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Partial or total replacement of fish meal in the diets of Florida pompano *Trachinotus carolinus*

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Abstract

Two trials were conducted to evaluate the efficacy of commercial enzyme-treated soy (ESBM) to replace the use of fish meal (FM) in practical diets for Florida pompano *Trachinotus carolinus*. The reference diet which has been run in numerous trials for Florida pompano was formulated using 150, 466 and 80 g/kg of FM, soybean meal and corn protein concentrate respectively. In trial 1, test diets were produced by replacing FM with 30.8, 61.4 and 92.1 g/kg of ESBM. In trial 2, test diets were produced by replacing FM with 28.9, 89.8, 120.1 g/kg of ESBM. Triplicate group of fish in trial 1 (mean weight = 13.05 ± 0.34 g) and trial 2 (mean weight = 18.45 ± 0.49 g) was fed these diets to apparent satiety for 8 weeks. Growth performance was affected as the dietary FM was replaced with ESBM. In trial 1, final weight (FW), percentage weight gain and thermal growth coefficient were lower in 6 g/kg of FM compared to 15 g/kg, while feed conversion ratio significantly higher in fish fed the lowest inclusion level of FM (6 g/kg). In trial 2, FW was significantly lower when FM completely replaced by ESBM and no significant differences in other growth parameters. In all trials, no significant differences were observed in terms of crude protein, moisture, fat, crude fibre, dry matter and ash content of the fish. No significant differences in serum levels of total protein, albumin, alkaline phosphatase, alanine transaminase, aspartate transaminase, glucose and bile acids were observed in either trial. However, in trial 1, serum cholesterol level was higher in fish fed 150 g/kg FM compared to other dietary treatments. The histomorphological structure of liver and distal intestine was slightly affected by lower inclusion level of FM. Overall, there was a decreasing trend in pompano growth performance as the inclusion of FM decreased. All parameters indicated that ESBM could be used to reduce the inclusion of FM from 150 to 90 g/kg.

KEYWORDS
distal intestine, enzyme activities, enzyme-treated soybean meal, Florida pompano, growth parameters, liver, serum

1 | INTRODUCTION

Efforts to formulate economical feed with low level of fish meal (FM) in some aquaculture species have achieved considerable success (Boonyaratpalin, Suraneiranat, & Tunpibal, 1998; Davis & Arnold, 2000; Hardy, 2010; Kaushik, Coves, Dutto, & Blanc, 2004; NRC, 2011). Several alternatives have been used to substantially decrease dietary level of FM in feed formulation, including the use of plant-protein sources that appear to be the most sustainable and renewable ingredient (Alexis & Nengas, 2001; Hardy, 2010;

Kaushik et al., 2004). Among the plant-protein sources, soybean meal (SBM) is the most prominent alternative because of its constant availability, favourable amino acid profile, cost effectiveness and high digestibility properties (Lemos, Ezquerra, & Garcia-Carreno, 2000; NRC, 2011; Suárez et al., 2009). However, deficiency in sulphur amino acids and the presence of anti-nutrients, such as proteinase inhibitors, phytic acid, saponins and anti-vitamins, limits the use of this ingredient and may have detrimental effects on the digestive process and growth of fish (NRC, 2011; Tibaldi et al., 2006). Therefore, the use of highly processed soy protein sources (e.g., de-hulled, solvent-extracted) by commercial feed manufacturers that can meet the dietary requirement has become a norm for many aquaculture species.

Studies conducted with Florida pompano *Trachinotus carolinus* indicated that the combination of solvent-extracted SBM with soy protein concentrate (SPC) was able to reduce the FM level from 30% to 15% without deleterious effect on growth performance (Quintero, Davis, & Rhodes, 2012). The results suggest that blending of conventional SBM with advanced soy products is likely to be a viable strategy to improve the nutritional value of plant-based diet (Lin & Mui, 2017; Rombenso, Crouse, & Trushenski, 2013). Beside SPC, several advanced products of soy protein – such as fermented soybean meal (FSBM) or enzyme-treated soybean meal (ESBM) – have also become commercially available. These products usually contain higher levels of protein, lower oligosaccharides and minimal levels of soy anti-nutritional factors (ANFs) compared to commodity SBM (Novriadi & Davis, 2018a). Thus, proper inclusion of highly processed soy protein could be considered as an alternative to reduce the dietary level of FM in diet formulations.

We have verified that 15% FM protein to maintain 40% crude protein (CP) level as the reference in the development of practical diet for Florida pompano may be considered as the minimum inclusion level that still supports optimum growth of this fish (Quintero et al., 2012; Rossi & Davis, 2012). Continued reduction in FM levels will likely reduce the growth performance of this species (Cook, Zhou, Rhodes, & Davis, 2016). Similarly, partial replacement of FM with conventional SBM reduced the growth of red sea bream *Pagrus major* (Biswas, Kaku, Ji, Seoka, & Takii, 2007). Interestingly, the latter author also indicated that phytase supplementation on SBM-based diet improved feed consumption and growth, as well as yielded similar growth with fish fed FM-based diet. Moreover, Tibaldi et al. (2006) and Refstie, Storebakken, and Roem (1998) indicated that using ESBM to replace dietary FM in practical diets for European sea bass *Dicentrarchus labrax* and Atlantic salmon *Salmo salar* resulted in better growth performance and nutrient utilization in comparison to the use of conventional SBM to replace the use of FM. Hence, such novel ingredients could be useful in further reducing the FM inclusion in the diets.

