Assessing the nutritional value of an enzymatically processed soybean meal in early-stage juvenile red drum, *Sciaenops ocellatus* L.

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ABSTRACT

We assessed the nutritional value of an enzymatically processed soybean meal (HP300, Hamlet Protein Inc., Findlay, Ohio, USA; hereafter ESBM) in the diet of red drum, *Sciaenops ocellatus.* A digestibility and two growth trials (Trials I and II) were conducted to: i) assess the *in vivo* digestibility of ESBM and commodity soybean meal (SBM); ii) assess the maximum replacement of the digestible protein (DP) from fishmeal (FM) with ESBM (MAX $REPL_{ESBM}$); iii) validate the estimated MAX $REPL_{ESBM}$ and compare it against SBM. All experimental diets were formulated to contain 32% DP, 12% lipid, and an estimated 13.8 kJ g^{-1} digestible energy. Seven experimental diets were designed in Trial I: a FM reference diet (FM-100), designed to contain all its protein from Special Select menhaden FM; and six test diets designed to replace the DP from FM in the FM-100 diet on a isonitrogenous basis, at 15% incremental levels (ESBM-15 to ESBM-90). Each diet was fed to juvenile red drum (5.72 g) in duplicate aquaria connected as a closed recirculating system. After 6 weeks of feeding, responses of red drum fed the various diets differed considerably, with survival ranging from 78 to 95%, final weight from 24.5 to 40.0 g, weight gain (% of initial) from 330 to 597%, and feed efficiency (FE) from 0.7 to 1.0. Second order polynomial regression analysis of weight gain and FE data indicated a MAX $REPL_{ESBM}$ of 72 and 63%, respectively. After feeding the FM-100, ESBM-70, and a SBM-70 diets to juvenile red drum (1.7 g) in Trial II (8 weeks, n=3), no differences in production performance of fish were found among treatments. These results indicated that either ESBM or SBM can replace up to 70% of FM DP in the diet of early-stage juvenile red drum without detrimental effects on production performance.

Keywords: Aquaculture, Soybean, Nutritional value, Fishmeal, Digestibility, Red drum

1. Introduction

Despite aquafeeds representing less than 10% of the total global animal feed production, aquaculture currently consumes over 70% of global fishmeal (FM) production (Tacon and Metian, 2015). Concurrently, the fast growth of global aquaculture aligned with the steady production and increasing prices of FM have challenged the aquaculture industry in finding alternative protein sources for the production of cost-effective and sustainably sound aquafeeds (Gatlin et al., 2007; Barrows et al., 2008).

The utilization of plant-protein (PP) feedstuffs as surrogates for FM in aquafeeds has been intensified in recent years (Tacon and Metian, 2015) and among the plant feedstuffs commercially available, soybean meal (SBM) is the foremost ingredient substituting for FM in aquafeeds (Troell et al., 2014). Approximately 190 million metric tons (mmt) of SBM were produced globally in 2013-2014 (www.fas.usda.gov/commodities) and it comprises the main PP source used in the animal feed industry. Soybean meal is a high-quality protein feedstuff with a reasonably balanced amino acid profile. Nevertheless, a series of bioactive compounds broadly categorized as anti-nutritional factors (ANF) present in commodity SBM may cause adverse effects on the digestive system and the overall physiological status of fish (Francis et al., 2001), thereby limiting its inclusion in aquafeeds.

To overcome limitations imposed by the presence of ANF and improve the nutritional value SBM to monogastric animals, novel processing technologies have been incorporated into the manufacturing of SBM and new soybean products (SPs) have been introduced to the feed industry. One such promising technology involves the treatment of commodity SBM with a blend of enzymes leading to a reduction in the level of carbohydrates, antigens, and oligosaccharides (Zhu et al., 1998). The resulting SP, containing diminished levels of ANF,

higher crude protein and amino acid contents compared to commodity SBM (Zhu et al., 1998; Cervantes-Pahm et al., 2010), has been proven efficient at improving the nutritional value and utilization of SBM in swine as well as in Atlantic salmon, *Salmo Salar* (Refstie et al., 1998), a carnivorous teleost highly sensitive to ANF present in SBM.

Although red drum have been demonstrated to be relatively more tolerant to ANF present in SBM compared to some carnivorous teleosts, experimental diets high in SBM have been shown to negatively affect its production performance (Rossi et al., 2013). Therefore, the objective of the present study was to evaluate the nutritional value of an enzymatically processed SBM in the diet of early-stage juvenile red drum.

2. Material and methods

A digestibility and two independent growth trials were conducted to thoroughly evaluate two soybean products (SPs) relative to menhaden FM in the diet of red drum. The SPs evaluated consisted of a conventionally processed, commodity soybean meal (SBM; designated SBM) and an enzymatically processed SBM (HP300, Hamlet Protein Inc., Findlay, Ohio, USA; designated ESBM), both of which were dehulled and solvent-extracted meals.

2.1 Fish

Three batches of red drum were obtained in different time periods from the Sea Center Texas Marine Aquarium, Fish Hatchery and Nature Center operated by Texas Parks and Wildlife Department in Lake Jackson, TX, and transported to the Aquacultural Research and Teaching Facility of Texas A&M University. Fish were maintained in quarantine for 4 weeks acclimating to local conditions until adequate size for the growth trials was attained. Over this period, red

drum were fed at a rate approaching apparent satiation with a 40% crude protein (CP) and 12% crude fat commercial diet (Rangen, Inc., Angleton, TX).

2.2 Digestibility Trial

A digestibility trial was conducted to determine the apparent digestibility coefficient (ADC) of organic matter, crude protein, lipid, and energy of each SP in red drum. The proximate composition and the content of trypsin inhibitor (TI), β-conglycinin, glycinin, oligosaccharides, and galactose of each test ingredient are presented in Table 1.

