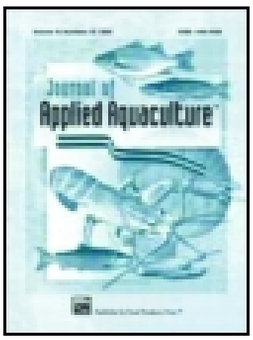

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Growth, body composition, and distal intestine histology of Florida pompano *Trachinotus carolinus* in response to dietary inclusion of hydrolyzed salmon meal and pH adjustment

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ABSTRACT

In this study, we aimed to investigate the inclusion effect of hydrolyzed salmon meal (HSM) in combination with enzyme-treated soybean meal (ESBM) with or without pH adjustment to completely replace dietary FM on the growth, body composition, and the distal intestine condition of the fish. For that purpose, four experimental diets were formulated to be iso-nitrogenous and iso-lipidic and to contain 400 g kg^{-1} crude protein and 80 g kg^{-1} lipid. A reference diet contained 150 g kg^{-1} FM, 450 g kg^{-1} soybean meal (SBM), and 39.5 g kg^{-1} ESBM, and the second diet was formulated replacing FM with 190.6 g kg^{-1} ESBM. The third diet was produced by supplementing 40 g kg^{-1} hydrolyzed salmon meal (4% HSM) into the diet formulation at the expense of ESBM, and the fourth diet was produced similar with the third diet, but the pH of the diet was adjusted to match the value in the reference diet (4% HSMA). All test diets were supplemented with taurine at the level of 5 g kg^{-1}. Diets were fed to apparent satiation to triplicate groups of Florida pompano juveniles (mean weight 11.88 ± 0.18 g). After 8 weeks of growth, there were no significant differences in the final weight, percentage weight gain, thermal growth coefficient, feed intake, survival rate, and feed conversion ratio in fish offered the various diets. Nor were significant differences observed in the dry matter composition of the fish in all treatments and only minor changes in the histomorphological of distal intestine as the FM was replaced by plant ingredients. Overall, the adjustment of pH level of the diet containing 4% HSMA did not have any significant effect on the growth and physiological function of fish compared to the group of fish fed with diet without pH adjustment.

KEYWORDS

Enzyme-treated soy; Florida pompano; growth parameters; distal intestine; pH adjustment

Introduction

Recently, increasing attention has been paid to the role of soybean meal (SBM) as an alternative to expensive marine ingredients, such as fish meal (FM), in the development of practical diets for most species in the aquaculture industry

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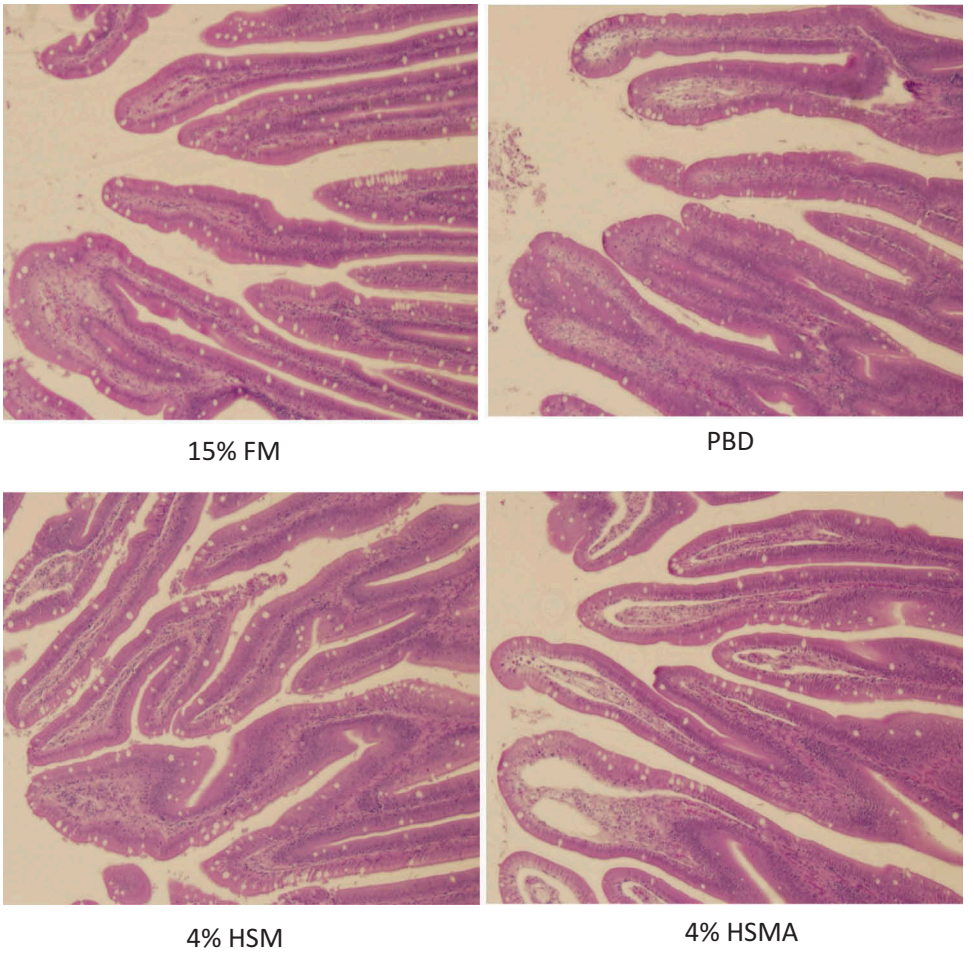

Figure 1. Representative histomorphological images from hematoxylin and eosin-stained sections of the distal intestine of pompano depicting the different degree of cellular infiltration (CI), lamina propria structure, and number of goblet cells in pompano fed the reference and test diet. Abbreviations: 15% FM = 15% fish meal diet; PBD = plant-based diet, complete replacement of FM with ESBM, 4% HSM, 4% inclusion level of hydrolyzed salmon meal to PBD diet, and 4% SPHA, 4% inclusion level of hydrolyzed salmon meal to PBD diet with pH adjustment.