Other than growth parameters, haematological and histopathological condition of liver and distal intestine of fish can provide valuable information about the physiological effects of specific ingredients (Bonvini et al., 2018; Krogdahl, Penn, Thorsen, Refstie, & Bakke, 2010; S. Lin & Luo, 2011; Zhang et al., 2018). From the work of Lin and Luo (2011) the experimental results indicate that high inclusion level of soy protein in fish diet may affect the normal level of glutamyl oxaloacetic transaminase and glutamyl pyruvic transaminase in the liver. In contrast, Ye, Liu, Wang, and Wang (2011) did not find any significant differences in the serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in Japanese flounder *Paralichthys olivaceus* fed with moderate and high inclusion level of SBM to replace the dietary FM. Overall these findings showed an inconsistency in enzyme activities when dietary FM is replaced by SBM. In addition, the presence of several ANFs in SBM is known to cause alterations in the histomorphological condition of fish liver (Kokou et al., 2015; López, Flores-Ibarra, Bañuelos-Vargas, Galaviz, & True, 2015; Robaina et al., 1998) and intestine (Heikkinen et al., 2006; Krogdahl, Bakke-McKellep, & Baeverfjord, 2003). Previous studies characterized the serum biochemistry, liver and distal intestine histology of Florida pompano as the effect of poultry by-product meal (PBM) and conventional SBM replacement with advanced soy products (Novriadi, Rhodes, Powell, Hanson, & Davis, 2017; Novriadi, Spangler, Rhodes, Hanson, & Allen Davis, 2017). However, to our knowledge, the effects of dietary FM substitution with advanced soy product have not been described in Florida pompano. Thus, the objective of the present study was to analyse the effect of partial and complete replacement of dietary FM with various inclusion levels of ESBM on growth performance, proximate composition of the whole body, amino acids profile, serum and enzyme activities and histomorphological condition of liver and distal intestine of Florida pompano.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

All diets were formulated to contain approximately 400 g/kg crude protein and 80 g/kg lipid. The reference diet in trial 1 was produced utilizing 150 g/kg of FM, 466.0 g/kg defatted SBM and 80 g/kg corn protein concentrate (CPC) based on previous work in developing low-FM diets for this species. Three experimental diets were formulated to include increasing levels of ESBM to reduce dietary FM down to 120, 90 and 60 g/kg (labelled as 12, 9 and 6% FM, Table 1). Diets in trial 2 were formulated based on the results of trial 1: four experimental diets were produced to include the increasing levels (30, 90, 120 and 150 g/kg) of ESBM to partially and completely replace dietary FM in the diet (Table 1). All experimental diets were produced at the aquatic animal nutrition laboratory at the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University (Auburn, AL) using the standard procedures. Briefly, diets were made by mixing pre-ground dry ingredients and fish oil in a food mixer (Hobart, Troy, OH) for approximately 15 min. Boiling water was then blended into the mixture to help ensure the appropriate consistency of the mix for pelleting. The mash from each diet was passed through a 3 mm die in the meat grinder and the pellets were then placed in forced air-drying oven (<45°C) overnight until moisture content of each diet was less than 10%. Subsequently, the diets were crumbled, sieved, packed in plastic bags and stored in freezer (−20°C) until needed.

TABLE 1 Composition (g/kg *as is*) of diets containing various levels of enzyme-treated soy (ESBM) used in both growth trial

	Trial 1				Trial 2			
Ingredients (g kg^{-1}, *as is*)	**15% FM**	**12% FM**	**9% FM**	**6% FM**	**12% FM**	**6% FM**	**3% FM**	**0% FM**
Menhaden Fishmeal[a]	150.0	120.0	90.0	60.0	120.0	60.0	30.0	0.0
Soybean meal[b]	466.0	466.0	466.0	466.0	466.0	466.0	466.0	466.0
Enzyme-treated soy[c]	0.0	30.8	61.4	92.1	28.9	89.8	120.1	150.5
Corn protein concentrate[d]	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
Menhaden Fish Oil[a]	50.0	52.9	55.8	58.7	50.2	54.7	57.0	59.3
Corn Starch[e]	38.5	31.4	24.5	17.5	36.0	23.8	17.3	11.3
Whole wheat[e]	180.0	180.0	180.0	180.0	180.0	180.0	180.0	180.0
Trace Mineral premix[f]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin premix[g]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride[e]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Stay C 35%[h]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic[e]	15.0	18.0	21.0	24.0	18.0	24.0	27.5	30.5
Lecithin (soy commercial)[i]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Methionine[e]	0.0	0.2	0.4	0.6	5.2	5.6	5.8	5.9
Taurine[e]	5.0	5.2	5.4	5.6	0.2	0.6	0.8	1.0
Proximate analyses (g kg^{-1}, *as is*)								
Phosphorus	13.2	12.0	12.3	12.5	11.0	10.8	10.8	10.9
Crude Protein	398.2	385.0	395.0	413.2	417.2	418.8	431.6	425.0
Moisture	64.1	96.1	80.9	81.7	75.9	77.8	60.7	63.9
Crude Fat	96.9	82.4	84.8	82.9	96.3	80.8	87.3	87.4
Crude Fibre	28.9	25.5	26.5	28.8	28.2	28.2	29.7	31.8
Ash	78.6	75.6	71.3	69.3	71.8	67.3	67.0	65.0

[a]Omega Protein Inc., Huston TX, USA. [b]De-hulled Solvent Extracted Soybean Meal, Bunge Limited, Decatur, AL, USA. [c]Nutrivance^{TM}, Midwest Ag Enterprises, Marshall, MN, USA. [d]Empyreal 75^{TM} Cargill Corn Milling, Cargill, Inc., Blair, NE, USA. [e]MP Biomedicals Inc., Santa Ana, CA, USA. [f]ASA Premix (g 100g^{-1} premix): cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250, ferrous sulphate heptahydrate, 4.0, manganous sulphate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193 and α-cellulose 81.826. [g]ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU/g), 2.40; vitamin D_3 (400,000 IU/g), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258. [h]Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA. [i]The Solae Company, St. Louis, MO, USA.

The composition of experimental diets was analysed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate analysis (Table 1) and amino acid profile (Table 2).

