

*Research Article*

## Partial replacement of fishmeal with meat and bone meal and tuna byproducts meal in practical diets for juvenile spotted rose snapper *Lutjanus guttatus*

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**ABSTRACT.** A 120 days feeding trial was conducted to evaluate diets in which fish meal (FM) was replaced with meat and bone meal (MBM) or tuna byproduct meal (TBM) on growth performance, apparent digestibility and hematological parameters of juvenile spotted rose snapper (SRS) *L. guttatus*. Three isonitrogenous compounds (47.6-49.0%) and isoenergetic (20.9-22.9 kJ g<sup>-1</sup>) diets were formulated. A control diet contained FM as a main protein source (D-FM) and two diets with 35% of fish meal protein replaced by MBM or TBM protein (D-MBM, D-TBM). Each diet was fed to triplicate groups of 20 SRS juvenile (initial weight 8.2 ± 0.02 g) to apparent satiation three times a day. Growth performance, hematological parameters and apparent digestibility of SRS fed D-MBM or D-TBM diets were not significantly different from D-FM diet. However, the whole body crude protein was significantly higher in D-MBM group than D-TBM group, and the values were comparable to D-FM group. Based on these results, the meat and bone meal is an economical and viable option, as tuna byproduct meal in practical diets for juvenile spotted rose snapper.

**Keywords:** *Lutjanus guttatus*, snapper, growth, animal protein, aquaculture.

## Reemplazo parcial de la harina de pescado con harina de carne y hueso, y harina de subproductos de atún en dietas para juveniles de pargo lunarejo *Lutjanus guttatus*

**RESUMEN.** Se realizó un experimento de alimentación durante 120 días para evaluar dietas en las cuales la harina de pescado (FM) fue reemplazada por la harina de carne y hueso (MBM) o la harina de subproductos de atún (TBM) sobre el rendimiento productivo, parámetros hematológicos y digestibilidad aparente en juveniles de pargo lunarejo (SRS) *L. guttatus*. Se formularon tres dietas compuestas isonitrogenadas (47,6-49,0% CP) e isoenergéticas (20,9-22,9 kJ g<sup>-1</sup>). La dieta control fue elaborada con (FM) como principal fuente de proteína (D-FM) y dietas con el 35% de la proteína de la FM reemplazada por la proteína de MBM o TBM (D-MBM, D-TBM). Cada dieta fue ofrecida a grupos por triplicado de 20 juveniles de SRS (promedio de 8,2 ± 0,02 g) a saciedad aparente, tres veces al día. El rendimiento productivo, parámetros hematológicos y digestibilidad aparente de SRS alimentados con las dietas D-MBM o D-TBM no fueron significativamente diferentes de la dieta D-FM. Sin embargo, la proteína cruda del cuerpo fue significativamente menor en el grupo de D-TBM y más alta en el grupo D-MBM; los valores fueron comparables al grupo de D-FM. Sobre la base de estos resultados, la harina de carne y hueso es una opción económica y viable así como la harina de subproductos de atún en dietas prácticas para juveniles de pargo lunarejo.

**Palabras clave:** *Lutjanus guttatus*, pargo lunarejo, crecimiento, proteína animal, acuicultura.

### INTRODUCTION

The spotted rose snapper (SRS) *Lutjanus guttatus* is a carnivorous marine fish found along the Pacific coast from the Gulf of California to Peru, including the Galapagos Islands (Allen, 1985). It is an economically important artisanal fishery along the northwest coast of

Mexico. Protocols for SRS reproduction in captivity, larval rearing and commercial grow out using aquaculture production system (*e.g.*, floating sea cages) have been developed and tested (Castillo-Vargasmachuca *et al.*, 2007; Ibarra-Castro & Alvarez-Lajonchère, 2011; Hernández *et al.*, 2015). Despite the knowledge gained on this species, it is well known that