(Gallagher 1994; Lim, Lee, and Webster 2008; Tacon and Metian 2008; Watanabe 2002). According to the meta-analysis study carried out by Sales (2009), up to 40% of FM protein can be replaced by soy protein without any deleterious effect on the growth of fish. However, because the expansion of the aquaculture industry has also been accompanied by an increasing demand for aqua feed (Gatlin et al. 2007), and because there is a limited supply of FM in the market (Hardy 2010), incorporation of higher inclusion levels of plant ingredients, especially soy protein, is necessary to produce sustainable and economically sound practical diets in the future (Gatlin et al. 2007; Rumsey 1993). Considering the anti-nutritional factors (ANFs) that might produce negative effects on the

growth, feed efficiency, digestive process, and health status of fish (NRC 2011), soy protein needs to be further processed to remove endogenous substances and to enhance the bioavailability of their nutritious components.

Advanced soy products, which are produced through a further processing of SBM, flakes, cake, or chips by using several extraction processes or by microbial fermentation, have been well investigated and are known to have a lower level of ANFs and better nutritional quality in the finished products than conventional soy (Bi et al. 2015; Chi and Cho 2016; Kumar et al. 2002). Several advanced soy products are currently being used in the development of aqua feeds, including soy protein isolates (SPI), soy protein concentrates (SPC), fermented soybean meal (FSBM), and enzyme-treated soy (ESBM) (Novriadi et al. 2018a, 2017; Salze et al. 2010; Tibbetts, Milley, and Lall 2006). In our laboratory, replacement of FM in the practical diets designed for Florida pompano has been conducted with several advanced soy products, including SPC, FSBM, and ESBM. Results from Quintero, Davis, and Rhodes (2012) demonstrated that the initial replacement of FM with SPC from 30% to 15% did not cause any significant effect on the growth of pompano. However, the later author Quintero, H.E., Davis, D.A., and Rhodes (2012) also mentioned that further replacement of dietary FM with SPC below 15% negatively affects the growth performance of pompano. Likewise, in our recent comparative study, the complete replacement of FM and dietary poultry by-product meal (PBM) with ESBM also yielded a decreasing trend in the growth of pompano (Novriadi et al. 2019, 2017). However, in the PBM study, the inclusion of squid hydrolyzates (SH) at the level of 4% into the plant-based diet was able to improve the nutritional quality and palatability of the diet and yielded similar growth performance as compared to fish fed with 15% PBM (Novriadi et al. 2017).

There are yet no clear explanations about the role of hydrolyzed protein in enhancing the growth performance of the fish, but it has been used as an intact protein in aquaculture feeds mainly to balance the levels of essential amino acids and to enhance the attractability of the diet and growth of fish (Kotzamanis et al. 2007; Moon and Gatlin 1989). In addition, protein hydrolyzates derived from fish by-products have also shown numerous bioactivities such as anti-oxidative activities and antihypertensive, antithrombotic, and immunomodulatory functions (Bogwald et al. 1996; Choi et al. 2000). The study of Gbogouri et al. (2004) indicated that salmon by-product hydrolyzates obtained by proper enzymatic treatment could also be used in a variety of food formulations due to the high solubility properties that lead to greater emulsification capacity, emulsion stability, and fat absorption. Since the active peptides from the hydrolyzation process of salmon meal also showed anti-inflammatory activity (Ahn, Je, and Cho 2012), the use of this ingredient might have potential benefits to prevent alteration in the digestive system of fish.

As for fish health, several studies revealed that the use of a high inclusion level of soy protein might induce inflammatory reactions in the intestine of some species of fish (Baeverfjord and Krogdahl 1996; Knudsen et al. 2007; Olsen et al. 2007; Urán et al. 2008; Van den Ingh et al. 1991). However, the severity of this inflammation, which is characterized by widening and shortening of the mucosal folding and infiltration of inflammatory cells in the lamia propria (LP) (Knudsen et al. 2007; Refstie et al. 2000), differs between species (Urán et al. 2008). Our findings with pompano indicate that the severity might depend on the type and inclusion level of soy protein. The inflammatory reactions will gradually decrease as the inclusion of fermented soybean meal (FSBM) increases (Novriadi et al. 2018a), and the use of high inclusion levels of ESBM was able to prevent alterations in the distal intestine and have a similar condition with fish fed 15% animal meal (Novriadi, Spangler, and Allen Davis 2018b). However, based on our unpublished work, the inclusion of ESBM and FSBM could considerably shift the pH of the feed. Since pH has been shown to have an effect on nutrient utilization (Wilson, Harding, and Garling 1977), proper adjustment of pH levels could become one strategy to improve diet quality.

Until now, the effect of combining hydrolyzed salmon meal (HSM), ESBM, and amino acid supplementation in pompano has not been established yet. Furthermore, there is limited information on the effects of pH adjustment in this type of diet on the growth and health status of pompano. The objectives of this study were to (1) validate the potential of a plant-based diets; and (2) investigate the efficacy of ESBM supplemented with HSM with or without pH adjustment to completely replace dietary FM on the growth, body composition, and distal intestine histomorphological condition of the fish.