2.2 | Growth trials

Both growth trials were carried out at the Claude Peteet Mariculture Center (CPMC), Gulf Shores, AL, USA. Florida pompano fingerlings were obtained from Troutlodge Marine Farms LLC, (Proaquatix) Vero Beach, Florida, USA, nursed in an indoor recirculating system facility of CPMC and fed with commercial diet (FF Starter, Zeigler Bros., Inc. Gardners, PA) until reaching a suitable size. All trials were conducted in a recirculating system consisting of 12 culture open-top circular tanks with volume ±700 L/tank equipped with reservoir tank, biological filter, supplemental aeration (provided using a central line, regenerative blower and air diffusers) and circulation pump. At the start of the trials, 20 fish with average initial weight of 13.05 ± 0.34 g for the first trial and 18.45 ± 0.49 g for the second trial were stocked into each tank and each trial consisted of four treatments each with three replicates in a completely randomized design. Fish from all trials were maintained under a natural photoperiod for 8 weeks. During the trials, fish were fed four times per day on a per cent body weight basis. Fish were bulk-weighed every other week to monitor growth and adjust feeding rations. During the sampling, fish were dipped in chloroquine phosphate (MP Biomedicals, Solon, OH) as a bactericide at the concentration of 60 mg/L followed with a freshwater dip for approximately 1 min to reduce the possibilities of parasitic infection. In both trials, dissolved oxygen (DO), pH, temperature and salinity were measured twice daily by using a water quality multi-parameter instrument (ProPlus, YSI Inc., Yellow Springs, OH). Total ammonia nitrogen was measured two times per week using an

TABLE 2 Amino acid (AA) profile (g/kg, *as is*) of experimental diets for both trials

	Trial 1				Trial 2			
Composition	**15% FM**	**12% FM**	**9% FM**	**6% FM**	**12% FM**	**6% FM**	**3% FM**	**0% FM**
Taurine	7.3	6.1	7.0	6.5	7.1	7.1	7.3	7.1
Hydroxyproline	2.2	1.8	3.8	1.2	1.5	1.9	1.5	0.8
Aspartic acid	36.1	36.8	37.1	39.8	37.5	38.3	40.2	40.2
Threonine	14.1	14.2	14.1	14.8	14.9	14.7	15.2	15.2
Serine	15.7	15.5	16.0	17.0	17.1	17.5	18.3	18.4
Glutamic acid	71.4	70.6	73.1	76.8	76.2	77.4	80.3	80.9
Proline	24.3	22.5	24.2	24.8	25.7	25.3	25.4	25.5
Glycine	19.2	18.7	18.6	18.4	19.1	17.3	17.4	16.7
Alanine	21.7	20.9	20.6	20.3	22.1	20.8	20.8	20.7
Cysteine	5.1	5.2	5.4	5.7	5.2	5.5	5.7	5.7
Valine	19.3	19.2	19.4	20.1	20.5	20.3	21.1	20.3
Methionine	7.3	7.3	7.5	7.4	7.1	7.2	7.3	7.1
Isoleucine	16.8	16.8	17.2	18.1	18.1	18.2	18.8	18.8
Leucine	34.4	33.6	34.2	35.1	37.7	37.2	38.0	38.5
Tyrosine	12.3	10.2	12.8	13.6	15.2	14.6	15.3	15.6
Phenylalanine	19.0	19.1	19.4	20.4	21.2	21.4	22.2	22.4
Hydroxylysine	0.8	0.7	0.7	0.5	1.1	0.9	0.8	0.8
Ornithine	0.3	0.5	0.3	0.3	0.3	0.3	0.2	0.2
Lysine	21.3	21.1	20.9	22.0	22.4	21.9	22.7	22.2
Histidine	9.7	9.5	9.7	10.1	10.0	10.0	10.3	10.2
Arginine	23.2	22.6	24.1	25.6	24.7	24.9	26.2	26.0
Tryptophan	5.1	5.0	5.2	5.6	5.1	5.2	5.6	5.3

ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA) and nitrate-nitrogen was measured once a week using colorimetric test kits (La Motte Chemicals, Chestertown, MD). At the end of the growth trial, fish were fasted overnight, then counted and batch-weighed to calculate the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR), feed intake (FI), protein efficiency ratio (PER) and thermal-unit growth coefficient (TGC).

$$\text{PWG} = \frac{(\text{average individual final weight} - \text{average individual initial weight})}{(\text{average individual initial weight})} \times 100$$

$$\text{FCR} = \frac{\text{feed given (as is g)}}{\text{live weigh gain (as is g)}}$$

$$\text{SR} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

$$\text{FI} = \frac{\text{feed intake (g)}}{\text{fish}}$$

$$\text{TCG} = \frac{\text{FBW}^{1/3} - \text{IBW}^{1/3}}{\sum_d T} \times 100$$

where FBW is final body weight, IBW is initial body weight, T is water temperature (°C) and D is number of trial days.

2.3 | Body composition analysis

Upon termination of all growth trials, four fish from each tank were randomly sampled and stored at −80°C for body composition analysis. Prior to proximate and amino acid analysis, whole body of fish were blended and chopped in a mixer according to the standard methods established by Association of Official Analytical Chemists (AOAC, 2006). Proximate composition and mineral contents of whole pompano body were analysed by Midwest Laboratories (Omaha, NE).

2.4 | Serum biochemistry analysis

At the end of all growth trials, four fish per tank were euthanized after an overnight fast with Tricaine-S (MS-222, tricaine methanesulfonate salt, Western Chemical, Inc., Ferndale, WA) and blood samples were taken from the caudal vein. Blood samples were collected using anticoagulant-free centrifuge tubes. Serum was obtained by centrifugation of blood at 3,000 rpm for 10 min and stored at −80°C pending analysis. Biochemical parameters in the serum samples were analysed using an automatic chemistry analyzer (Cobas C311, Roche

Diagnostics, IN) for total protein, albumin, activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, cholesterol and bile acid concentrations.