limitation in marine based protein sources exist in the world and the reducing fish meal in fish diets may increase the profitability of aquaculture operations. Diet costs generally constitute up to 60% of the total farm production costs. Therefore, it is important for researchers to identify some less expensive and more sustainable ingredients to utilize in SRS diets, and these diets must have an equal or even better nutritional quality compared to diets based mainly on fish meal. Previous study in SRS juvenile, showed that FM can be replaced by tuna by products meal (TBM) up to 30%, in 8 weeks trial, where a long-term growth studies is recommended to confirm this conclusion (Hernández *et al.*, 2014a). Although TBM it is a fishery by products and is still fishery dependent protein source, locally constant supply exist. On the other hand, render product such as poultry by products (PBM) in SRS showed that fish meal can be replaced up to 50% by feed grade PBM, or up to higher level with PBM pet grade that presents higher nutritional value, while fish meal can be replaced up to 90% in SRS diets (Hernández *et al.*, 2014b, 2014c). Another potential render ingredient that could be alternative ingredients to partial replace FM in practical diets for SRS is meat and bone meal (MBM) as previous reported for other marine fishes (Robaina *et al.*, 1997; Ai *et al.*, 2006; Rossi & Davis, 2014). Thus, the purpose of this study was to evaluate the growth performance, protein efficiency, body composition, apparent digestibility and hematological parameters of *L. guttatus* juveniles fed practical diets containing MBM or TBM meal as a partial replacement of fish meal.

## MATERIALS AND METHODS

### Fish and growth trial

SRS juveniles were produced in a pilot-scale hatchery at Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD), Mazatlán, Mexico, following the established protocols for spawning and larval rearing according to Abdo de la Parra *et al.* (2010). The fish were randomly distributed at a stocking density of 20 fish (weight  $8.2 \pm 0.02$  g) per tank among nine tanks (volume 350 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  $1.5 \text{ L min}^{-1}$ . Triplicate groups were fed by hand to apparent satiation three times a day (07:00, 13:00 and 17:00 h) during 120 days. Uneaten feed 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  $60^\circ\text{C}$ . Feed intake was calculated as the amount of feed supplied minus the amount of unconsumed feed.

Over the duration of the study, these water quality parameters average ( $\pm$ SD): water temperature,  $24.8 \pm 2^\circ\text{C}$ ; dissolved oxygen,  $6 \pm 0.5 \text{ mg L}^{-1}$ ; salinity,  $34.6 \pm 0.4$ ; pH,  $8.1 \pm 0.3$ .

The fish were weighed every two weeks to calculate 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  $0.3 \text{ mL}^{-1}$ . Then the specimens were weighed individually on a digital scale (accurate to  $\pm 0.01$  g).

The growth and economic performance and feed efficiency of the fish were assessed by calculating the weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), survival (SUR), feed intake (FI), protein efficiency ratio (PER), and profit index (PI), as follows:

$$\text{WG}\% = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{weight gain (g)}}$$

$$\text{SGR} = \left[ \frac{\ln \text{ final weight} - \ln \text{ initial weight}}{\text{number of days}} \right] \times 100$$

$$\text{Survival} = \left( \frac{\text{final number}}{\text{initial number}} \right) \times 100$$

$$\text{FI} = \sum_i n \left[ \frac{\text{total feed consumption (g)}}{\text{number of fish}} \right] / \text{number of days}$$

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

The economic performance of the diets was calculated from the method of Vincke (1969) as

$$\text{PI} = \frac{\text{value of fish (kg)}}{\text{cost of feed (US\$)}}$$

### Digestibility determination

Almost at the end of the growth trial, the same three replicates with experimental animals were used to measure the apparent digestibility coefficient of dry matter and nutrients for each of the experimental diets. The fish were adapted to the marked diets (with chromic oxide) for 15 days before the collection of feces. Fecal samples were collected 5 h after feeding by the stripping technique (Austreng, 1978), every three days until sufficient feces were collected to analysis. Chromic oxide concentration of the feed and feces samples was measured using the acid digestion technique (Furukawa & Tsukahara, 1996). The absorbance was read on a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 350 nm, after colorimetric reaction.

