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 fishmeal (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⁻¹) 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.
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
replace 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

**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 ± 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 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°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 (±SD): water temperature, 24.8 ±
2°C; dissolved oxygen, 6 ± 0.5 mg L⁻¹; salinity, 34.6 ±
0.4; pH, 8.1 ± 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-phenoxethanol (Sigma®, St. Louis, MO, USA) at a
concentration of 0.3 mL⁻¹. Then the specimens were
weighed individually on a digital scale (accurate to
±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:

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

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

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

\[
\text{Survival} = \frac{\text{final number}}{\text{initial number}} \times 100
\]

\[
\text{PER} = \frac{\text{weight gain}}{\text{protein intake}}
\]

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

**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):

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

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

**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⁻¹. 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 Soxtex Avanti 2050 Automatic System (Foss Soxtex, Hoganas, 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⁻¹ of 2-phenoxethanol, 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 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 K₂ 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.

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

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 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. Tryptophan was not determined by the analytical method used; †Whole body composition of spotted rose snapper provided for comparison.

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

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 hemacrit, hemoglobin (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 g kg\(^{-1}\) without compromising the health or growth performance of the spotted rose snapper juveniles. 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 g kg\(^{-1}\) in practical diets (Rossi & Davis, 2014), rainbow trout, Oncorhynchus mykiss accepted 240 g kg\(^{-1}\) of MBM in diets (Bureau et al., 2000), cuneate drum, Nibea mitchthioides was able to accept 105 g kg\(^{-1}\) in practical diets (Guo et al., 2007), Korean rockfish Sebastes schlegeli accepted 123 g kg\(^{-1}\) substitution of MBM by FM (Yan et al., 2014), juvenile gibel carp Carassius auratus gibelio accepted 110 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 g kg\(^{-1}\)) (Lee et al., 2012), while yellowtail Seriola quinquemaculata showed reduced growth performance when fed with practical diets of

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

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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D-FM</th>
<th>D-MBM</th>
<th>D-TBM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>84.4 ± 0.5</td>
<td>76.6 ± 1.5</td>
<td>79.3 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crude protein</td>
<td>83.3 ± 2.5</td>
<td>84.0 ± 0.6</td>
<td>85.3 ± 0.5</td>
<td>0.496</td>
</tr>
<tr>
<td>Energy</td>
<td>85.4 ± 1.6</td>
<td>85.5 ± 0.7</td>
<td>86.2 ± 1.1</td>
<td>0.761</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial</th>
<th>D-FM</th>
<th>D-MBM</th>
<th>D-TBM</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>69.5 ± 0.2</td>
<td>63.10 ± 0.1</td>
<td>63.90 ± 0.1</td>
<td>63.33 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>7.31 ± 0.1</td>
<td>12.63 ± 0.03</td>
<td>10.05 ± 0.2</td>
<td>12.53 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.09 ± 0.1</td>
<td>5.24 ± 0.1</td>
<td>5.37 ± 0.1</td>
<td>4.96 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>17.10 ± 0.3</td>
<td>18.43 ± 0.02</td>
<td>19.42 ± 0.01</td>
<td>18.1 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

192 g kg⁻¹ 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⁻¹ 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⁻¹ (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⁻¹ (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⁻¹ 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.
ences 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⁻¹, 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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