2.5 | Histological analysis

Histological analysis was only carried out in trial 1 and samples were randomly collected after an overnight fast with three fish per each treatment tank. Fish were individually dissected to collect the liver and distal intestine. Both organs of approximately 0.5 cm were immediately fixed in Bouin's solution (picric acid-formalin-acetic acid mixture, Ricca Chemical, Arlington, TX) for 20 hr at room temperature and then transferred to 70% ethanol solution (VWR, Radnor, PA) until processed by standard histological analysis procedures. Samples were dehydrated through a standard ethanol series to 100%, embedded in paraffin wax and sectioned at 4 μm intervals for staining with Haematoxylin-Eosin (H&E) stain (Merck, Darmstadt, Germany). Double blinded evaluation with a grading system of 1 (healthy) to 5 (degraded) was used to evaluate the distal intestine condition according to Novriadi, Rhodes et al. (2017). The following parameters were evaluated for distal intestine analysis: the number of goblet cells (GC), level of cellular infiltration (CI) and thickness of the lamina propria within the intestinal folds (WLP). Meanwhile, for the liver, evaluation was focused on presence of hepatocyte vacuolization, change in nucleus and granulation. Histomorphological images were acquired using a microscope (Olympus BX41, Olympus Optical Co., Ltd., Tokyo, Japan).

2.6 | Statistical analysis

Growth parameters, proximate composition and amino acids profile of the whole body, serum levels and enzyme activities between dietary groups for both trials were analysed using regression and one-way analysis of variance (ANOVA) to determine significant differences among treatments followed by Tukey's multiple comparison test to determine the difference between treatment means in each trial. Score data for histomorphological condition of the liver and distal intestine were treated as categorical data, tested for normality and homoscedasticity and subsequently analysed using linear regression model. Analysis of covariance (ANCOVA) was performed to investigate the interaction between FM replacement level and trial. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC).

3 | RESULTS

3.1 | Water quality

For water quality data (mean ± standard deviation) in trial 1, temperature, D.O., salinity, pH, TAN and nitrate-nitrogen were maintained at 27.07 ± 1.99°C, 5.52 ± 0.55 mg/L, 33.73 ± 2.58 ppt, 7.76 ± 0.25,

TABLE 3 Growth performance of juvenile Florida pompano fed experimental diets for 56 days for the first trial (Mean initial weight 13.05 ± 0.09 g) and second trial (Mean initial weight 18.45 ± 0.49 g)

Diet	Final weight (g)	Weight Gain (%)	TGC	Feed intake (g fish^{-1})	FCR	Survival (%)
Trial 1						
15% FM	70.34^{a}	432.73^{a}	0.1183^{a}	96.39	1.69^{a}	98.33
12% FM	70.73^{a}	444.13^{a}	0.1188^{a}	97.07	1.68^{a}	100.00
9% FM	66.33^{ab}	411.98^{ab}	0.1100^{ab}	94.51	1.77^{a}	91.67
6% FM	62.04^{b}	376.45^{b}	0.1070^{b}	90.74	1.86^{b}	95.00
PSE[1]	1.6356	12.2549	0.0022	2.0130	0.0401	2.7639
p-Value	0.0177	0.0201	0.0206	0.1947	0.0445	0.2272
Trial 2						
12% FM	100.24^{a}	442.41	0.1230	138.55^{a}	1.70	98.33
6% FM	92.92^{ab}	405.65	0.1163	132.80^{b}	1.78	100.00
3% FM	91.88^{ab}	393.16	0.1145	132.65^{b}	1.81	100.00
0% FM	88.08^{b}	382.90	0.1118	128.03^{b}	1.83	98.33
PSE	2.2011	18.7670	0.0054	1.1389	0.0516	1.1785
p-Value	0.0260	0.2056	0.1092	0.0014	0.3644	0.5957
Trial effect	<0.0001	0.3512	0.2208	<0.0001	0.2854	0.0785
FM level effect	<0.0001	0.0006	0.0001	<0.0001	0.0022	0.4483
Trial x FM level	0.5426	0.3212	0.1809	0.9762	0.1668	0.1076

Note. Values represent the mean of four replicates. Results in the same columns with different superscript letter are significantly different ($p < 0.05$) based on analysis of variance followed by the Tukey's multiple comparison test. Interaction FM replacement level x trial for both trials was obtained based on ANCOVA analysis.
PSE = Pooled standard error.

TABLE 4 Proximate composition (g/kg, *as is*) of whole body of pompano fed experimental diets for 8 weeks. Results in the same columns with different superscript letter are significantly different ($p < 0.05$) based on analysis of variance followed by the Tukey's multiple comparison test

Treatment	Crude protein	Moisture	Fat	Crude fibre	Dry matter	Ash
First trial[a]						
15% FM	180.5	709.8	67.5	0.60	n. p	30.5
12% FM	179.1	707.8	76.6	0.60	n. p	29.5
9% FM	175.4	716.9	64.3	0.90	n. p	34.8
6% FM	179.8	712.3	64.0	1.00	n. p	33.4
PSE	0.3011	0.5239	0.5967	0.0181	–	0.2149
p-Value	0.6479	0.6573	0.4512	0.2713	–	0.3389
Second trial[b]						
12% FM	178.0	700.0	70.3	n. p	30.00	36.8
6% FM	196.0	708.7	70.1	n. p	29.13	23.5
3% FM	178.7	723.3	66.1	n. p	27.67	25.4
0% FM	194.7	707.0	60.7	n. p	29.30	29.9
PSE	0.5313	1.0621	0.6017	–	1.0621	0.5994
p-value	0.0727	0.5037	0.6545	–	0.5037	0.4526

Note. n. p = analysis was not performed.
[a]Analysed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). [b]Analysed at Midwest Laboratories, Inc. (Omaha, NE, USA).