The ADC of dry matter, protein or energy was calculated as the ratio of nutrients and markers in the feed and feces (Maynard & Loosli, 1969):

$$\text{ADC dry matter (\%)} = 100 - \left[ \left( \frac{\% \text{ Cr}_2\text{O}_3 \text{ in feed}}{\% \text{ Cr}_2\text{O}_3 \text{ in feces}} \right) \times 100 \right]$$

$$\text{ADC nutrients (\%)} = 100 - 100 \left[ \left( \frac{\% \text{ Cr}_2\text{O}_3 \text{ in feed}}{\% \text{ Cr}_2\text{O}_3 \text{ in feces}} \right) \times \left( \frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in feed}} \right) \right]$$

### Ingredients and experimental diets

The Monterrey sardine (*Sardinops sagax caerulea*) fishmeal was produced by Selecta de Guaymas, S.A. de C.V., Guaymas, Sonora, Mexico. Meat and bone meal (MBM) was obtained from a rendering plant (Griffin Industries, Cold Spring, KY, USA). Tuna by-product meal (TBM) was locally sourced for this study (PINSA, S.A de C.V., Mazatlán, Sin., Mexico). Biochemical analyses of these meals are presented in Table 1.

Three diets were formulated to be complete with regard to known the nutrient requirements of SRS (Abdo de la Parra *et al.*, 2010), contained 47.6-49.0% crude protein, and gross energy 20.9-22.9 kJ g<sup>-1</sup>. The control diet (D-FM) had 52.6% sardine fish meal as described Silva-Carrillo *et al.* (2012). In the experimental diets, 35% of FM was replaced with MBM or TBM (D-MBM, D-TBM) (Table 2). The experimental diets were balanced for essential amino acids using *L. guttatus* whole body amino acids profile as a target value (Table 3). The dietary levels of other feed ingredients (squid meal, krill meal, carophyll pink, antioxidants, soy lecithin, sodium alginate and vitamin and mineral premixes) were held constant, while corn flour was used to adjust to 100%. Chromic oxide (0.5%) was used as an indigestible marker into the control and experimental diets for the evaluation of apparent digestibility. 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 A-200 Troy, OH, USA) followed by micro ingredients mix and fish oil and then boiling water were added until a homogeneous mixture was obtained. The chromic oxide was added manually before fish oil and boiling water. The resulting mash was passed through a meat grinder (Tor-rey ® Model 22) to produce pellets. The moist pellets were dried in a forced air oven at a temperature of 38°C for about 12 h. Subsequently, the pellets were crumbled and sieve to the desired size before use. The pellets were stored in labeled, sealed containers and were held at -20°C until utilization.

### Chemical analysis

Ten randomly 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 group. Moisture, protein, lipid and ash levels of test ingredients, diets, carcasses and fecal samples were determined using standard methods (AOAC, 2000).

The samples were homogenized and dried at 105°C for 24 h prior to 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). The amino acid composition of ingredients, experimental diets and whole-body of the fish was quantified following Vázquez-Ortiz *et al.* (1995) by high performance liquid chromatography (HPLC, Varian 9012, Walnut Creek, CA, USA).

### Blood chemistry parameters

At the end of the feeding experiment, the fish were carefully handled to minimize stress, anesthetized with 0.3 mL L<sup>-1</sup> of 2-phenoxyethanol, and in less than 3 min, blood samples were collected from the caudal vein using 1 mL non-anticoagulant insulin syringes 21 G x 32 mm (Terumo Mexico, DF, Mexico). Three fish were selected randomly from each tank (nine fish for each treatment group for blood sampling). A volume of 400 µL of blood from each fish was extracted and placed in placed into two Eppendorf tubes. The first tube, with no anticoagulant, was immediately centrifuged for 10 min at 7000 rpm in a Clay-Adams micro centrifuge, and the serum was stored in a -20°C freezer for further analysis of the total protein concentration and glucose levels. The second tube included K<sub>2</sub> EDTA (BD Microtainer, Franklin Lakes, NJ, USA) to prevent coagulation. This tube was used to determine the hematocrit and hemoglobin concentrations.

To calculate the hematocrit levels, tubes were placed for 10 min in a microhematocrit centrifuge (SOL-BAT 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 erythrocytes was determined using the cyanmethemoglobin method (HemogloWiener reactive, Wiener Lab., Riobamba, Rosario, Argentina) following the manufacturer's instructions.