An indirect method was utilized for the determination of digestibility coefficients and chromic oxide (Cr_2O_3) was used as the non-digestible marker. A pre-existing FM reference diet (Rossi et al., 2015) containing 46% CP and 12.2% lipid was utilized as the digestibility reference diet (REF). For the manufacture of REF, the original diet was hammer-milled followed by the inclusion (on a dry matter basis) of carboxymethyl cellulose at 4% for appropriate binding and chromic oxide at 1%. After mixing these components in an industrial mixer for 45 min, the mixture was divided into three fractions for the manufacture of the REF and test diets. The test diets were manufactured by combining and mixing the REF and each SP in a 70:30% ratio according to previously described methodologies (Cho et al., 1982; Wilson and Poe, 1985). Resulting diets were blended with water using a secondary industrial mixer with a meat grinder attachment and pelleted with a 5-mm die. After drying under forced air for 24 h at 25° C, pelleted diets were stored at – 20° C until fed. The ingredient composition of the REF and the analyzed composition of the REF and test diets are presented in Table 2. The inclusion of chromic oxide and carboxymethyl cellulose into REF slightly reduced dietary CP and lipid

values relative to the original diet (Rossi et al., 2015). The analyzed chromic oxide level of the REF and test diets was 0.98 and 0.72%, respectively.

Groups of 35 advanced-juvenile red drum (~ 200 g) were stocked into six, 1000-L circular-fiberglass tanks operating as a recirculating system. Water quality was maintained within adequate ranges for red drum [(mean ± standard deviation (SD)]: salinity = 6.0 ± 0.5 g L^{-1}; dissolved oxygen = 6.1 ± 0.3 mg L^{-1}; temperature = 27.4 ± 1.0° C; pH = 7.8 ± 0.1; total ammonia nitrogen (TAN) = 0.2 ± 0.1 mg L^{-1}). Each diet was randomly assigned to two tanks and the fish were fed their assigned diet once daily to satiation. A total of three fecal samplings were performed in each replicate tank within a 3-week period. On fecal-sampling days, feed was provided to each group of fish 10-min apart in sequential tanks to ensure that all experimental fish were sampled at approximately 5-h postprandial. Fecal contents from fish's lower intestine was manually stripped (Austreng, 1978) and pooled by tank at each sampling. Before being returned to their respective culture tanks, fish were immersed for 5 min in a bath containing nitrofurazone (5-nitro-2-furaldehyde semicarbazone, Sigma-Aldrich, Co., St. Louis, MO, USA) at 8 mg L^{-1}, as a preventive measure against bacterial infections.

Immediately following collection, pooled fecal samples from each tank were partially dried at 60° C for 24 h, and for an additional 3 h at 135° C before chemical analyses were conducted. Based on those analyses the apparent digestibility coefficients (ADCs) were computed for each SP so that diets in the growth trials could be formulated on a digestible protein (DP) basis. The DP of FM (82%) was not determined in the current study and was based on a previous determination (unpublished) using the same methodology.

2.3 Growth Trials

Two growth trials were conducted to: Trial I) evaluate the nutritional value of the ESBM in the diet of red drum by estimating its maximum replacement value (MAX $REPL_{ESBM}$) for dietary FM on a DP basis; Trial II) validate the MAX $REPL_{ESBM}$ and compare it against a SBM-based diet formulated to replace the DP from FM at the estimated MAX $REPL_{ESBM}$. The MAX REPL was defined as the maximum replacement of DP from FM at which growth performance of red drum would not change relative to the fish fed the FM reference diet (designated FM-100).

All experimental diets were formulated to contain 32% DP and 12% lipid. Seven diets were formulated for Trial I including the FM-100, designed to contain all its protein from menhaden FM, and six ESBM-test diets (Table 3). The latter were designed to replace the DP from FM in the FM-100 diet on an isonitrogenous basis, at 15% incremental levels (ESBM-15 to ESBM-90). Three experimental diets were utilized in Trial II: the FM-100 diet, manufactured as for Trial I; an ESBM-based diet, formulated to validate the estimated MAX $REPL_{ESBM}$ in Trial I; and a SBM-based diet, designed to compare both SP as surrogates for FM DP (Table 4). All experimental diets were supplemented with glycine at 2% for enhanced palatability (McGoogan and Gatlin, 1997) and with taurine at 1% (Velasquez et al., 2015). In addition to lysine-HCl, DL-methionine and dicalcium phosphate were supplemented to all test diets to ensure that lysine, total sulfur amino acids, and total phosphorus requirements were met (Davis and Robinson, 1987; Moon and Gatlin, 1991; Craig and Gatlin, 1992). The analyzed values of moisture, crude protein, lipid, ash, total phosphorus, gross energy, and amino acids were all consistent across treatments, except for a slightly lower lipid level in the SBM-70 diet in Trial II (Tables 3 and 4).

Trials I and II were conducted in sequence for 6 and 8 weeks, respectively, utilizing 110-L aquaria operated as a recirculating system. All monitored water quality parameters in Trials I and II were maintained within adequate ranges for red drum (respectively, mean ± SD): salinity

133 (g L^{-1}) = 6.8 ± 0.2 and 7.7 ± 0.6; dissolved oxygen (mg L^{-1}) = 6.2 ± 0.3 and 7.41 ± 0.5;
134 temperature (°C) = 27.2 ± 0.7 and 26.3 ± 0.2; pH = 7.9 ± 0.1 and 8.0 ± 0.1; and TAN (mg L^{-1}) =
135 0.5 ± 0.33 and 0.2 ± 0.1). A 12h light: 12h dark cycle was maintained using fluorescent lighting
136 controlled by timers. On the day red drum were transferred from the quarantine tanks to the
137 aquaria, a sample of 15 fish was collected from the population and frozen (- 20° C) for
138 subsequent analyses of initial whole-body composition. Twenty red drum juveniles initially
139 weighing (mean ± SD) 5.7 ± 0.04 and 1.7 ± 0.04 g were stocked in each aquarium of Trial I and
140 Trial II, respectively. After a 1-week conditioning period in which fish was fed with the ESBM-
141 45 diet (50/50 FM/ESBM) prior to the commencement of each growth trial, each experimental
142 diet was randomly assigned to duplicate aquaria in Trial I and to triplicate aquaria in Trial II. In
143 Trial I, fish were feed to complete satiation in the mornings to assess potential differences in
144 intake across treatments, and at a fixed rate in the afternoons [3.5 to 2.0% of tank biomass
145 (beginning and end; adjusted weekly)]. In the complete satiation feeding, each aquarium was
146 visited (by the same person) three times within a 30- to 45-min period and fish were fed in
147 accordance to the visual assessment of their appetite. Red drum in Trial II were fed twice daily at
148 a fixed rate ranging from 10 to 3.5% of tank biomass (beginning and end; adjusted weekly).