TABLE 5 Amino acids analysis (g/kg, *as is*) of whole fish from trial 1

Composition[a]	Initial	15% FM	12% FM	9% FM	6% FM	PSE	*P*-value
Taurine	1.2	3.7	3.8	3.8	3.8	0.0050	0.5570
Hydroxyproline	3.2	3.4	3.9	4.0	4.5	0.0323	0.2185
Aspartic acid	13.0	15.5	15.5	15.0	15.0	0.0268	0.4076
Threonine	6.0	7.2	7.2	6.9	7.0	0.0117	0.3319
Serine	5.4	6.3	6.4	6.2	6.2	0.0091	0.6996
Glutamic acid	19.1	22.5	22.7	22.1	21.9	0.0388	0.4737
Proline	7.7	8.4[b]	9.4[ab]	9.1[ab]	9.8[a]	0.0268	0.0325
Glycine	13.4	13.2	15.1	14.4	16.4	0.0752	0.0925
Alanine	9.6	11.1	11.8	11.3	12.0	0.0310	0.2267
Cysteine	1.3	1.5	1.5	1.5	1.5	0.0041	0.6265
Valine	6.9	8.5	8.6	8.2	8.2	0.0139	0.1723
Methionine	4.0	4.7	4.7	4.6	4.7	0.0091	0.6996
Isoleucine	6.1	7.3	7.2	6.9	6.8	0.0155	0.1904
Leucine	10.0	12.3	12.0	11.6	11.5	0.0234	0.1370
Tyrosine	33.	5.3	4.9	4.9	4.8	0.0164	0.1942
Phenylalanine	5.7	6.8	6.7	6.5	6.5	0.0099	0.2428
Hydroxylysine	0.5	0.5	0.6	0.6	0.6	0.0033	0.2679
Lysine	10.9	13.4	13.4	13.0	12.9	0.0250	0.3267
Histidine	2.9	3.8	3.7	3.6	3.5	0.0058	0.0639
Arginine	9.1	10.9	11.3	11.0	11.5	0.0230	0.2590
Tryptophan	1.1	1.7	1.7	1.6	1.6	0.0053	0.9375

Note. Values represent the mean of four replicates. Results in the same row with different superscript letter are significantly different ($p < 0.05$) based on analysis of variance followed by the Tukey's multiple comparison test.
[a]Analysed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

TABLE 6 Effect of different diets on serum levels and enzyme activities in Florida pompano in all trials

Diets	Total protein (g dL^{-1})	Albumin (g dL^{-1})	ALP (U L^{-1})	ALT (U L^{-1})	AST (U L^{-1})	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Bile acid (mg dL^{-1})
First trial								
15% FM	4.61	1.52	46.07	<5.00	69.00	214.67	236.33[a]	4.10
12% FM	4.21	1.36	35.53	<5.00	65.00	239.67	217.33[ab]	3.73
9% FM	3.92	1.27	40.70	<5.00	30.00	232.67	192.67[b]	3.80
6% FM	4.29	1.39	41.57	<5.00	87.00	215.00	211.67[ab]	3.67
PSE	0.1982	0.0638	3.3131	>0.0000	13.0356	20.9649	7.6014	0.6183
p-Value	0.1800	0.1295	0.2438	>0.0000	0.0764	0.7811	0.0230	0.9603
Second trial								
12% FM	4.33	1.37	28.87	15.67	99.67	219.00	176.33	3.47
6% FM	4.27	1.33	26.67	10.33	87.33	198.33	166.33	3.20
3% FM	4.37	1.33	25.00	12.00	113.00	207.00	158.00	3.93
0% FM	4.33	1.33	28.17	19.67	174.67	231.33	170.00	5.60
PSE	0.1453	0.0527	1.7166	3.3666	31.2352	18.1082	8.0037	1.4136
P- value	0.9672	0.9578	0.4414	0.2825	0.2773	0.6158	0.4760	0.6442

Note. Values represent the mean of four replicates. Results in the same columns with different superscript letter are significantly different ($p < 0.05$) based on analysis of variance followed by the Tukey's multiple comparison test.
ALP = Alkaline phosphatase; ALT = Alanine transaminase; AST = Aspartate transaminase; PSE = Pooled standard error.

0.13 ± 0.23 mg/L and 57.69 ± 48.48 mg/L respectively. In trial 2, temperature, D.O, salinity, pH, TAN and nitrate-nitrogen were maintained at 28.65 ± 1.15°C, 5.53 ± 0.39 mg/L, 33.04 ± 2.56 ppt, 7.83 ± 0.19, 0.05 ± 0.05 mg/L and 74.80 ± 29.84 mg/L respectively.

3.2 | Growth trials

For trial 1, survival was over 90% and no significant differences were observed in terms of feed intake. However, fish fed the lowest inclusion level of FM (FM 6%) had a significantly lower FW, PWG and TGC. In addition, fish fed 6% FM had the highest value for FCR (Table 3). For trial 2, there were no significant differences in terms of survival, PWG, TGC and FCR. However, fish fed with completely free FM had the lowest final weight compared to other dietary treatment (Table 3).

3.3 | Body composition analysis

There were no significant effects of dietary FM replacement on crude protein, fat content, crude fibre, dry matter, moisture and ash content for both trials (Table 4). Meanwhile for the amino acids profile in trial 1, as the dietary FM decreased, the level of proline in the whole body of pompano tended to increase (Table 5).