Material and methods

Experimental diets

The diets were produced at the Laboratory of Aquatic Animal Nutrition, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, AL, USA. All diets were formulated to contain approximately 400 g kg^{-1} crude protein and 80 g kg^{-1} lipid (Table 1). All diets contained 450 g kg^{-1} dehulled solvent-extracted SBM (Bunge Limited, Decatur, AL, USA), 80 g kg^{-1} corn protein concentrate (CPC, Empyreal 75^{TM}, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA), and 140 g kg^{-1} of whole wheat (MP Biomedicals Inc., Santa Ana, CA, USA). The combination of 150 g kg^{-1} of FM (Omega Protein Inc., Houston, TX, USA) and 39.5 g kg^{-1} ESBM (NutriVance^{TM}, Midwest Ag Enterprises, Marshall, MN, USA) was used as the primary protein source in the reference diet (15% FM). Dietary FM was replaced by ESBM to produce a plant-based diet (PBD) containing

Table 1. Composition (g kg^{-1} as is) of experimental diets fed to juvenile Florida pompano for 56 days.

Ingredients (g kg^{-1}, as is)	15% FM	PBD	4% HSM	4% HSMA
Menhaden fish meal[1]	150.0	0.0	0.0	0.0
Hydrolyzed salmon meal[2]	0.0	0.0	40.0	40.0
Soybean meal[3]	450.0	450.0	450.0	450.0
Enzyme-treated soy[4]	39.5	190.6	151.2	151.2
Corn protein concentrate[5]	80.0	80.0	80.0	80.0
Menhaden fish oil[1]	54.7	66.1	66.7	66.7
Corn starch[6]	43.3	9.8	9.4	9.4
Whole wheat[6]	140.0	140.0	140.0	140.0
Trace mineral premix[7]	2.5	2.5	2.5	2.5
ASA vitamin premix w/o choline[8]	5.0	5.0	5.0	5.0
Choline chloride[6]	2.0	2.0	2.0	2.0
Stay C 35%[9]	1.0	1.0	1.0	1.0
CaP-dibasic[6]	22.0	40.0	40.0	40.0
Lecithin (soy commercial)[10]	5.0	5.0	5.0	5.0
Lysine[6]	0.0	1.7	1.2	1.2
Methionine[6]	0.0	1.3	1.0	1.0
Taurine[6]	5.0	5.0	5.0	5.0
Proximate analyses (g kg^{-1}, as is)				
Crude protein	421.4	423.8	420.7	420.5
Moisture	66.9	58.6	64.2	59.7
Crude fat	88.1	88.0	89.6	89.8
Crude fiber	27.0	31.7	28.1	31.1
Ash	78.2	70.4	73.3	77.4

[1]Omega Protein Inc., Houston, TX, USA.
[2]Amino salmon P60, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.
[3]De-hulled Solvent Extracted Soybean Meal, Bunge Limited, Decatur, AL, USA.
[4]Nutrivance™, Midwest Ag Enterprises, Marshall, MN, USA.
[5]Empyreal 75™ Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
[6]MP Biomedicals Inc., Santa Ana, CA, USA.
[7]ASA Premix (g 100 g^{-1} premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250, ferrous sulfate heptahydrate, 4.0, manganous sulfate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193, and α-cellulose 81.826.
[8]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^{-1}), 2.40; vitamin D_3 (400,000 IU g^{-1}), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.
[9]Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.
[10]The Solae Company, St. Louis, MO, USA.

190.6 g kg^{-1} of ESBM. The next two diets were formulated to contain 40 g kg^{-1} of hydrolyzed salmon meal (HSM, Amino Salmon P60, Fiordo Austral Company, Cardonal, Puerto Montt, Chile), designated as 4% HSM, at the expense of ESBM. Additionally, the pH of the fourth diet was adjusted to match the value in the reference diet of 15% FM (pH: 6.47) with an addition of sodium hydroxide (NaOH, MP Biomedicals Inc., Santa Ana, CA, USA) solution and designed as 4% HSMA, with no pH adjustment in diet 3 (4% HSM, pH: 6). All diets were supplemented with 5 g kg^{-1} of taurine, and three experimental diets were supplemented with various levels of L-lysine (Lys) and DL-methionine (Met) (MP Biomedicals Inc., Santa Ana, CA, USA) to match the calculated levels in 15% FM as the reference diet. The diets were

prepared by mixing preground dry ingredients along with Menhaden fish oil (MFO) in a food mixer (Hobart, Troy, OH, USA) for approximately 15 min. Boiling water was then blended into the mixture to attain an appropriate consistency for pelleting. The moist mash from each diet was passed through a 3.0-mm die in a grinder. Wet diets were then placed into a fan-ventilated oven (<45°C) overnight to attain a moisture content of less than 10%. Diets were stored at −20°C. Proximate (Table 1) and AA profile (Table 2) of the diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Experimental conditions

Florida pompano (*Trachinotus carolinus*) juveniles were obtained from brood stock spawned at the Claude Peteet Mariculture Center (CPMC), Gulf Shores, AL, USA. The fish were acclimatized for 3 weeks to the experimental facilities at CPMC and fed with commercial diet (FF Starter, Zeigler Bros., Inc. Gardner's, PA, USA) until reaching a suitable size. Triplicate tanks of fish were fed one of four experimental diets during the growth trial for 56 days. At the start of the trial, 20 fish (mean individual weight = 11.88 ± 0.18 g) were randomly distributed in 12 tanks (volume 1,000 L) in a semi-recirculation system equipped with reservoir

Table 2. Amino acid (AA) profile (g kg^{-1}, as is) of experimental diets. Since only pH adjustment was applied between third and fourth diet, the AA profile of both diets were represented as 4% SPH.