149 *2.4 Data Acquisition and Analyses*

150 *2.4.1 Digestibility Trial*:

151 All samples of test ingredients, diets, and fecal material were stored at -20 C and were dried
152 (135° C for 3 h, AOAC, 1990) prior to the chemical analyses. Chromic oxide concentrations in
153 diets and fecal samples was determined according to the method described by Furukawa and
154 Tsukahara (1966), in which, after a colorimetric reaction, marker concentrations were measured

spectrophotometrically as values of absorbance at 540 nm. Ash and organic matter were determined after ashing samples at 650° C for 3 h (AOAC, 1990). Crude protein (N × 6.25) was determine by the Dumas method (AOAC, 2005), and crude lipid was determined according to Folch et al. (1957). Gross energy was determined in a bomb calorimeter (Parr 6200; Parr Instrument Company, Moline, IL). The apparent digestibility coefficient (ADC) of a nutrient or gross energy in the diets (1) or in the test ingredients (2) was calculated using standard formulas (NRC, 2011):

1)

$$ADC = \frac{Cr_2O_3 \text{ in feed}}{Cr_2O_3 \text{ in feces}} \times \frac{\text{Nutrient in feces}}{\text{Nutrient in feed}}$$

2)

$$ADC_{\text{test ingredient}} = ADC_{\text{test diet}} + ((ADC_{\text{test diet}} - ADC_{\text{ref. diet}}) \times (0.7 \times D_{\text{ref.}} \div 0.3 \times D_{\text{ingredient}}))$$

where $D_{ref.}$ is the percent of nutrient or kcal g^{-1} gross energy (GE) of the reference diet, and $D_{ingredient}$ is the percent of nutrient or kcal g^{-1} GE of the test ingredient (NRC, 2011).

2.4.2 Growth Trials:

At the end of the growth trials, fish in each aquarium were group-weighed and sampled after an overnight fast. Three representative fish from each tank were euthanized with an overdose of tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA), frozen at -20° C and subsequently homogenized for proximate analysis to determine crude protein, lipid, moisture, and ash in whole-body tissues acording to the previously cited procedures. Subsequently, bled fish were dissected to obtain liver and intraperitoneal fat (IPF) for computing

hepatosomatic index (HSI) and IPF-ratio values, respectively, then filleted to obtain muscle for computing muscle yield values. The gastrointestinal tract (GIT) from each fish was excised and the intestine flash frozen in liquid nitrogen for the determination of trypsin and chymotrypsin activities as described by Anson (1938) and Ásgeirsson and Bjarnason (1991), respectively. Enzyme extraction procedure prior to quantification was performed as described by Castillo et al. (2014).

2.5 Calculations and Statistical Analyses

The fish performance parameters utilized to compare treatments in the growth trials were calculated as follow:

- Weight gain, % = [(Final weight – initial weight)/(initial weight)] × 100;
- Feeding rate, % of body weight per day (BW d^{-1}) = [dry feed intake (g)/($\sqrt{\text{initial body weight} \times \text{final body weight (g)}}$)/days on feed] × 100;
- Feed efficiency ratio (FE) = [weight gain (g)/ dry feed consumed (g)];
- Protein retention efficiency, % = {[(final body weight (g) × final body protein (%)) – (initial body weight (g) × initial body protein (%))]}/(protein intake (g) x 100;
- Energy retention efficiency, % = {[(final body weight (g) × final body energy (%)) – (initial body weight (g) × initial body energy (%))]}/(energy intake (g) × 100;
- Muscle yield, % = [fillet muscle weight (g)/body weight (g)] × 100;
- Viscerosomatic indices (HSI and IPF ratio), % = [Liver or intraperitoneal fat weight (g)/body weight (g)] × 100.

In all statistical analyses, $\alpha < 0.05$ was used as the significance level. The normality and homogeneity of variances of resulting data were validated by Shapiro-Wilk and Bartlett's tests,

respectively. Digestibility data were subjected to Student's t-test to detect significant differences between ADC values of ESBM and SBM. Data resulting from Trial I were subjected to regression analyses to fit the model that best explained red drum's responses to the replacement of DP from FM by ESBM. The one-way ANOVA was used to detect significant differences in the resulting data of Trial II. When significant differences were detected, orthogonal contrasts were used for the separation of treatment or treatment-group means. The adjusted R^2 (Adj. R^2) was calculated as previously described by Kvalseth (1985). All statistical analyses were carried out using the SAS® software package (SAS Institute Inc., Cary, NC USA).

3. Results

The ADC of organic matter, lipid, and gross energy was significantly higher in the ESBM than in SBM. In contrast, the ADC of CP was significant higher in SBM than ESBM (Table 5).