**Table 1.** Chemical composition and concentrations of essential amino acids (% AA per 100 g of protein) of the tested ingredients: sardine fishmeal (FM), meat and bone meal (MBM) and tuna by product meal (TBM). \*Essential amino acids.

Proximate analysis (% dry matter)	Ingredients		
	FM	MBM	TBM
Crude protein Nx6.25	67.4	49.0	58.0
Crude fat	10.0	13.0	12.0
Ash	15.4	28.0	25.0
Amino acid (AA%/100 g protein)			
Alanine	7.5	7.1	7.3
Arginine*	6.6	6.7	9.1
Aspartic acid	9.2	7.0	7.0
Glutamic acid	16.6	12.0	12.2
Glycine	11.5	12.3	12.1
Histidine*	3.5	2.0	2.0
Isoleucine*	4.9	3.2	4.8
Leucine*	6.5	6.1	6.5
Lysine*	6.7	5.8	5.9
Methionine*	2.4	1.5	2.6
Phenylalanine*	3.9	2.9	6.1
Serine	3.2	2.4	2.4
Threonine*	2.3	3.2	4.3
Tyrosine	3.6	3.0	6.4
Valine*	4.5	4.2	3.9

### Statistical analysis

The data for each parameter were tested for normality and homoscedasticity. Percentage data were arcsine-transformed before one-way analysis of variance (ANOVA) was used for all parameters with diet as the independent variable. 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 SigmaPlot 11.0 software.

## RESULTS

### Growth performance and nutrient utilization

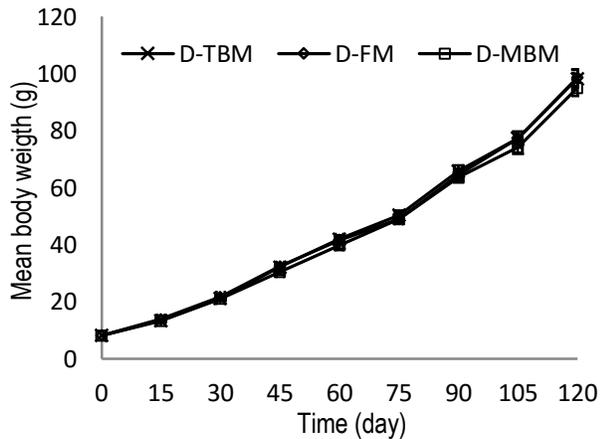
The growth performance and feed efficiency of SRS juveniles fed the control and experimental diets are presented in Figure 1 and Table 4. Survival ranged between 89.7-98.3% for all diets showing no significant differences ( $P > 0.05$ ). The partial replacement of FM with MBM or TBM did not affect weight gain (WG%), specific growth rate (SGR), feed intake (FI), protein efficiency ratio (PER) or feed conversion ratio (FCR) ( $P > 0.05$ ) among treatment groups. Profit index (PI) showed that replacing FM with MBM or TBM lowered the cost diets, therefore, the profit indices of the fish fed these animal proteins increased (Table 4).

**Table 2.** Ingredient and proximate composition of experimental diets for the spotted rose snapper *L. guttatus*. <sup>a</sup>Fish meal was obtained from Selecta de Guaymas, S.A. de C.V., Guaymas, Sonora, México, <sup>b</sup>This product was imported by Proteínas marinas y Agropecuarias, S.A. of C.V., Guadalajara, Jalisco, México, <sup>c</sup>Maz Industrial, S.A. de C.V., Mazatlan, Sinaloa, México, <sup>d</sup>PROAQUA, S.A. de C.V., Mazatlán, Sinaloa, México, <sup>e</sup>Droguería Cosmopolita, S.A. de C.V., México D.F., México, <sup>f</sup>Sigma-Aldrich Chemical, S.A. de C.V. Toluca, Mexico State, Mexico, <sup>g</sup>Trouw Nutrition México S.A. de C.V. (by courtesy), <sup>h</sup>Vitamins premix composition: Vitamin A, 10,000,000 IU o mg g<sup>-1</sup>; 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; Folic Acid, 4.00 g; 80 mg; Biotin, 500 mg; Vitamin C, 100.00 g; Choline 300.00 g. <sup>g\*\*</sup>Mineral premix composition: Manganese, 100 g; Zinc, 160 g; Iron, 200 g; Copper, 20 g; Iodine, 5 g; Selenium, 400.00 mg; Cobalt 600.00 mg. <sup>h</sup>DSM Nutritional Products Mexico S.A. de C.V., El Salto, Jalisco, México. <sup>i</sup>Butyl hydroxytoluene (Dresen, Quimica, S.A. de C.V.). <sup>j</sup>Sigma-Aldrich Chemical, S.A. C.v. Toluca, Mexico State, Mexico. <sup>k</sup>Nitrogen-free extract (including fiber) = 100 - (% protein + % lipid + % ash).