Composition	15% FM	PBD	% HSM*
Taurine	0.70	0.61	0.60
Hydroxyproline	0.16	0.03	0.05
Aspartic Acid	3.91	4.15	4.04
Threonine	1.53	1.53	1.52
Serine	1.69	1.79	1.76
Glutamic Acid	7.42	7.98	7.79
Proline	2.41	2.44	2.43
Glycine	0.00	0.22	0.22
Alanine	1.97	1.64	1.69
Cysteine	2.26	2.05	2.10
Valine	0.58	0.64	0.62
Methionine	2.00	2.02	2.00
Isoleucine	0.79	0.80	0.79
Leucine	1.88	1.94	1.91
Tyrosine	3.69	3.76	3.74
Phenylalanine	1.51	1.63	1.63
Hydroxylysine	2.12	2.24	2.19
Ornithine	0.11	0.09	0.10
Lysine	0.03	0.02	0.03
Histidine	2.35	2.37	2.33
Arginine	1.04	1.04	1.02
Tryptophan	2.53	2.69	2.62

*Hydrolyzed salmon meal.

tank, biological filter, supplemental aeration (provided using a central line, regenerative blower, and air diffusers), and circulation pump. The tanks were supplied with sea water at temperatures of 28.62 ± 1.21 C, pH 7.62 ± 0.25, dissolved oxygen 5.33 ± 0.47, salinity 32.16 ± 4.72‰, nitrite 0.64 ± 0.49 mg L^{-1}, nitrate 117.70 ± 102.08 mg L^{-1}, and ammonia 0.04 ± 0.07 mg L^{-1}. Fish were exposed to a natural photoperiod of 14 h light and 10 h darkness, and a subsample of fish from the initial stocking was retained for proximate, amino acid, and mineral profile analysis. During the trial, fish were fed four times per day, and the daily ration was adjusted based on a percentage of body weight after sampling the fish every two weeks. During the sampling period, fish were dipped in Chloroquine phosphate (MP Biomedicals, Solon, OH) as a bactericide at the concentration of 60 mg L^{-1} followed with freshwater dip for approximately one minute to reduce any possibilities for parasitic infection. At the end of 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), voluntary feed intake (VFI), protein efficiency ratio (PER), and thermal unit growth coefficient (TGC):

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

$$\mathrm{FCR} = \frac{feed\,given\,(g)}{alive\,weight\,gain\,(g)}$$

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

$$\mathrm{VFI} = \frac{feed\,intake\,(g)}{fish}$$

$$\mathrm{TGC} = \frac{FBW^{\frac{1}{3}} - IBW^{\frac{1}{3}}}{\sum TD} \times 100$$

where FBW is final body weight, IBW is initial body weight, T is water temperature (^{0} C), and D is number of trial days.

Body composition analysis

At the end of the growth trials, four fish from each tank or 12 fish per dietary treatment were randomly sampled and stored at –80°C for body composition

analysis. Prior to dry matter and amino acids (AA) analysis, dried whole fish were rigorously blended and chopped in a mixer according to the standard methods established by the Association of Official Analytical Chemists (AOAC, 1990). The AA profile of whole pompano was analyzed by Ajinomoto Heartland, Inc. (Eddyville, IA, USA).

Histopathology analysis

Randomly, samples of distal intestine were taken from four selected fish per tank at the end of the experiment to assess the histomorphological condition, fixed in Bouins solution (picric acid-formalin-acetic acid mixture, Ricca Chemical, Arlington, TX, USA) for 20 h at room temperature, and then transferred to a 70% ethanol solution (VWR, Radnor, PA, USA) until processed by standard histological analysis procedures. The blocks of designed sample were dehydrated through a standard ethanol series to 100%, embedded in paraffin wax, and sectioned at 4 μm intervals for staining with Hematoxylin-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 et al. (2018b). The following parameters were taken into account for distal intestine analysis: the appearance of goblet cells (GC), cellular infiltration (CI), and widening of the lamina propria within the intestinal folds (WLP).

Statistical analysis

All growth parameters, proximate composition, and amino acids profile of the whole body of pompano were analyzed using one-way analysis of variance (ANOVA) to determine the significant differences among treatments followed by Tukey's multiple comparison tests to determine the difference between treatment means in each trial. Score data for histomorphological condition of the distal intestine were treated as categorical data, tested for normality and homoscedasticity, and subsequently analyzed using Kruskal-Wallis one-way analysis of variance followed by Tukey's multiple comparison tests to determine significant differences between treatments. All statistical analysis were conducted using the SAS system (V9.4., SAS Institute, Cary, NC, USA).

Results

Growth trial

No significant differences were found in final weight, PWG, TGC, VFI, SR, and FCR among fish fed the different dietary treatments ($P > 0.05$) (Table 3).

Table 3. Growth performance of juvenile Florida pompano (mean initial weight 11.88 ± 0.18 g) offered experimental diets for 56 d. 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.

Diet	Final mean weight (g)	Weight gain (%)	TGC	Feed intake (g/fish)	FCR*	Survival (%)
15% FM	81.83	590.98	0.1473	105.02	1.50	100.00
PBD	76.53	540.47	0.1398	103.34	1.60	98.33
4% HSM	76.74	549.96	0.1408	102.22	1.58	98.33
4% HSMA	75.01	529.90	0.1381	102.21	1.62	98.33
P value	0.145	0.154	0.155	0.223	0.160	0.802
PSE**	1.923	17.635	0.002	1.027	0.003	1.443

*FCR = Feed conversion ratio.
**PSE = Pooled standard error.