In Trial I, the relationship between dietary ESBM and the dependent variables final weight, feed efficiency, survival, protein and energy retention was best explained by second order polynomial (SOP) models, while no relationship ($P > 0.05$) was found for feeding rate (Table 6). The MAX $REPL_{ESBM}$ was estimated to be 71.8% for final weight and weight gain, 63.0% for feed efficiency, 64.2 and 54.1% for protein and energy retention efficiencies, respectively. A MAX $REPL_{ESBM}$ for survival was not estimated as with the exception of the ESBM-45 treatment, all treatments had > 80% survival. The effect of dietary ESBM on the composition indices and whole-body composition of red drum in Trial I also was best explained by SOP models for HSI and whole-body values of moisture, protein, and lipid (Table 7). A linear response was found for IPF ratio, which decreased as the level of ESBM increased in the diets, whereas no significant relationship was found for muscle yield and whole-body ash.

In Trial II, red drum fed the FM-100 diet showed a significantly higher protein retention efficiency compared to fish fed both SP diets (ESBM-70 or SBM-70), while no differences were found among treatments regarding the other performance parameters (Table 8). The HSI and IPF ratio of red drum fed the FM-100 diet was significantly higher than in fish fed either SP. A significantly higher HSI was displayed by red drum fed the ESBM-70 diet relative to those fed the SBM-70 diet. Whole-body ash content also was significantly higher in fish fed the FM-100 diet compared to fish fed either SP-based diet (Table 9).

Trypsin activity displayed a negative correlation with the level of ESBM in the diet of red drum in Trial I, decreasing linearly with incremental levels of ESBM in the diet (Fig. 1, A); whereas, no significant differences were found for trypsin activity among treatments in Trial II (Fig. 1, B). Following the same trend observed for trypsin, chymotrypsin activity (Fig. 1, C) displayed a linear decrease in response to dietary ESBM in Trial I; whereas, in Trial II, a significant lower activity of the enzyme was observed in red drum fed the SBM-70 diet (Fig. 1, D).

4. Discussion

Representing an addition to the increasing number of studies demonstrating the applicability and high nutritional value of SPs for red drum and other carnivorous species, the present findings showed that up to 70% of the DP in the diet of early-stage juvenile red drum can be derived from either ESBM or SBM without negatively affecting fish performance. Once reproduced under commercial settings, these results will aid in the development of more sustainable and cost-effective aquafeeds for red drum.

Although the commodity SBM utilized in this study was not exactly the same as that used in the manufacture of the ESBM evaluated, it is reasonable to assume that the enzymatic treatment used in the manufacturing of ESBM can remarkably reduce the level of ANF in commodity SBM, as shown before (Zhu et al., 1998; Cervantes-Pahm et al., 2010). Besides the reduction of important bioactive compounds known for causing adverse physiological effects in the GIT of monogastric animals, including fish (Francis et al., 2001), the enzymatic process also leads to an increased protein and amino acid concentration in the final product (Cervantes-Pahm et al., 2010), thereby reducing the need for supplementing crystalline amino acids in the diet to meet established requirements.

With the exception of CP, the ADC values of organic matter, lipid, and GE of ESBM were significantly higher than in SBM. However, despite the statistical significances found, the narrow differences observed may have limited practical significance. In addition, regardless of the type of SP, these results agreed fairly well with those reported by Gaylord and Gatlin (1996) with the exception of a higher ADC for lipid in the present study (92.3 and 95% vs. 62.7%). The low ADC values observed for organic matter and energy also are in agreement with those previously found for solvent extracted, dehulled SBM (Gaylord and Gatlin, 1996), and reflect the poor digestibility of the carbohydrate fraction by red drum.

According to the SOP model, 72% of the DP provided by FM can be substituted with ESBM in the diet of red drum before reductions in growth occur relative to that supported by the FM-100 diet (Fig. 2). This estimate closely agrees with the recently reported 75% FM CP replacement with SPs in the diet of red drum and shortfin corvina, *Cynoscion parvipinnis* (Minjarez-Osorio et al., 2016). On the other hand, a more conservative level of DP replacement (60%) was estimated by fitting a quadratic broken-line model (adapted from Robbins et al.,

2006). Considering that the performance of red drum was largely unaffected in Trial II wherein ESBM was used to replace 70% of the DP provided by FM in the FM-100 diet, the SOP model was more efficient in predicting the MAX $REPL_{ESBM}$. In addition, the similar performance of red drum observed in Trial II indicates that either ESBM or SBM can be used at this level of FM DP replacement.

The observed similar performance of ESBM and SBM as surrogates for FM DP in the diet of red drum is potentially due to the increasingly evident higher tolerance of this sciaenid to ANF present in SBM compared to other carnivorous teleosts such as Atlantic salmon, well-known for being highly sensitive (Francis et al., 2001; Krogdahl et al., 2003). For instance, Atlantic salmon fed a bioprocessed SBM replacing 40% of the FM protein outperformed fish fed diets in which SBM was the alternative protein source (Refstie et al., 1998); whereas, no improvements were observed in Atlantic cod, *Gadus morhua* (Refstie et al., 2006a,b). Therefore, even though the enzymatic treatment used in the manufacture of ESBM substantially diminishes the content of important ANF in SBM, it may not translate into improved performance in less sensitive fish species.

Of the ANF found in SBM, protease inhibitors have been studied extensively. The Kunitz soybean TI reduces the activity of trypsin by combining with the enzyme and forming a stable compound whose concentration is directly related to the amount of TI; whereas, chymotrypsin is only slightly inhibited by Kunitz TI (Kunitz, 1947). On the other hand, a molecule of Bowman-Birk trypsin- and chymotrypsin- inhibitor can inhibit one molecule of trypsin and one molecule of chymotrypsin simultaneously (Birk, 1985). Although heat treatment can effectively reduce inhibitor activity in SBM (Kunitz, 1947; Wilson and Poe, 1985; Friedman et al., 1991), the low heat provided by the cold-pelleting of experimental diets evaluated in our study suggests no TI