Ingredients* (% as feed basis)	Diet		
	D-FM	D-MBM	D-TBM
Fish meal (sardine) <sup>a</sup>	52.6	34.5	34.5
Meat & bone meal <sup>b</sup>	-	25.2	-
Tuna meal by-product <sup>c</sup>	-	-	21.3
Squid meal <sup>b</sup>	6	6	6
Krill meal <sup>d</sup>	7.5	7.5	7.5
Fish oil <sup>e</sup>	8.7	8.2	7.8
Dextrine <sup>e</sup>	17.14	10.54	14.84
Alginate <sup>f</sup>	3.0	3.0	3.0
Wheat gluten <sup>f</sup>	2.0	2.0	2.0
Vitamin premix <sup>g*</sup>	0.6	0.6	0.6
Minerals premix <sup>g**</sup>	0.23	0.23	0.23
Carotenoids <sup>h</sup>	0.08	0.08	0.08
Soybean lecithin (70%) <sup>e</sup>	1.5	1.5	1.5
Vitamin C <sup>h</sup>	0.1	0.1	0.1
Antioxidants <sup>i</sup>	0.05	0.05	0.05
Chromic oxide <sup>j</sup>	0.5	0.5	0.5
Composition (% as feed basis)			
Moisture	6.53	5.71	7.32
Crude protein	48.98	48.12	47.63
Crude lipid	15.99	17.29	17.41
Ash	12.24	13.69	13.38
NFE <sup>k</sup>	22.79	20.9	21.58
P/E	23.7	23.7	23.8
Cost of feed (US\$)	1.74	1.72	1.59

**Table 3.** Amino acid content of experimental diets (% AA per 100 g of protein) for juvenile spotted rose snapper *L. guttatus* containing fishmeal (FM), meat and bone meal (MBM) or tuna by-product meal (TBM). \*Essential amino acids. <sup>a</sup>Tryptophan was not determined by the analytical method used; <sup>b</sup>Whole body composition of spotted rose snapper provided for comparison.

Amino acid <sup>a</sup>	Body <sup>b</sup> D-FMD-MBMD-TBM			
Alanine	6.1	6.4	6.6	6.4
Arginine*	5.9	6.1	7.0	6.3
Aspartic acid	9.3	8.9	9.4	8.9
Glutamic acid	13.1	15.2	17.5	15.0
Glycine	9.8	10.6	14.4	12.9
Histidine*	1.9	2.3	2.3	2.5
Isoleucine*	4.3	4.4	4.9	4.7
Leucine*	7.0	7.8	8.1	7.5
Lysine*	6.0	7.7	5.2	8.4
Methionine*	2.4	2.6	2.3	2.4
Phenylalanine*	5.4	4.7	4.6	4.3
Serine	1.4	2.9	2.4	2.9
Threonine*	2.3	3.6	4.1	4.0
Tyrosine	1.5	3.3	5.1	5.0
Valine*	4.9	4.1	4.3	4.3



**Figure 1.** Growth of spotted rose snapper juvenile fed the experimental diets over a 120-day trial.

### Digestibility determination

The ADCs of the experimental diets are listed in Table 5. The replacement of FM with MBM or TBM did not affect the dry matter digestibility coefficients of the diet ( $P < 0.05$ ). The ADCs for protein or energy were not affected ( $P > 0.05$ ).