The survival rate in all treatments was above 98%, and there was no palatability issue in any dietary treatment.

Body composition

Amino acids (AA) depositions of whole body of pompano were shown in Table 4. None of the dietary treatments had any significant effect on the dry matter composition of the fish. However, for the AA deposition, Arginine (Arg, P value = 0.052), Aspartic acid (Asp, P value = 0.007), Cysteine (Cys, P value = 0.037), Glutamic acid (Glu, P value = 0.007), Histidine (His,

Table 4. Dry matter[1] and amino acids[2] analysis (g kg^{-1}, as is) of whole body of pompano. Values represent the mean of three replicates. Results are reported on "as fed" basis, and numbers in the same row with different superscript letters are significantly different (P < 0.05) among the fish fed with experimental diets (excluded the initial) based on analysis of variance followed by the Tukey's multiple comparison test.

Composition* Amino acids (g kg^{-1}, dry matter)	Initial	15% FM	PBD	4%HSM	4% HSMA	P value	PSE
Alanine	3.053	3.371	3.573	3.412	3.219	0.090	0.083
Arginine	2.678	3.092^{ab}	3.333^{a}	3.146^{ab}	2.967^{b}	0.052	0.076
Aspartic acid	4.462	4.845^{b}	5.473^{a}	4.808^{b}	4.750^{b}	0.007	0.114
Cysteine	0.371	0.459^{ab}	0.512^{a}	0.435^{b}	0.430^{b}	0.037	0.018
Glutamic acid	6.448	6.879^{b}	7.772^{a}	6.877^{b}	6.728^{b}	0.007	0.164
Glycine	3.285	3.598	3.549	4.004	3.345	0.416	0.267
Histidine	0.946	1.105^{b}	1.263^{a}	1.067^{b}	1.061^{b}	0.007	0.032
Isoleucine	2.052	2.214^{b}	2.474^{a}	2.024^{b}	2.134^{b}	0.008	0.067
Leucine	3.597	3.806^{b}	4.290^{a}	3.612^{b}	3.690^{b}	0.008	0.105
Lysine	3.616	3.866^{b}	4.446^{a}	3.798^{b}	3.747^{b}	0.008	0.114
Methionine	1.188	1.287^{b}	1.415^{a}	1.268^{b}	1.217^{b}	0.015	0.033
Phenylalanine	2.011	2.126^{b}	2.369^{a}	2.112^{b}	2.037^{b}	0.004	0.045
Proline	1.852	2.121	2.167	2.332	1.978	0.340	0.128
Serine	2.015	2.107^{b}	2.366^{a}	2.120^{b}	2.053^{b}	0.004	0.044
Threonine	2.015	2.185^{b}	2.467^{a}	2.176^{b}	2.132^{b}	0.004	0.048
Tyrosine	1.243	1.223	1.281	1.087	0.978	0.151	0.089
Valine	2.345	2.563^{b}	2.817^{a}	2.350^{b}	2.471^{b}	0.009	0.070
Tryptophan	0.547	0.597	0.612	0.613	0.595	0.989	0.049

*Analyzed by Ajinomoto Heartland, Inc., Eddyville, IA, USA.

P value = 0.007), Isoleucine (Iso, P value = 0.008), Leucine (Leu, P value = 0.008), Lysine (Lys, P value = 0.008), Methionine (Met, P value = 0.015), Phenylalanine (Phe, P value = 0.004), Serine (Ser, P value = 0.004), Threonine (Thr, P value = 0.004), and Valine (Val, P value = 0.009) were affected by dietary treatments. The deposition of all AA was found to be higher in fish fed PBD compared to all dietary treatments, and the adjustment of pH level on the diet of 4% HSMA did not have any significant effect on the utilization enhancement of AA compared to fish fed with 4% HSM.

Distal intestine histology

Overall, the distal intestine of fish fed the different types of diets displayed a normal histomorphological condition. However, statistically, the number of goblet cells in the distal intestine of fish fed the 15% FM diet was significantly lower than fish fed plant-protein sources. The numbers of goblet cells in the two groups of fish fed with 4% HSM and 4% HSMA were significantly higher than in the other group (Table 5). The cellular infiltration and the thickness of lamina propria showed a higher score for the group of fish fed with 4% HSM and 4% HSMA compared to fish fed 15% FM. Regarding the pH adjustment, there were no significant differences in any distal intestine histomorphological structures observed between the 4% HSM group and the 4% HSMA group.

Discussion

Boonyaratpalin, Suraneiranat, and Tunpibal (1998) and Chong, Hashim, and Ali (2003) found that the palatability issue on the use of soy protein may limit their wider use in formulating aquafeed for some species of fish. However, in the present study, we did not observe any palatability problems with high inclusion levels of soy protein in fish-meal-free diets, and there was no significant difference in feeding intake between fish fed the PBD diet and fish fed 15% FM. Moreover, among the dietary treatments, we did not observe any significant differences in any growth parameters during the

Table 5. Score analysis comparison between different diet treatments and distal intestine condition. Results presented as the average score of microscopic observation (n = 20).