284 deactivation. In such a case, the estimated TI concentration of the experimental diets ranged from
285 0.14 (ESBM-15) to 0.85 mg g^{-1} (ESBM-90) in Trial I, and were 0.7 and 1.7 mg g^{-1} in the ESBM-
286 70 and SBM-70 diets of Trial II, respectively. These estimated levels were substantially lower
287 than those found detrimental to the performance of channel catfish, *Ictalurus punctatus* (> 2.2
288 mg g^{-1}; Wilson and Poe, 1985), or Atlantic salmon (~5.0 mg g^{-1}; Olli et al., 1994). Therefore,
289 despite the observed linear reduction in the activity of trypsin and chymotrypsin in response to
290 increased dietary levels of ESBM (Fig. 1, A and B), it is questionable whether the observed
291 reductions in red drum performance beyond the MAX $REPL_{ESBM}$ was due to diminished trypsin
292 and chymotrypsin activities. In fact, growth performance of red drum in the SBM-70 treatment
293 of Trial II was unaffected despite of the significantly lower chymotrypsin activity observed.
294 Both β-conglycinin and glycinin are major storage proteins in soybean and are known for
295 being allergenic to animals and humans (Garcia et al., 1997); and their detrimental effects also
296 have been observed in fish (Rumsey et al., 1994; Gu et al., 2014). However, as in the case of TI,
297 the levels of β-conglycinin and glycinin evaluated in those studies were much higher than those
298 estimated for this study: ranging from < 0.01 mg g^{-1} in all ESBM diets to 5 (β-conglycinin) and
299 20.5 (glycinin) in the SBM-70 diet. Thus, in sum, our findings suggest a weak link between red
300 drum performance beyond the MAX $REPL_{ESBM}$ and the estimated levels of ANF in the
301 experimental diets.
302 Despite that both SP-based diets formulated to replace 70% of FM DP supported similar
303 ($Pr > t = 0.106$) protein retention in Trial II, replacing FM DP beyond 64.2% (ESBM inclusion
304 levels of 43%) was detrimental for protein retention in Trial I according to the SOP model. Even
305 less FM DP could be replaced (54.1%) to support similar energy retention - although in this case,
306 dietary DE was estimated base on standard physiological fuel values of 4, 4, and 9 kcal/g for

carbohydrate, protein and lipid, respectively. Nevertheless, these observed responses suggest that assuming constant nutrient and/or energy digestibility based on a previous *in vivo* digestibility assessment may not hold true when dietary levels of a SP far exceeds 30%. Unfortunately, neither nutrient nor energy digestibility of the experimental diets was assessed in Trial I.

In conclusion, the present study demonstrated that either ESBM or SBM can be used in the diet of juvenile red drum replacing up to 70% of FM DP without compromising the overall production performance of the fish. Further research is warranted to improve the nutritional value of PP-based diets in early-stage juvenile red drum.

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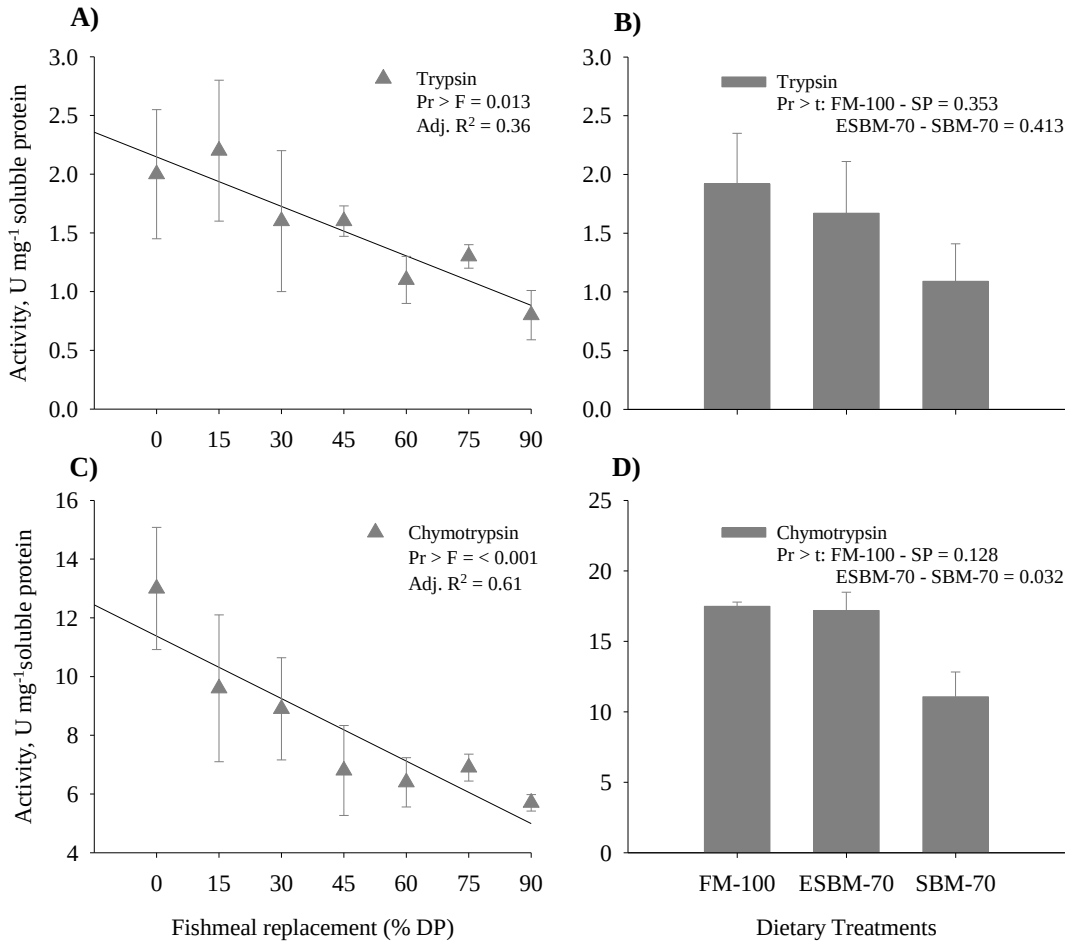
Figure1. Trypsin (A and B) and chymotrypsin (C and D) activities of red drum in Trials I and II, respectively. FM = fishmeal; ESBM = enzymatically processed soybean meal; SBM = soybean meal. Error bars represent standard error (SE).