3.4 | Serum biochemistry analysis

Table 6 shows results of serum levels and enzyme activities as the effect of dietary FM replacement in both trials. In trial 1, there were no significant effects of dietary FM replacement with ESBM on the level of total protein in the serum, albumin, glucose, bile acids, ALP, ALT and AST activities. However, the 15% FM had the highest total

TABLE 7 Linear regression relationship between different inclusion of FM and liver and distal intestine condition. Results presented as the average score of microscopic observation ($n = 3$)

	Liver			Distal intestine		
Diets	Granulation	Inflammation	Nuclear change	Number of goblet cells	Cellular infiltration	Thickness of Lamina propria
15% FM	2.674	2.604	2.875	2.889	2.882	2.958
12% FM	2.715	2.674	2.750	2.799	2.931	3.021
9% FM	2.799	3.118	2.938	3.174	3.257	3.688
6% FM	3.104	3.063	3.021	3.278	3.528	3.271
p- value	0.0206	0.0805	0.2104	0.0719	0.0008	0.0667
Slope	−0.0458	−0.0607	−0.0208	−0.0514	−0.0755	−0.0535
Intercept	3.3044	3.5016	3.1146	3.5743	3.9419	3.7959

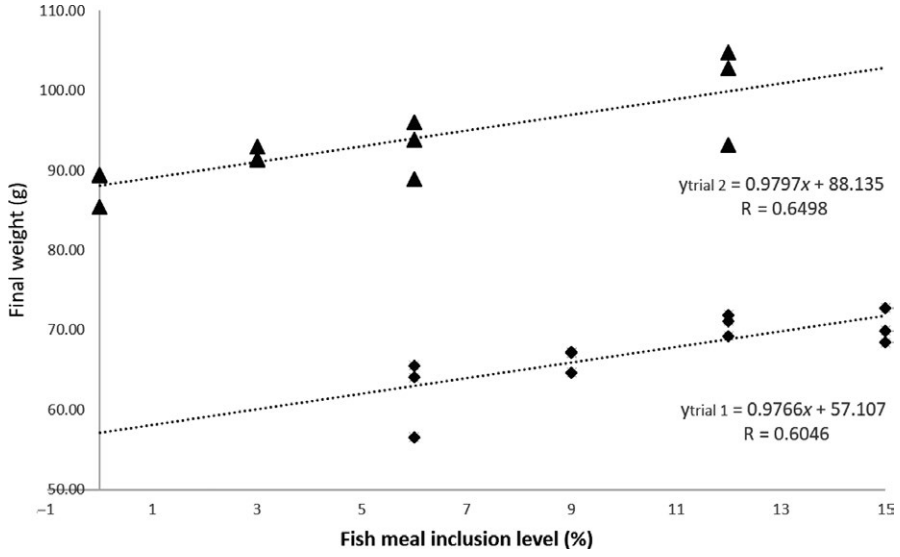

FIGURE 1 Relationship between final weight (y) and replacement level of FM (x) in both trials. Trial 1 is described by $y = 0.9766x + 57.107$ and trial 2 with $y = 0.9797x + 88.135$

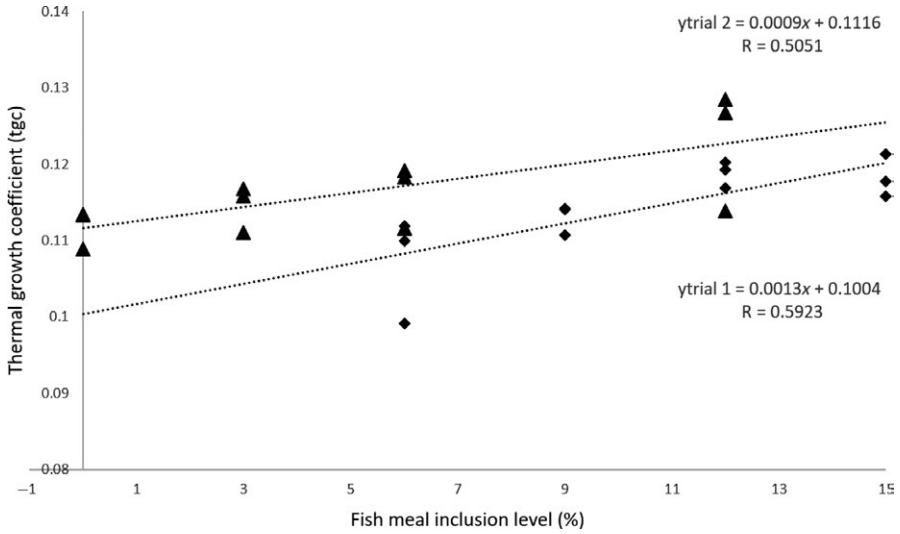

FIGURE 2 Relationship between thermal growth coefficient (y) and replacement level of FM (x) in both trials. Trial 1 is described by $y = 0.0013x + 0.1004$ and trial 2 described by $y = 0.0009x + 0.1116$

cholesterol level compared to 9% FM. In trial 2, partial and complete replacement of FM with ESBM did not have a significant effect on serum levels and enzyme activities.

3.5 | Histological analysis

Sections of the liver and distal intestine of Florida pompano fed with various replacement level of FM with ESBM for 8 weeks are shown in Figures 3 and 4. Linear regression modelling suggested that severe condition of pompano liver as indicated by the increasing incidence of granulation and inflammation was present as the inclusion of dietary FM further replaced by ESBM (Table 7). A similar condition was also observed in the distal intestine of pompano where the level of cellular infiltration into lamia propria was higher in fish fed with lower level of dietary FM. Based on the microscopic observations, there is a tendency towards severe condition as the inclusion of FM further replaced by EBM.

3.6 | Regression and ANCOVA analysis

Replacement of dietary FM was negatively correlated with FW and TGC in trial 1 and 2. The regression line for FW in trial 1 described by $y = 0.9766x + 57.107$ ($R^2 = 0.6046$, p-value = 0.0177) and trial 2 $y = 0.9797x + 88.135$ ($R^2 = 0.6498$, p-value = 0.0260) (Figure 1). Meanwhile for TGC, regression line for trial 1 described by $y = 0.0013x + 0.1004$ ($R^2 = 0.5923$, p-value = 0.0206) and trial 2 with $y = 0.0009x + 0.1116$ ($R^2 = 0.5051$, p-value = 01,092) (Figure 2). Based on the ANCOVA results, there were no significant interactions between the FM replacement level and different trial on the growth performance of fish (Table 3).