	Distal intestine condition		
Diets	Number of goblet cells	Cellular infiltration	Thickness of lamina propria
15% FM	4.003^{a}	3.667^{a}	3.600^{a}
PBD	4.100^{b}	3.833^{ab}	3.867^{ab}
4% HSM	4.233^{c}	3.967^{b}	3.933^{b}
4% HSMA	4.333^{c}	3.933^{b}	3.900^{b}
P value	0.047	0.006	0.026
PSE	0.396	0.681	0.556

growth trials, indicating that the proper combination of plant protein can be beneficial in supplying the required nutrients for the growth of the fish.

In line with our previous studies, despite the fish fed the basal diet—which is a combination of 148 g kg^{-1} ESBM and 472.1 g kg^{-1} dehulled solvent extracted SBM to replace all dietary animal meal—having the lowest growth parameters compared to 15% animal meal, the supplementation of 4% squid hydrolyzates into the basal diet was able to yield a similar growth performance compared to fish fed with 15% animal meal (Novriadi et al. 2017). However, in this study, the use of 190.6 g kg^{-1} ESBM in combination with 450 g kg^{-1} SBM (PBD diet) to replace all dietary FM had similar growth performance with fish fed with 15% FM. Despite no statistical differences in terms of growth, biologically, the final weight of pompano fed with PBD was 11.5% lower than fish fed 15% FM. Variations in fish growth at the end of the trial within the PBD group seems to be related to this no-differences condition. Different sensitivity to the palatability and anti-nutritional factors (ANF) contained within the plant-protein sources could become the primary factor for the growth variation.

Data for the whole-body analysis presented in Table 4 indicate that the dietary treatments influenced the whole-body AA composition of the fish, but there was no appreciable effect on the dry matter content of pompano. The AA deposition was higher in the whole-body pompano fed with PBD, but statistically there is no significant effect on the live weight gain among the dietary treatments. The results obtained here demonstrated that the AA profiles in all experimental diets may adequately meet the requirements of pompano, and the minor excess found in the PBD group will only force their deamination and catabolism process (NRC 2011). Interestingly, the adjustment of pH level on the 4% HSMA did not cause any significant effect on the AA deposition as compared to the 4% HSM. According to NRC (2011), AA deposition is determined by genetic, endogenous (life stage), and exogenous factors, such as environment and feed. As would be expected from the digestive system physiology point of view, the utilization and metabolism of AA could be improved by adjusting the dietary pH to 5 or higher using NaOH solution (Nose et al. 1974). In this study, despite pH in the 4% HSMA diet having increased from 6 to 6.47 or similar with the pH level in 15% FM, it had no effect on the AA deposition and growth. This finding is similar to the study conducted by Murai et al. (1983), where no appreciable effects were seen on the protein profile of the whole body of fingerling carp after the fish were fed with different levels of pH for 6 weeks. More studies are still needed for physiological and biochemical interpretation of these results, especially related to the diffuse nature of the exogenous pancreas and the buffering capacity of the intestine that might affect the chemical condition of the diet.

To make a solid assessment about the functional effect of plant-based diets on the distal intestine morphology of pompano, several studies have been

performed to evaluate the efficacy of ESBM and FSBM to partially or completely replace the use of animal meal (Novriadi et al. 2018a, 2019; Novriadi, Spangler, and Allen Davis 2018b; Novriadi et al. 2017). Recent study on the use of different levels of FSBM to replace the use of dehulled SBM showed that higher inclusion levels of FSBM partially prevent the development of marked histological alteration in the distal intestine (Novriadi et al. 2018a). Furthermore, proper combination of ESBM and 4% squid protein hydrolyzates partly prevented the alteration of distal intestine histomorphology and yielded similar condition with fish fed 15% FM (Novriadi et al. 2017). In this study, despite the histology score analysis showing significant differences in the thickness of lamina propria, cellular infiltration, and the number of goblet cells, the histomorphological abnormalities tended to be minor, indicating that the use of ESBM with more functional properties to replace the dietary use of FM do not cause any tremendous effect on the digestive function of pompano. Many articles have reported different sensibility of some species of fish to the anti-nutritional factors (ANFs) present in SBM—such as soybean trypsin inhibitor, saponins, and lectins—that lead to physiological changes in distal intestine, indicated by the widening of central stroma within the mucosal folding with increased amount of connective tissue, infiltration of inflammatory cells in the lamina propria, and an increase number of eosinophilic granular cells (Baeverfjord and Krogdahl 1996; Ferrara et al. 2015; Refstie et al. 2001; Van den Ingh et al. 1991; Van den Ingh, Olli, and Krogdahl 1996). However, in this study, the use of advanced soy products (ESBM) does not seem to cause any detrimental effects on the histomorphological condition of pompano (Figure 1). This could be due to the lower level of ANFs and low level of oligosaccharide (raffinose 0.2% and stachyose 0.5%) in the final product of ESBM, which can enhance better digestion of nutrients in the intestine (Amezquita and Arana 2015). Interestingly, the image analysis showed insignificant differences in terms of intestine histomorphology between 4% HSM and 4% HSMA. Combination effect of protein hydrolyzates and the reduction of ANFs level in ESBM could potentially enhance the digestive function of pompano (Novriadi et al. 2017). Therefore, slight differences in pH level seem to have little effect on the morphological changes in the distal intestine of pompano.