Figure 2. Regression models of red drum weight gain in response to the replacement of fishmeal (FM) digestible protein (DP) with the enzymatically processed soybean meal (ESBM). Solid line = second order polynomial model ($Pr > F = 0.006$, Adj $R^2 = 0.50$); dashed line = quadratic broken line model ($Pr > F = 0.025$, $R^2 = 0.51$). The maximum replacement value of ESBM for FM DP was estimated in 72 and 60% by each regression model, respectively. Error bars represent standard error (SE).


A)
B)
C)
D)
Trypsin
Pr > F = 0.013
Adj. R2 = 0.36
Trypsin
Pr > t: FM-100 - SP = 0.353
ESBM-70 - SBM-70 = 0.413
Chymotrypsin
Pr > F = < 0.001
Adj. R2 = 0.61
Chymotrypsin
Pr > t: FM-100 - SP = 0.128
ESBM-70 - SBM-70 = 0.032
Activity, U mg-1 soluble protein
Activity, U mg-1soluble protein
Fishmeal replacement (% DP)
FM-100
ESBM-70
SBM-70
Dietary Treatments

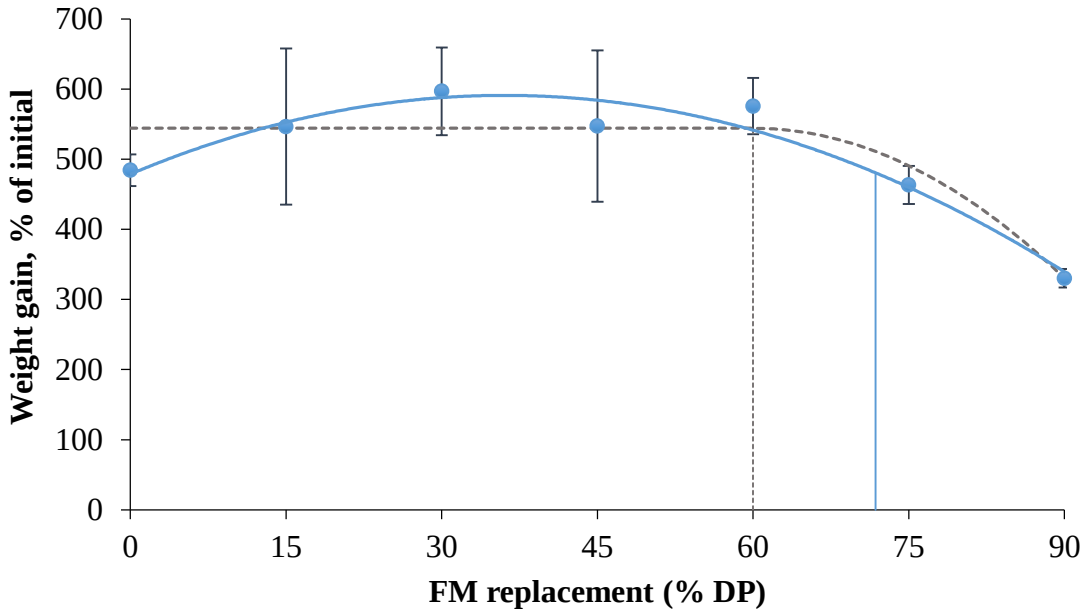

700
600
500
400
300
200
100
0
Weight gain, % of initial
0
15
30
45
60
75
90
FM replacement (% DP)

Table 1 Proximate composition and analyzed anti-nutritional factor content of the soybean products evaluated.

	Proximate composition					Anti-nutritional factors				
	M	OM	CP	L	GE	TI	BC	G	OS	Gal
	%	%	%	%	kJ g^{-1}	mg g^{-1}	ppm	ppm	%	%
		dry matter basis								
ESBM[a]	7.0	93.5	59.8	4.1	15.9	1.4	1.1	2.2	0.9	0.6
SBM[b]	7.5	93.8	53.8	4.7	19.7	3.4	9885.9	41063.2	8.2	-

M = moisture; OM = organic matter; CP = crude protein; L = lipid; GE = gross energy; TI = trypsin inhibitor; BC = beta conglycinin; G = glycinin; OS = oligosaccharides; Gal = galactose.

[a] Enzymatically processed soybean meal; HP300, Hamlet Protein Inc., Findlay, OH, USA. Crude protein = 581.4 g kg^{-1}; Lipid = 41.1 g kg^{-1} (dry-matter basis).

[b] Soybean meal; Producers Coop. Association, Bryan, Texas, USA. Crude protein = 500.8 kg^{-1}; Lipid = 60.2 kg^{-1} (dry-matter basis).

Table 2 Composition of the reference and test diets[1] used in the digestibility trial.

	REF	ESBM	SBM
Ingredients	*% of dry matter[2], otherwise noted*		
Menhaden fishmeal[a]	55.4		
Wheat starch[b]	10.8		
Menhaden oil[a]	3.3		
Vitamin premix[f,g]	2.9		
Mineral premix[f,g]	3.9	70	70
Carboxymethyl cellulose[j]	4.0		
Chromic oxide[h]	1.0		
Celufil[j]	18.7		
ESBM[i]		30	
SBM[k]			30
Analyzed composition			
Moisture	12.5	14.6	12.8
Organic matter	85.8	87.6	88.1
Protein	44.0	49.3	47.3
Lipid	10.6	9.2	9.5
Gross energy (kJ g^{-1})	18.0	18.4	19.2
Ash	15.9	14.4	13.4
Chromic oxide	0.98	0.72	0.72

[1] REF = reference, ESBM = enzymatically processed soybean meal, SBM = soybean meal.
[2] Except moisture.
[a – j] Rossi et al. (2015).
[k] same as [b] in Table 1.
[h] Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.
[i] same as [a] in Table 1.

