ab}	1.38 ± 0.09^{b}	0.07
SR (%)	97.80 ± 3.85^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	1.92
ANU (%)	55.70 ± 4.81^{a}	52.31 ± 1.06^{a}	44.97 ± 0.78^{b}	40.82 ± 2.03^{b}	2.47
PI (US $)[3]	4.76	5.39	6.11	7.12	

[1]Means in the same row that are followed by the same superscript letter are not significantly different ($P > 0.05$).
[2]Standard error of the mean.
[3] Price of 1 kg of fish is fixed at US$ 8.

substitute up to 50% of FM protein without reducing fish performance, but higher substitutions may affect the protein and energy digestibility.

PBM meal may have differing constituents (*e.g.*, bone, meat and blood), nutrient compositions, processing methods and digestibility (Nengas *et al*., 1999; Webster *et al*., 2000). If high-quality PBM is used, many species tolerate replacement levels up to 100%. Steffens (1994) reported that poultry offal meal could be successfully used as the sole animal protein source in the diet of salmonids provided that amino acid supplementation was included.

In this study, the use of PBM-PG reduced the inclusion of fish meal dietary protein from 52.6% to

Table 4. Whole body composition of juvenile spotted rose snapper *L. guttatus,* that were fed experimental diets for 83 days[1].

Diet	Moisture	Lipid	Ash	Protein
Initial	67.79	4.95	5.07	16.21
PBM-FG0	69.7 ± 0.85[a]	6.93 ± 1.02[a]	5.23 ± 0.23[a]	17.83 ± 1.06[a]
PBM-FG25	69.6 ± 1.51[a]	6.19 ± 3.83[a]	5.0 ± 0.33[a]	17.41 ± 1.34[a]
PBM-FG50	70.6 ± 1.23[a]	6.52 ± 2.53[a]	5.5 ± 0.08[a]	17.15 ± 0.16[a]
PBM-FG75	70.1 ± 1.48[a]	6.33 ± 8.88[a]	5.2 ± 076[a]	17.02 ± 0.32[a]
SEM[2]	0.86	1.72	0.21	0.87

[1]The values in the same column that are followed by the same superscript letter are not significantly different ($P > 0.05$).
[2]Standard error of the mean.

Table 5. Hematological parameters of spotted rose snapper *L. guttatus* fed experimental diets for 83 days[1].

	Diet				
	PBM-FG0	PBM-FG25	PBM-FG50	PBM-FG75	SEM[3]
Hematocrit (%)	44.33 ± 4.8[a]	44.50 ± 2.9[a]	44.37 ± 4.5[a]	46.50 ± 4.7[a]	4.23
Hemoglobin (g dL^{-1})	9.32 ± 2.20[a]	9.71 ± 1.36[a]	9.82 ± 0.85[a]	8.95 ± 1.2[a]	1.48
CHCM (g dL^{-1})[2]	51.60 ± 5.2[a]	48.31 ± 3.4[a]	46.94 ± 6.3[a]	46.58 ± 8.7[a]	4.22
Total protein (g L^{-1})	51.60 ± 3.2[a]	48.31 ± 3.71[a]	46.94 ± 2.32[a]	46.58 ± 2.46[a]	0.91
Triglycerides (mg dL^{-1})	212.22 ± 46.8[a]	189.87 ± 37.2[a]	203.44 ± 57.7[a]	243.96 ± 47.55[a]	47.86
Glucose (mg dL^{-1})	76.91 ± 9.20[a]	65.88 ± 0.47[ab]	61.98 ± 13.47[ab]	39.79 ± [b]	9.91

[1]The values (mean ± SD with n in parentheses) with different superscripts denote significant differences between the treatments ($P < 0.05$) using evidence from the Tukey test.
[2]CHCM = [(Concentration of hemoglobin x 100) / (% microhematocrit)].

Table 6. Coefficients of apparent digestibility of protein, lipids, energy and dry matter of the experimental diets for juvenile spotted rose snapper *L. guttatus*; diet of fish meal (PBM-FG0) and diets based on poultry products (PBM-FG0, PBM-FG25, PBM-FG50 and PBM-FG75)[1]. SEM: standard error of the mean.