### Whole-body composition and hematological characteristics

The whole-body proximate composition of the fish is shown in Table 6. There were significant differences ( $P < 0.05$ ) in protein, lipid, moisture and ash contents of

the fish fed different diets, where MBM showed higher protein values and lower lipid values than other diets. The measured blood parameters, including hematocrit, hemoglobin ( $\text{g dL}^{-1}$ ) and total protein did not differ significantly among the treatments ( $P > 0.05$ ), except glucose (Table 7).

## DISCUSSION

Following the trend of replacing FM in fish feeds to support sustainable growth of the aquaculture industry (Tacon & Metian, 2008), this study provides useful information regarding the replacement of FM by MBM and TBM in 35% of ingredient in feeds for the spotted rose snapper. FM was reduced from 526 to 345  $\text{g kg}^{-1}$  without compromising the health or growth performance of the spotted rose snapper juvenile. Previous studies in SRS by 8 weeks trial (Hernandez *et al.*, 2014a), support the use of TBM up to 30% of ingredient, therefore, the present study confirm and improve previous reports, accepting plus 5% of TBM in diets during longer trial (120 days).

The amino acid profile of the MBM revealed lower levels of methionine, isoleucine and phenylalanine compared to FM and TBM. Nevertheless, partial substitution of FM with MBM or TBM, did not affect final profile of amino acids in diets, thus, the diets meet with the amino acid pattern of the whole body tissue of *L. guttatus*. MBM is generally considered to be an inferior animal protein source to fishmeal in the diet for fish culture (Lee *et al.*, 2012), however, in the present study similar weight gain and SGR of SRS fed the D-MBM diet compared to D-FM or D-TBM diets is obtained, showing a good balance of nutrients in all diets.

The potential to utilize MBM ingredient as FM substitutes in fish diet varies among fish species. Meat and bone meal is commonly successfully use in low levels inclusion and/or in combination with other protein sources, without affecting growth parameters, where Florida pompano, *Trachinotus carolinus* L. accepted MBM inclusions of 100  $\text{g kg}^{-1}$  in practical diets (Rossi & Davis, 2014), rainbow trout, *Oncorhynchus mykiss* accepted 240  $\text{g kg}^{-1}$  of MBM in diets (Bureau *et al.*, 2000), cuneate drum, *Nibea miichthioides* was able to accept 105  $\text{g kg}^{-1}$  in practical diets (Guo *et al.*, 2007), Korean rockfish *Sebastes schlegeli* accepted 123  $\text{g kg}^{-1}$  substitution of MBM by FM (Yan *et al.*, 2014), juvenile gibel carp *Carassius auratus gibelio* accepted 110  $\text{g kg}^{-1}$  of FM by substitution of MBM (Hu *et al.*, 2008), olive flounder *Paralichthys olivaceus* was able to substitute 20% of fish meal (120  $\text{g kg}^{-1}$ ) (Lee *et al.*, 2012), while yellowtail *Seriola quinqueradiata* showed reduced growth performance when fed with practical diets of

**Table 4.** Growth and feed performance indices of juvenile spotted rose snapper *L. guttatus* fed experimental diets for 120 days. IBW: initial body weight, FBW: final body weight, WG: weight gain, FI: feed intake, FCR: feed conversion ratio, SGR: specific growth rate, SUR: survival, PER: protein efficiency ratio; PI: profit index. Price of 1 kg of fish is fixed at US\$ 8.00.

Parameter	Diet			P-value
	D-FM	D-MBM	D-TBM	
IBW (g)	8.23 ± 0.01	8.23 ± 0.02	8.23 ± 0.02	0.76
FBW (g)	98.61 ± 2.1	95.04 ± 6.5	98.32 ± 0.8	0.52
WG (%)	1098.58 ± 23.7	1054.54 ± 8.5	1095.34 ± 8.4	0.1
FI (g fish <sup>-1</sup> )	121.92 ± 6.9	133.98 ± 5.6	126.89 ± 8.7	0.64
FCR	1.35 ± 0.08	1.54 ± 0.2	1.41 ± 0.1	0.24
SGR (% day <sup>-1</sup> )	2.07 ± 0.02	2.04 ± 0.1	2.07 ± 0.01	0.49
SUR (%)	98.33 ± 0.9	89.67 ± 18.9	91.67 ± 7.6	0.52
PER	1.51 ± 0.09	1.36 ± 0.1	1.48 ± 0.11	0.30
PI (US\$) <sup>c</sup>	4.59	5.03	4.65	-

**Table 5.** Coefficients of apparent digestibility of dry matter, crude protein and energy of the experimental diets for juvenile spotted rose snapper *L. guttatus*. The values in the same row (mean ± SD) with different superscripts denote significant differences among the treatments ( $P < 0.05$ ) using evidence from the Tukey's HSD test.