Table 3 Composition of experimental diets[1] fed to red drum for 6 weeks in Trial I.

	FM-100	ESBM-15	ESBM-30	ESBM-45	ESBM-60	ESBM-75	ESBM-90
Ingredients	*% of dry matter[2], otherwise noted*						
Menhaden fishmeal[a]	56.0	47.6	39.2	30.8	22.4	14.0	5.6
ESBM[b]		10.1	20.1	30.2	40.3	50.3	60.4
Menhaden oil[c]	5.5	6.4	7.3	8.2	9.1	10.0	11.0
Dextrinized corn starch[d]	13.2	11.5	10.0	8.4	6.8	5.2	3.6
Carboxymethyl cellulose[e]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix[d,e]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix[d,e]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Dicalcium phosphate[f]		0.03	1.4	2.9	4.3	5.7	6.6
Glycine[g]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Taurine[g]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lysine HCl[g]		0.08	0.13	0.19	0.24	0.30	0.35
DL-Methionine[g]		0.09	0.16	0.23	0.29	0.36	0.43
Celufil[g]	13.3	12.2	9.6	7.1	4.6	2.1	0.0
Analyzed proximate composition							
Moisture	12.5	11.0	10.7	11.3	10.8	11.3	11.9
Protein	44.5	44.0	43.8	44.0	43.9	44.1	44.2

Lipid	14.1	13.8	14.3	14.2	14.2	14.1	14.2
Ash	14.6	13.5	13.9	14.0	14.0	14.3	14.1
Total phosphorus	2.8	2.4	2.5	2.6	2.7	2.8	2.6
Gross energy (kJ g^{-1})	20.1	20.1	20.1	20.1	20.5	19.7	20.5
Analyzed amino acid composition							
Arg	3.1	3.3	3.7	3.1	3.2	3.7	3.6
His	1.4	1.4	1.6	1.3	1.3	1.5	1.5
Ile	2.4	2.5	2.7	2.2	2.2	2.6	2.4
Leu	4.2	4.4	4.7	3.8	3.9	4.5	4.2
Lys	2.9	3.2	3.4	2.6	2.5	2.9	2.4
Met	1.5	1.4	1.5	1.1	1.0	1.1	0.9
Phe	2.3	2.4	2.7	2.3	2.4	2.9	2.9
Tau	1.8	1.9	1.9	1.4	1.4	1.3	1.4
Thr	2.2	2.3	2.4	1.9	1.9	2.2	2.0
Val	2.7	2.8	3.0	2.4	2.5	2.8	2.6

[1] FM = fishmeal; ESBM = enzymatically processed soybean meal.

[2] Except moisture.

[a] Special Select [TM] Omega Protein Inc., Abbeville, LA, USA. Crude protein = 700.8 g kg^{-1}; Lipid = 116.4 g kg^{-1} (dry matter basis).

[b] Same as [a] in Table 1.

[c] Omega Protein, Reedville, VA, USA.

[d] MP Biomedicals, Solon, OH, USA.

[e] Moon and Gatlin (1991).

[f] Fisher Scientific, Pittsburg, PA, USA.

[g] USB, Cleveland, OH, USA.

Table 4 Composition of experimental diets[1] fed to juvenile red drum for 8 weeks in Trial II.

	FM-100	ESBM-70	SBM-70
Ingredients		*% of dry matter*[2]	
Menhaden fishmeal[a]	57.7	17.1	17.1
ESBM[b]		47.0	
SBM[c]			50.0
Menhaden oil[d]	5.3	9.2	7.2
Dextrinized corn starch[e]	13.2	5.7	5.2
Carboxymethyl cellulose[f]	2.0	2.0	2.0
Vitamin premix[e,f]	3.0	3.0	3.0
Mineral premix[e,f]	4.0	4.0	4.0
Dicalcium phosphate[g]		5.7	5.0
Glycine[h]	2.0	2.0	2.0
Taurine[h]	1.0	1.0	1.0
Lysine HCl[h]		0.3	0.5
DL-Methionine[h]		0.5	0.5
Celufil[h]	11.7	2.6	2.6
Analyzed proximate composition			
Moisture	6.2	5.7	6.0
Protein	43.7	43.6	42.0
Lipid	12.9	13.1	10.7
Ash	15.6	15.7	15.0
Analyzed amino acid composition			
Arg	3.0	4.1	3.6
His	1.4	1.7	1.5
Ile	2.3	2.8	2.5
Leu	4.1	4.9	4.4

Lys	3.0	3.1	3.1
Met	1.4	1.4	1.3
Phe	2.3	3.1	2.7
Tau	1.9	1.6	1.6
Thr	2.2	2.4	2.1
Val	2.7	3.1	2.8

[1] FM = fishmeal; ESBM = enzymatically processed soybean meal; SBM = soybean meal.

[2] Except moisture.

Superscripts: a, d, e = same as in Table 3; b and c = a and b in Table 1; f, g and h = e, f, and g in Table 3.

Table 5 Apparent digestibility coefficient values of the diets and the soybean products.

	Organic Matter	Crude Protein	Lipid	Gross Energy
Diets		*ADC, % of dry matter*		
REF	57.6	80.0	91.7	65.7
ESBM	60.4	80.4	92.1	65.5
SBM	57.8	80.6	91.8	64.7
Ingredients				
ESBM	59.8	81.3	95.0	62.9
SBM	51.7	82.0	92.3	60.9
PSE	*2.420*	*0.818*	*1.373*	*2.890*
Student's t-test (Pr > t)	*<0.001*	*<0.001*	*<0.001*	*<0.001*

ADC = apparent digestibility coefficient; REF = reference; ESBM = enzymatically processed soybean meal; SBM = soybean meal; PSE = pooled standard error of treatment means (n = 2).

Table 6 Growth, feed efficiency, survival, protein and energy retention efficiencies of red drum after 6 weeks of feeding the experimental diets in Trial I.