Diet	Protein	Lipid	Energy	Dry matter
PBM-PG0	86.70 ± 0.47[a]	97.73 ± 0.28[ab]	82.65 ± 0.64[a]	79.43 ± 0.71[a]
PBM-PG25	82.80 ± 1.76[a]	97.92 ± 0.14[ab]	82.34 ± 1.12[a]	80.64 ± 2.16[a]
PBM-PG50	74.52 ± 1.55[b]	97.36 ± 0.19[a]	77.65 ± 1.38[b]	72.98 ± 1.65[b]
PBM-PG75	57.97 ± 2.63[c]	98.22 ± 0.29[b]	65.17 ± 1.98[c]	58.36 ± 2.34[c]
SEM	1.77	0.23	1.36	1.82

[1]Values in the same column with the same superscript are not significantly different ($P > 0.05$).

18.27% without compromising the health or growth performance of the snapper, showing a capacity similar to that of other species, such as the red sea bream, gilthead seabream and humpback grouper, when fed diets with a high inclusion of PBM-FG. Likewise, freshwater species such as tilapia, gibel carp and mahseer were able to digest a diet with up to 100% inclusion of PBM without a significant reduction in growth performance (Nengas *et al*., 1999; Takagi *et al*., 2000; Yang *et al*., 2006; Shapawi *et al*., 2007; Ismail *et al*., 2012).

The findings of this study are in agreement with the conclusions of previous research studies indicating that PBM could be successfully applied at 25% for silver seabream (El-Sayed, 1994), 21% for Australian snapper (Quartararo *et al*., 1998), 14% for red drum (Kureshy *et al*., 2000) and 45% for cobia *Rachycentron canadum* (Zhou *et al*., 2011). The reduction in digestibility of the snapper fed PBM-FG may be due to: i) limited amino acid content (histidine, methionine, isoleucine, lysine and phenylalanine) (Tacon & Jackson, 1985; Nengas *et al*., 1999) and ii) feathers,

connective tissue and skin in the PBM, which are considered difficult for fish to digest (Hassan *et al*., 1997).

The reduced nutrient digestibility found in the present study is closely related to the deficiencies of essential amino acids in experimental diets. The amino acid contents of the experimental diets are lower than the muscle amino acid values. This result is consistent with findings of Shapawi *et al*. (2007), who showed that dry matter and apparent protein digestibility coefficients of diets decreased with increasing dietary PBM, due to the limited content of lysine and methionine. Zhou *et al*. (2004) reported that the cobia *R. canadum* fed PBM-based diets showed higher values (90.5%) of energy ADC with PBM than those in the present study. Likewise, Bureau *et al*. (1999) found higher energy ADC values (87%) for the rainbow trout *Oncorhynchus mykiss*.

The replacement of fish meal with PBM in the gilthead seabream has been found to produce low SGR values, and similar results have been reported in the turbot and humpback grouper (Nengas *et al*., 1999; Yigit *et al*., 2006; Shapawi *et al*., 2007).

However, the quantitative amino acid requirement for the snapper is unknown. Further information may be required in the future to define the essential amino acid requirements and the potential interactions among amino acids.

Nevertheless, it is well known that the total protein, hemoglobin, hematocrit and red blood cell concentration may be used to evaluate the physiological state and immune response of fish (De Pedro *et al*., 2005). In particular, high hemoglobin and red blood cell counts can serve to indicate good health (Zhou *et al*., 2005). In the present study, the blood parameter values of fish receiving the different diets indicated that the condition and health status were considered normal for fish fed food-grade PBM diets (Del Rio-Zaragoza *et al*., 2011). This study showed that the body composition of the snapper was not affected by the replacement of fish meal with PBM meals. In contrast, previous findings have shown that the fat content of trout fed PBM was higher than that fed on a control diet (Steffen, 1994).

Economic evaluation of the feeding trials after 83 days (Table 3) showed that replacing FM with food grade PBM lowered the cost of diets, therefore, the profit indices of the fishes fed PBM-FG diets increased. Based on the economic performance of the snapper fed with the experimental diets, the replacement of FM with PBM-FG is recommended.

In conclusion, the results of the present study showed that in the snapper, up to 50% of the fish meal protein in formulated diets can be replaced by PBM food grade, without any negative effects on health and growth performance. The present study represents the first research conducted on the nutritional capacity of the snapper and may serve as a basis for future studies.

ACKNOWLEDGMENTS

This research was co-funded by FORDECYT Project No. 147325: "Development of technology for on-growing snapper in floating cages: a productive alternative to the shores of the Mexican northwest" and SAGARPA-CONACYT Project No. 164673: "Development of formulated feeds nutritionally efficient for profitable culture of fish". Any mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not indicate a recommendation or endorsement by CIAD, A.C.

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Received: 11 May 2013; Accepted: 11 December 2013

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