Conclusions

The results obtained in this study indicated that the proper combination of plant-protein sources—450.0 g kg^{-1} of SBM, 190.6 g kg^{-1} of ESBM, and 80 g kg^{-1} of CPC—supplemented with Lys and Met could be used to replace the 150.0 g kg^{-1} of FM in the development of practical diets for pompano. The inclusion of 40 g kg^{-1} of hydrolyzed salmon meal (HSM) did not cause any significant effect on the growth performance of the fish, and the adjustment of the pH level in the diet supplemented with HSM did not cause any

significant effect on the physiological function of the fish. It should be noted that more thorough studies are necessary to clarify the role of pH adjustment in the growth and physiological condition of pompano.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Ahn, C.-B., J.-Y. Je, and Y.-S. Cho. 2012. Antioxidant and anti-inflammatory peptide fraction from salmon byproduct protein hydrolysates by peptic hydrolysis. *Food Research International* 49:92–98. doi:10.1016/j.foodres.2012.08.002.

Amezquita, J., and E. Arana, 2015. Evaluation of SPC as a partial substitute for fish meal in the diet for Striped bass in Todos Santos Island, Baja California, Mexico. Technical report to U.S. Soybean Export Council.

Association of Official Analytical Chemists (AOAC). (1990) *Official methods of analysis*. Association of Official Analytical Chemists, Washington, D.C

Baeverfjord, G., and Å. Krogdahl. 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, Salmo salar L., distal intestine: A comparison with the intestines of fasted fish. *Journal of Fish Diseases* 19:375–87. doi:10.1111/j.1365-2761.1996.tb00376.x.

Bi, H., H. Zhao, F. Lu, C. Zhang, X. Bie, and Z. Lu. 2015. Improvement of the nutritional quality and fibrinolytic enzyme activity of soybean meal by fermentation of *Bacillus subtilis*. *Journal of Food Processing and Preservation* 39:1235–42. doi:10.1111/jfpp.12340.

Bogwald, J., R. Dalmo, R. M. Leifson, E. Stenberg, and A. Gildberg. 1996. The stimulatory effect of a muscle protein hydrolysate from Atlantic cod, *Gadus morhua* L., on Atlantic salmon, *Salmo salar* L., head kidney leucocytes. *Fish & Shellfish Immunology* 6:1–2. doi:10.1006/fsim.1996.0002.

Boonyaratpalin, M., P. Suraneiranat, and T. Tunpibal. 1998. Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. *Aquaculture* 161:67–78. doi:10.1016/S0044-8486(97)00257-3.

Chi, C.-H., and S.-J. Cho. 2016. Improvement of bioactivity of soybean meal by solid-state fermentation with *Bacillus amyloliquefaciens* versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*. *LWT-Food Science and Technology* 68:619–25. doi:10.1016/j.lwt.2015.12.002.

Choi, Y.-R., P.-J. Park, J.-H. Choi, S.-H. Moon, and S.-K. Kim. 2000. Screening of biofunctional peptides from cod processing wastes. *Applied Biological Chemistry* 43:225–27.

Chong, A., R. Hashim, and A. B. Ali. 2003. Assessment of soybean meal in diets for discus (*Symphysodon aequifasciata* HECKEL) farming through a fishmeal replacement study. *Aquaculture Research* 34:913–22. doi:10.1046/j.1365-2109.2003.00945.x.

Ferrara, E., A. Gustinelli, M. L. Fioravanti, B. Restucci, F. Quaglio, S. Marono, and G. Piccolo. 2015. Histological and micro-/macro-morphological evaluation of intestine in sharpsnout seabream (Diplodus puntazzo) fed soybean meal-based diets added with MOS and inulin as prebiotics. *Aquaculture International* 23:1525–37. doi:10.1007/s10499-015-9902-y.

Gallagher, M. L. 1994. The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (*Morone saxatilis*× *M. chrysops*). *Aquaculture* 126:119–27. doi:10.1016/0044-8486(94)90253-4.

Gatlin, D. M., III, F. T. Barrows, P. Brown, K. Dabrowski, T. G. Gaylord, R. W. Hardy, E. Herman, G. Hu, Å. Krogdahl, and R. Nelson. 2007. Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research* 38:551–79. doi:10.1111/j.1365-2109.2007.01704.x.

Gbogouri, G., M. Linder, J. Fanni, and M. Parmentier. 2004. Influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates. *Journal of Food Science* 69: C615–C622. doi:10.1111/j.1365-2621.2004.tb09909.x.

Hardy, R. W. 2010. Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquaculture Research*. 41:770–76. doi:10.1111/j.1365-2109.2009.02349.x.

Knudsen, D., P. Urán, A. Arnous, W. Koppe, and H. Frøkiær. 2007. Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. *Journal of Agricultural and Food Chemistry* 55:2261–67. doi:10.1021/jf0626967.

Kotzamanis, Y., E. Gisbert, F. Gatesoupe, J. Z. Infante, and C. Cahu. 2007. Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to Vibrio anguillarum in European sea bass (*Dicentrarchus labrax*) larvae. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* 147:205–14. doi:10.1016/j.cbpa.2006.12.037.

Kumar, R., V. Choudhary, S. Mishra, I. K. Varma, and B. Mattiason. 2002. Adhesives and plastics based on soy protein products. *Industrial Crops and Products* 16:155–72. doi:10.1016/S0926-6690(02)00007-9.

Lim, C., C.-S. Lee, and C. D. Webster. 2008. *Alternative protein sources in aquaculture diets*. New York: Haworth Press.

Moon, H., and D. Gatlin III 1989. Amino acid nutrition of the red drum (*Sciaenops ocellatus*): Determination of limiting amino acids and development of a suitable amino acid test diet In The current status of fish nutrition in aquaculture. Proceedings of the Third International Symposium of Feeding and Nutrition in Fish, Toba, Japan, 201–08.