Parameter	Diet			P-value
	D-FM	D-MBM	D-TBM	
Dry matter	84.4 ± 0.5 <sup>a</sup>	76.6 ± 1.5 <sup>b</sup>	79.3 ± 0.3 <sup>b</sup>	<0.001
Crude protein	83.3 ± 2.5	84.0 ± 0.6	85.3 ± 0.5	0.496
Energy	85.4 ± 1.6	85.5 ± 0.7	86.2 ± 1.1	0.761

**Table 6.** Whole body composition of juvenile spotted rose snapper *L. guttatus* fed experimental diets for 120 days. The values in the same row (mean ± SD) with different superscripts denote significant differences among the treatments ( $P < 0.05$ ) using evidence from the Tukey's HSD test.

Parameter	Initial	Diet			P-value
		D-FM	D-MBM	D-TBM	
Moisture (%)	69.5 ± 0.2	63.10 ± 0.1 <sup>b</sup>	63.90 ± 0.1 <sup>a</sup>	63.33 ± 0.2 <sup>b</sup>	<0.001
Lipid (%)	7.31 ± 0.1	12.63 ± 0.03 <sup>a</sup>	10.05 ± 0.2 <sup>b</sup>	12.53 ± 0.1 <sup>a</sup>	<0.001
Ash (%)	6.09 ± 0.1	5.24 ± 0.1 <sup>a</sup>	5.37 ± 0.1 <sup>a</sup>	4.96 ± 0.1 <sup>b</sup>	<0.001
Protein (%)	17.10 ± 0.3	18.43 ± 0.02 <sup>ab</sup>	19.42 ± 0.01 <sup>a</sup>	18.1 ± 0.1 <sup>b</sup>	<0.001

192 g kg<sup>-1</sup> fish meal substituted by MBM (Shimeno *et al.*, 1993).

Nevertheless, other fish species have accepted higher inclusion of MBM in practical diets, such as large yellow croaker *Pseudosciaena crocea*, able to substitute 325 g kg<sup>-1</sup> of FM by MBM, representing 45% of protein (Ai *et al.*, 2006), juvenile hybrid striped bass *Morone chrysops* x *M. saxatilis* did not showed growth affections by inclusion of 450 g kg<sup>-1</sup> (Bharadwaj *et al.*, 2002), Sutchi catfish, *Pangasius hypophthalmus* was able to digest up to 67% of total protein concentrate substitution without hampering the growth and feed

utilization (Kader *et al.*, 2011) and gilthead seabream *Sparus aurata* was able to accept 280 g kg<sup>-1</sup> (40% replacement of FM) in their diet, however, fish where compromised in liver tissue alterations, recommending an inclusion of 20% of replacement (Robaina *et al.*, 1997). Therefore, the single level inclusion of MBM in diet of *L. guttatus* has been shown that it is a potential ingredient, up to 252 g kg<sup>-1</sup> inclusion in diets (35% of protein diet). Based on this observation, we suggest that is needed to be conducted a further study considering evaluate higher inclusion levels with amino acid balance to find the optimum level inclusion. Differen-

**Table 7.** Hematological parameters of spotted rose snapper *L. guttatus* fed experimental diets for 120 days. <sup>a</sup>The values in the same row (mean  $\pm$  SD) with different superscripts denote significant differences among the treatments ( $P < 0.05$ ) using evidence from the Tukey's HSD test.