Treatments[1]	Initial weight g	Final weight g	Weight gain %	FE	Feeding rate % BW d^{-1}	Survival %	PR %	ER %
FM-100	5.7	33.5	484	0.9	4.8	95.0	33.5	32.0
ESBM-15	5.8	37.2	547	1.0	4.8	90.0	35.2	31.7
ESBM-30	5.8	40.0	597	1.0	4.8	90.0	40.0	37.2
ESBM-45	5.8	37.2	547	1.0	4.9	77.5	35.1	31.1
ESBM-60	5.7	38.6	576	0.9	4.8	85.0	35.1	31.5
ESBM-75	5.8	32.4	464	0.8	4.8	85.0	29.9	24.1
ESBM-90	5.7	24.5	330	0.7	4.9	85.0	22.2	17.6
PSE	*0.009*	*1.616*	*26.975*	*0.035*	*0.018*	*1.817*	*1.619*	*1.947*
Regression (n = 2)								
Model		SOP	SOP	SOP	NR	SOP	SOP	SOP
Pr > F		0.006	0.006	0.002	0.562	0.029	0.002	0.005
Adj. R^2		0.51	0.50	0.64	-0.05	0.35	0.86	0.51
MAX REPL (%)		71.8	71.8	63.0			64.2	54.1

[1]FM = fishmeal; ESBM = enzymatically processed soybean meal.

FE = feed efficiency; BW d^{-1} = body weight per day; PR = protein retention; ER = energy retention; PSE = pooled standard error of treatment means (n = 2); SOP = Second order polynomial; NR = no relationship; MAX REPL = maximum replacement.

Table 7 Composition indices and whole-body composition of red drum after 8 weeks of feeding the experimental diets in Trial I.

	Composition Indices			Whole-body Composition[2]			
Treatments[1]	Muscle yield	HSI	IPF ratio	Moisture	Protein	Lipid	Ash
			%				
FM-100	36.4	2.03	0.92	74.6	16.0	5.8	3.7
ESBM-15	35.5	1.91	0.72	74.8	16.1	5.3	3.8
ESBM-30	37.2	1.68	0.90	73.9	16.8	5.9	3.9
ESBM-45	36.7	1.65	0.87	74.4	17.1	5.5	4.8
ESBM-60	38.0	1.57	0.34	74.7	16.3	5.4	4.0
ESBM-75	36.0	1.58	0.51	76.3	15.9	4.3	4.0
ESBM-90	36.7	1.88	0.24	76.8	15.2	4.3	3.8
PSE	*0.243*	*0.065*	*0.074*	*0.312*	*0.181*	*0.231*	*0.104*
Regression (N=2)							
Model	NR	SOP	L	SOP	SOP	SOP	NR
Pr > F	0.410	0.022	< 0.001	0.005	0.004	0.037	0.074
Adj. R^2	-0.02	0.38	0.60	0.51	0.53	0.33	0.24

[1]FM = fishmeal; ESBM = enzymatically processed soybean meal.

[2]Initial whole-body composition (%): Moisture = 74.4; Protein = 16.3; Lipid = 5.8; Ash = 3.6.

HSI = hepatosomatic index; IPF = intraperitoneal fat; FM = fishmeal; ESBM = enzymatically treated soybean meal; PSE= pooled standard error of treatment means (n = 2); NR = no relationship; SOP = second order polynomial; L = linear.

Table 8 Growth, feed efficiency, survival, protein and energy retention efficiencies of red drum after 8 weeks of feeding the experimental diets in Trial II.

Treatments[1]	Initial weight	Final weight	Weight gain	FE	Survival	PR	ER
	g	g	%		%	%	%
FM-100	1.7	33.3	1862	0.97	80.0	37.4	36.9
ESBM-70	1.7	33.5	1924	1.0	78.3	38.9	36.7
SBM-70	1.7	31.5	1764	0.97	81.7	40.7	34.5
PSE	*0.016*	*1.176*	*72.02*	*0.011*	*2.357*	*0.573*	*0.648*
Anova (Pr > F)		*0.795*	*0.718*	*0.560*	*0.880*	*0.037*	*0.277*
Contrasts (Pr > t)							
FM-100 – SP						*0.027*	
ESBM-70 – SBM-70						*0.106*	

[1]FM = fishmeal; ESBM = enzymatically processed soybean meal; SBM = soybean meal.
FE = feed efficiency; PR = protein retention; ER = energy retention; PSE = pooled standard error of treatment means (n = 3); SP = soybean products (ESBM and SBM).

Table 9 Composition indices and whole-body composition of red drum after 8 weeks of feeding the experimental diets in Trial II.

	Composition Indices			Whole-body Composition[2]			
Treatments[1]	Muscle yield	HSI	IPF ratio	Moisture	Protein	Lipid	Ash
				%			
FM-100	37.9	2.60	0.71	73.5	16.8	6.2	3.7
ESBM-70	36.4	2.10	0.47	74.4	17.0	5.7	3.8
SBM-70	35.1	1.55	0.25	73.8	17.5	5.0	3.8
PSE	*0.561*	*0.168*	*0.008*	*0.548*	*0.306*	*0.173*	*0.040*
Anova (Pr > F)	*0.097*	*0.006*	*0.035*	*2.889*	*1.673*	*2.581*	*0.295*
Contrasts (Pr > t)							
FM-100 – SP		*0.004*	*0.021*				*0.015*
ESBM-70 – SBM-70		*0.046*	*0.147*				*0.585*

[1] FM = fishmeal; ESBM = enzymatically processed soybean meal; SBM = soybean meal.
[2]Initial whole-body composition (%): Moisture = 77.0; Protein = 16.2; Lipid = 3.0; Ash = 3.9.
HSI = hepatosomatic index; IPF = intraperitoneal fat; PSE = pooled standard error of treatment means (n = 3); SP = soybean product (ESBM and SBM)