Murai, T., Y. Hirasawa, and T. Nose. 1983. Effects of dietary ph and electrolyte concentration on utilization of crystalline amino acids by fingerling carp. *Bulletin Of The Japanese Society Of Scientific Fisheries* 49 (9):1377-1380.

Nose, T., S. Arai, D. L. Lee, and Y. Hashimoto. 1974. A note on amino acids essential for growth of young carp. *Bulletin of the Japanese Society of Scientific Fisheries* 40:903–08. doi:10.2331/suisan.40.903.

Novriadi, R., E. Spangler, and D. Allen Davis. 2018b. Comparative effect of advanced soy products or corn protein concentrate with porcine meal on growth, body composition, and

distal intestine histology of Florida pompano, *Trachinotus carolinus*. *Journal of the World Aquaculture Society* 50(02): 433-447.

Novriadi, R., E. Spangler, M. Rhodes, T. Hanson, and D. A. Davis. 2017. Effects of various levels of squid hydrolysate and squid meal supplementation with enzyme-treated soy on growth performance, body composition, serum biochemistry and histology of Florida pompano *Trachinotus carolinus*. *Aquaculture* 481:85–93. doi:10.1016/j.aquaculture.2017.08.032.

Novriadi, R., G. Salze, A. Abebe, T. Hanson, and D. A. Davis. 2019. Partial or total replacement of fish meal in the diets of Florida pompano *Trachinotus carolinus*. *Aquaculture Research.* 50:1527–38. doi:10.1111/are.14029.

Novriadi, R., M. Rhodes, M. Powell, T. Hanson, and D. Davis. 2018a. Effects of soybean meal replacement with fermented soybean meal on growth, serum biochemistry and morphological condition of liver and distal intestine of Florida pompano *Trachinotus carolinus*. *Aquaculture Nutrition* 24:1066–75. doi:10.1111/anu.12645.

NRC. 2011. *Nutrient requirements of fish and shrimp*. Washington D.C: National Academic Press.

Olsen, R. E., A.-C. Hansen, G. Rosenlund, G.-I. Hemre, T. M. Mayhew, D. L. Knudsen, O. T. Eroldoğan, R. Myklebust, and Ø. Karlsen. 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) II—Health aspects. *Aquaculture* 272:612–24. doi:10.1016/j.aquaculture.2007.05.010.

Quintero, H. E., D. A. Davis, and M. A. Rhodes. 2012. Soy protein concentrate as an alternative ingredient in Florida pompano (*Trachinotus carolinus*) diets. *Journal of Applied Aquaculture* 24:247–61. doi:10.1080/10454438.2012.679164.

Quintero, H.E., D.A. Davis, and M.A. Rhodes. 2012. Soy protein concentrate as an alternative ingredient in florida pompano (trachinotus carolinus) diets. *Journal Of Applied Aquaculture.* 24: 247-261. doi: 159-261 doi:10.1080/10454438.2012.679164.

Refstie, S., Ø. J. Korsøen, T. Storebakken, G. Baeverfjord, I. Lein, and A. J. Roem. 2000. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture.* 190:49–63. doi:10.1016/S0044-8486(00)00382-3.

Refstie, S., T. Storebakken, G. Baeverfjord, and A. J. Roem. 2001. Long-term protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with partial replacement of fish meal by soy protein products at medium or high lipid level. *Aquaculture.* 193:91–106. doi:10.1016/S0044-8486(00)00473-7.

Rumsey, G. L. 1993. Fish meal and alternate sources of protein in fish feeds update 1993. *Fisheries* 18:14–19. doi:10.1577/1548-8446(1993)018<0014:FMAASO>2.0.CO;2.

Sales, J. 2009. The effect of fish meal replacement by soyabean products on fish growth: A meta-analysis. *British Journal of Nutrition* 102:1709–22. doi:10.1017/S0007114509991279.

Salze, G., E. McLean, P. R. Battle, M. H. Schwarz, and S. R. Craig. 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, Rachycentron canadum. *Aquaculture.* 298:294–99. doi:10.1016/j.aquaculture.2009.11.003.

Tacon, A. G., and M. Metian. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture* 285:146–58. doi:10.1016/j.aquaculture.2008.08.015.

Tibbetts, S. M., J. E. Milley, and S. P. Lall. 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, Gadus morhua (*Linnaeus*, 1758). *Aquaculture* 261:1314–27. doi:10.1016/j.aquaculture.2006.08.052.

Urán, P., A. Gonçalves, J. Taverne-Thiele, J. Schrama, J. Verreth, and J. Rombout. 2008. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish & Shellfish Immunology* 25:751–60. doi:10.1016/j.fsi.2008.02.013.

Van den Ingh, T., Å. Krogdahl, J. Olli, H. Hendriks, and J. Koninkx. 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): A morphological study. *Aquaculture.* 94:297–305. doi:10.1016/0044-8486(91)90174-6.

Van den Ingh, T., J. Olli, and Å. Krogdahl. 1996. Alcohol-soluble components in soybeans cause morphological changes in the distal intestine of Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* 19:47–53. doi:10.1111/j.1365-2761.1996.tb00119.x.

Watanabe, T. 2002. Strategies for further development of aquatic feeds. *Fisheries Science* 68:242–52. doi:10.1046/j.1444-2906.2002.00418.x.

Wilson, R. P., D. E. Harding, and D. L. Garling Jr. 1977. Effect of dietary pH on amino acid utilization and the lysine requirement of fingerling channel catfish. *The Journal of Nutrition* 107:166–70. doi:10.1093/jn/107.1.166.