Parameter	Diet			P-value
	D-FM	D-MBM	D-TBM	
Hematocrit (%)	69.6 $\pm$ 6.9	56.8 $\pm$ 7.4	50.6 $\pm$ 9.0	0.058
Hemoglobin (g dL <sup>-1</sup> )	14.6 $\pm$ 0.7	14.2 $\pm$ 1.9	13.0 $\pm$ 1.5	0.263
Total protein (g L <sup>-1</sup> )	56.6 $\pm$ 1.6	54.5 $\pm$ 1.3	54.7 $\pm$ 1.1	0.196
Glucose (mg dL <sup>-1</sup> )	69.6 $\pm$ 1.6 <sup>a</sup>	61.2 $\pm$ 2.3 <sup>b</sup>	70.4 $\pm$ 1.6 <sup>a</sup>	<0.001

ces in the results among species might be attributed to variations in the nutritional quality of the ingredient. As with other animal by-products, the nutritive value of meat and bone meal can be affected by variations of both, the raw materials used and processing conditions during rendering (Skurray & Herbert, 1974).

The apparent digestibility coefficients of dry matter showed a very high differences between D-FM diet and experimental diets (D-MBM, D-TBM), nevertheless ADC for crude protein and energy of D-MBM or D-TBM diets were similar than D-FM diet, therefore the quantity and chemical composition of the test ingredients of meals were adequate to feed SRS. Additionally, it should be noted in terms of absolute quantity, the difference of same protein digested coming from 84, 76 and 79% of dry matter digestibility. Reports in other species show that apparent ADC in diets for juvenile hybrid striped bass did not show differences up to 40% MBM inclusion (ADC of protein values of 81.2 %) (Bharadwaj *et al.*, 2002), while ADC of dry matter, protein and energy of diets (76.6%, 93.2% and 86.6% respectively) with MBM inclusions in Korean rockfish did not show differences in ADC compared with D-FM control diet (Yan *et al.*, 2014). Nevertheless, is reported for large yellow croaker that ADC values of dry matter, protein, lipid and energy for MBM (52.4, 82.3, 70.2 and 70.2% respectively) were significantly lower compared with those of FM (70.0, 92.4, 90.5 and 82.6% respectively) (Ai *et al.*, 2006). Therefore growth and feed efficiency depends on fish physiological and biochemical capacities to digest and absorb nutrients in diets (Furné *et al.*, 2008) where independent of fish habits, digest capacity is directly related to diet composition (Pérez-Jiménez *et al.*, 2009).

In the present study, the ash and protein of whole body composition were higher in fish fed D-MBM than D-TBM, but the fish fed D-MBM were comparable to D-FM diet. Ai *et al.* (2006) found that fish body composition of yellow croaker showed that the carcass protein had a decreasing trend (from 16.3 to 14.8%), and the ash had an increasing trend (from 3.5 to 3.6%) with increasing dietary MBM, but no significant

difference in the carcass protein and ash contents were observed among dietary treatments. This confirmed that there were not imbalances with this partial inclusion (35% of protein). A number of studies found a negative relationship between ash content and the digestibility dietary protein (or dry matter) (Bureau *et al.*, 1999). Based in this observation, Robaina *et al.* (1997) suggested that more than 12.5% ash content in the diets would lead to lower digestibility of protein. In the present study, ash content more than 12.5% resulted in protein digestibility around of 84%.

Furthermore, hematological parameters of fish receiving the different diets indicated that the condition and health status were comparable to those reported for clinically healthy snappers of the same species (Del Rio-Zaragoza *et al.*, 2011), where reduced hematocrit and hemoglobin concentration may be attributed to depression in erythropoiesis (McCue, 2010) and may impact the immune response of fish (Zhou *et al.*, 2005). Previous reports in the specie, show hemoglobin values ranging from 8.9 to 11.7 g dL<sup>-1</sup>, and hematocrit values ranging from 43 to 48% (Hernández *et al.*, 2014b, 2014c), those values are similar to the range of the present study.

SRS seems to be able to utilize good quality meat and bone meal, making it a promising alternative protein source in spotted rose snapper culture, with inclusions up to 35%, nevertheless, higher inclusion levels could be probe with inclusion of limiting amino acids to optimize the use of this ingredient. At same time, the present study confirm the viability of TBM substitution up to 35% in SRS diets, as an alternative byproduct source that partially replace high quality FM. From the economic standpoint, replacement of fish meal with cheaper animal byproduct meal in a practical diet for SRS can alleviate the problem of low fish meal availability and high cost.

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