4 | DISCUSSION

In this study, the reduced growth performance of Florida pompano when 15% inclusion level of FM further replaced with soy protein,

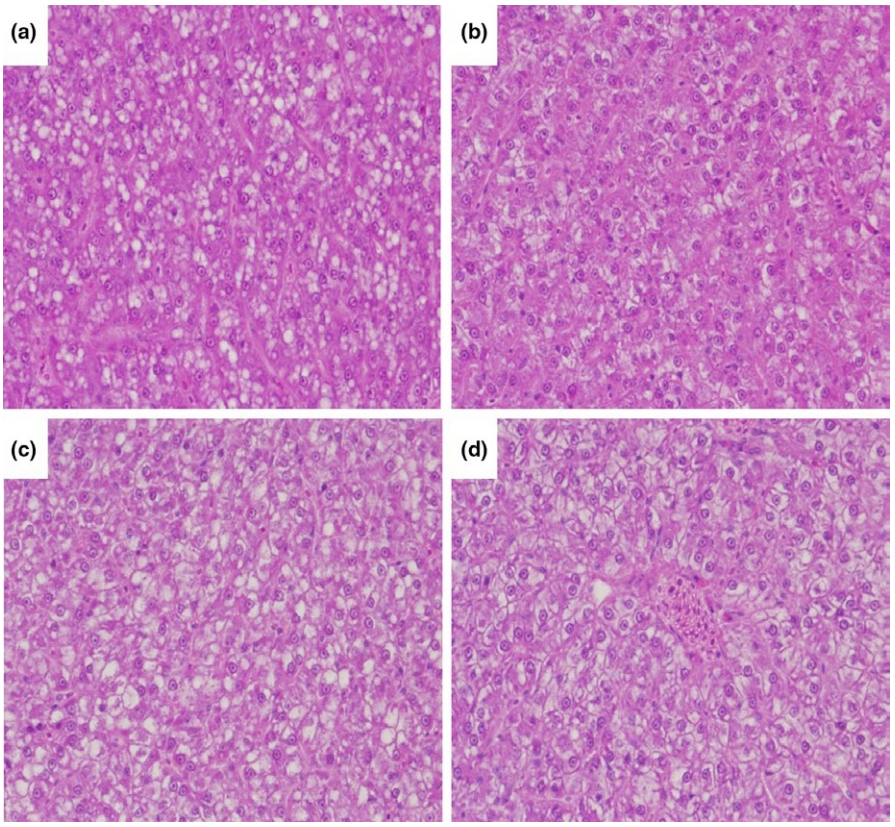

FIGURE 3 Representative histopathological images of haematoxylin and eosin-stained sections of liver from Florida pompano in trial 1 after 56 d of being fed with (a) 15% FM, (b) 12% FM, (c) 9% FM and (d) 6% FM

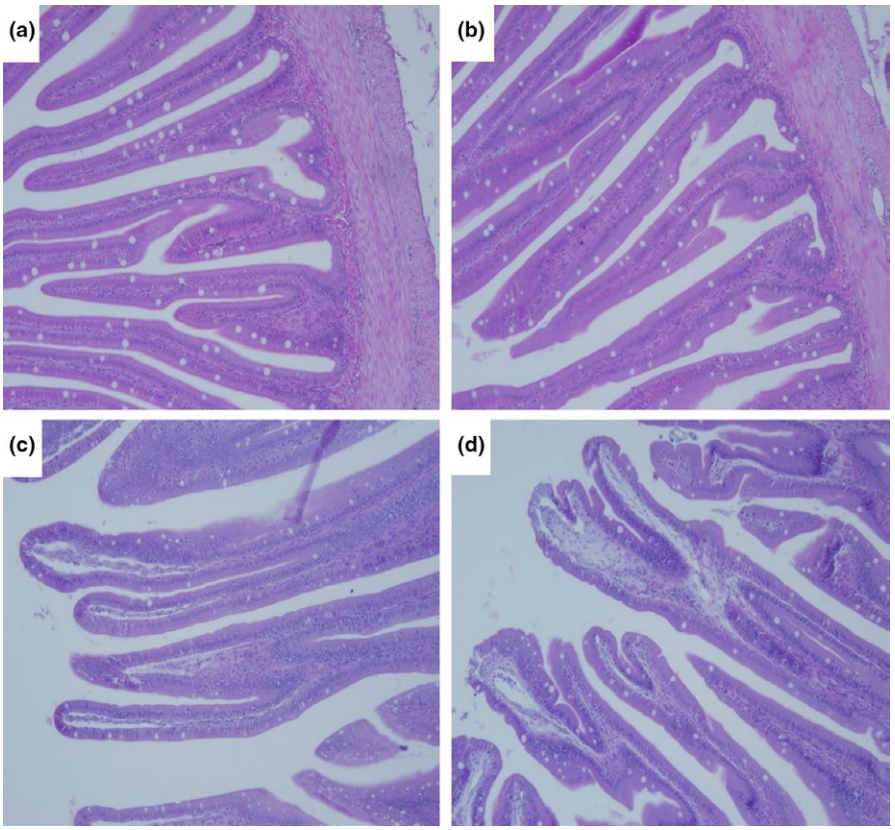

FIGURE 4 Representative histopathological images of haematoxylin and eosin-stained sections of distal intestine from Florida pompano in trial 1 after 56 d of being fed with (a) 15% FM, (b) 12% FM, (c) 9% FM and (d) 6% FM

confirmed the observations of the previous study as demonstrated by Quintero et al. (2012). In trial 1, a gradual reduction in FW is consistent with decreasing number of TGC and higher FCR. The linear regression confirmed this decreasing trend as the FM inclusion level reduces below 15%. Meanwhile, in trial 2, significant reduction in growth parameters only observed in FW when dietary FM was completely replaced with ESBM. High variation in growth outcomes resulted in the absence of significant differences among the dietary treatments in trial 2, including TGC. According to NRC (2011), reduction in growth performance is often associated with the lower feed intake (FI) as the effect of increasing plant-protein sources in the diet. However, in the present study, no significant difference was observed when dietary FM reduced from 15% to 6% in trial 1. Further reduction in FI only occurred in trial 2 when dietary FM was reduced from 12% to 6, 3 and 0%. The results suggest that at certain level, proper combination of ESBM and SBM still provides good palatability to the diets. Interestingly, we could observe a discrepancy between trials, where in trial 1 the decreasing trend occurs as the inclusion level of FM was decreased below 6%, while in trial 2, the significant reduction, especially in final weight, only occurred when dietary FM was completely replaced by ESBM. According to Dabrowski, Poczyczynski, Köck, and Berger (1989) initial size of the fish could influence the fish growth rate and optimum utilization of soy protein during the growth trial. In their trial, larger fish favour the utilization of SBM compared to small fish. Therefore, the difference in stocking size in the present study could explain the different growth response of pompano to the high inclusion level of SBM during the growth trial.

Several alternative protein sources have been evaluated in Florida pompano diet to gradually replace dietary FM, such as cottonseed products (Cook et al., 2016) and poultry by-product meal (Rossi & Davis, 2012), but none of them showed significant differences in terms of crude protein, crude fat, moisture and ash content in the whole body of pompano. In line with previous studies, various levels of ESBM to replace dietary FM in this study also did not show any significant differences in terms of proximate composition of the whole body of pompano. In addition, the dietary treatment in this study also did not have any significant effect on amino acid composition. This observation points out the need for further studies on the effect of plant-protein sources on the nutritional quality of fish beyond the typical growth period for pompano nutrition study.

Recently, blood biochemical evaluation has received considerable attention towards the development of aquafeed, especially for the clinical assessment of specific novel ingredients (Ilham & Fotedar, 2017; Norag et al., 2018; Wang et al., 2018). Previous findings when fish fed with various levels of ESBM (102.2–148 g/kg) to replace 150 g/kg dietary PBM supplemented with squid hydrolysates and squid meal did not show any clinical differences for total protein, albumin, glucose, cholesterol, bile acids, ALP, AST and ALT enzyme activities among the dietary treatments (Novriadi, Spangler et al., 2017). Additional studies with the replacement of conventional SBM with various level of commercial FSBM also did not show any significant differences in all observed serum and enzyme activities parameters (Novriadi, Rhodes et al., 2017). Our results in the present study largely confirm the PBM replacement study where the various replacement of dietary FM with ESBM did not cause any significant effect on the total protein, albumin, ALP, ALT, AST, glucose and bile acids contents. They only differed in blood cholesterol levels, where fish fed 15% dietary FM had significantly higher cholesterol level than fish fed higher inclusion level of soy protein. According to Zhu et al. (2017) the use of plant-protein sources to replace the animal meal in rainbow trout *Oncorhynchus mykiss* feed will greatly reduce the level of cholesterol supplied by the feed. In addition, there was a tendency towards the inhibition of cholesterol intestinal absorption due to the presence of phytosterols, which are predominant sterol forms in plant ingredients and thus leads to a lower cholesterol level in the plasma (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000; Thrall, Weiser, Allison, & Campbell, 2012). A similar result was obtained by Romarheim et al. (2006) where plasma cholesterol levels were lower in rainbow trout *Oncorhynchus mykiss* fed diets containing SBM and white flakes as an intermediate product in the production of SPC and soy protein isolates than in fish fed dietary FM. Based on these facts, lower cholesterol level in fish fed with high inclusion level of soy protein should not come as a surprise. However, in the second trial, none of these parameters showed any clinical differences among the dietary treatments. The results suggest that the use of ESBM in practical diet to replace FM did not cause any obvious alterations in the blood and serum composition of Florida pompano.

Several fundamental studies indicated that the higher inclusion of plant-based diet will probably cause morphological changes in the intestine and liver of farmed fish (Bureau, Harris, & Cho, 1998; Ostaszewska, Dabrowski, Palacios, Olejniczak, & Wieczorek, 2005; Sitjà-Bobadilla et al., 2005; Trushenski, 2015). In this study, as the fish received diets with high inclusion level of soy protein, there were marked increases in the frequency of granulation and liver inflammation (Figure 3). In terms of distal intestine structure, there were slight increases in the number of goblet cells and cellular infiltration in the lamina propria (Figure 4). These results tie well with previous studies wherein the complete replacement of 15% PBM with ESBM slightly increased the glycogen deposition and inflammation with nuclear change (Novriadi, Spangler et al., 2017). In addition, still from the same study, the number of goblet cells slightly increased in the distal intestine of pompano fed with plant-based diet with an increase in cellular infiltration compared to fish fed 15% PBM. However, the nutritional effect of soy protein to the distal intestine of pompano is not clear since the complete replacement of animal meal and conventional SBM with ESBM in the comparative effect study did not cause any significant alterations compared to the reference diet (Novriadi, Spangler, & Allen Davis, 2018b).

5 | CONCLUSION

Integrated evaluation of novel ingredients by means of growth trial, proximate and amino acid composition of the whole body, serum and enzyme activities in the blood of fish together with microscopical

methods for liver and distal intestine tissue analysis provides a comprehensive and reliable assessment of its nutritional effect. In this study, ESBM can be used to reduce the dietary FM from 15 to 9 g/kg in the development of practical diet for pompano containing 466 g/kg of conventional SBM and 80 g/kg CPC. However, further studies are required aiming to investigate the inclusion effect of ESBM to replace dietary FM for long-term growth period beyond the current growth trial.

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