Received: 14 February 2018 | Revised: 21 May 2018 | Accepted: 7 June 2018

DOI: 10.1111/jwas.12547

WILEY Journal of the World Aquaculture Society

FUNDAMENTAL STUDIES

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*

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Funding information
Fulbright Scholar Program , Grant/Award Number: 15150910; Hatch Funding Program of Alabama Agriculture Experiment Station

The present study was designed to investigate the effects of diets containing advanced soy products (enzyme-treated soy and fermented soy) or corn protein concentrate (CPC) in combination with porcine meal (PM) to completely replace poultry byproduct meal (PBM) on growth performance, body composition, and distal intestine histology of Florida pompano, *Trachinotus carolinus*. Four experimental diets were formulated to be isonitrogenous and isolipidic, to contain 400 g/kg crude protein and 80 g/kg lipid. A reference diet (PBM diet [PBMD]) contained 150 g/kg PBM and 495 g/kg soybean meal (SBM), and three test diets were formulated replacing PBM with 15 g/kg of CPC (CPC diet [CPCD]) or replacing all SBM and PBM with 535 g/kg fermented soy (fermented soybean meal diet [FSBMD]) or 451.3 g/kg enzyme-treated soy (enzyme-treated soybean meal diet [ESBMD]). All three test diets were supplemented with 38 g/kg of PM. Diets were fed based on a percentage of bodyweight adjusted after sampling the fish every 2 weeks to triplicate groups of Florida pompano juveniles (mean weight 8.06 ± 0.22 g). After 8 weeks of feeding, fish fed CPCD and ESBMD performed equally well in terms of final body weight, thermal growth coefficient, and percentage weight gain in comparison to fish fed PBMD. In all cases, feeding FSBMD resulted in poor feed conversion and lower feed intake compared to other treatments. Protein retention efficiency, whole-body proximate composition, phosphorus, sulfur, potassium, magnesium, calcium, sodium, and zinc contents were not significantly influenced by the dietary treatments. The results obtained in the present histological study showed no significant differences in the thickness of serous layer, muscular layer, and submucosal layer of the intestine among treatments. Fish fed CPCD showed a significant widening of the lamina propria with an increase of cellular infiltration and higher presence of goblet cells compared to other dietary treatment. Based on these results, 451 g/kg ESBM or combination of 150 g/kg of CPC and 495 g/kg SBM supplemented with 38 g/kg PM can be utilized to develop a practical diet for juvenile Florida pompano without impacting growth, nutritive parameters, and several distal intestine health parameters.

KEYWORDS

corn protein, distal intestine, enzyme-treated soy, fermented soy, Florida pompano, growth, porcine meal

1 | INTRODUCTION

The development of practical diets for juvenile Florida pompano, *Trachinotus carolinus*, has been evaluated by using several plant protein sources, such as soybean meal (SBM), corn gluten meal, and cottonseed meal products, and led to an optimized crude protein and lipid level of 40–44% and 7–12%, respectively (Cook, Zhou, Rhodes, & Davis, 2016; Lazo, Davis, & Arnold, 1998; Lech & Reigh, 2012; Novriadi, Spangler, Rhodes, Hanson, & Davis, 2017; Rhodes, Zhou, Salze, Hanson, & Davis, 2017; Riche, 2014; Williams, Lovell, & Hawke, 1985). Proper combinations of these plant protein sources supplemented with limiting amino acids (AAs) also reduce the inclusion of animal meal up to 15%, without negatively affecting the growth parameters of pompano (Quintero, Davis, & Rhodes, 2012). However, as the inclusion of plant-protein sources, especially SBM, continues to increase and levels of animal meals decrease, the wider inclusion is generally hindered by some limitations associated with the imbalance of AA profiles (Aragao et al., 2003; Fagbenro & Davies, 2001) and the presence of anti-nutritional factors (ANFs), such as lectins, phytic acid, saponins, phytosterols, and possible allergens (National Research Council, 2011). The cumulative effects can be responsible for the decreased growth performance (Tibaldi et al., 2006), feed efficiency (Olli, Krogdahl, & Våbenø, 1995), and possible histomorphological change in the distal intestine of some species of fish (Nordrum, Bakke-McKellep, Krogdahl, & Buddington, 2000; Rumsey, Siwicki, Anderson, & Bowser, 1994).

Recent work with various advanced soy products, such as fermented soybean meal (FSBM) and enzyme-treated soybean meal (ESBM), seems promising due to their ability to improve nutritional value of soy protein and substitute for the use of animal meal in fish diet formulation (Barnes, Brown, Bruce, Sindelar, & Neiger, 2014; Lim & Lee, 2011; Novriadi, 2017; Novriadi, Rhodes, Powell, Hanson, & Davis, 2018). Fermentation processes that allow microorganisms to degrade macromolecules to low molecular weights have been reported to have numerous benefits, including the degradation of soy immunoreactivity (Song, Frias, Martinez-Villaluenga, Vidal-Valdeverde, & de Mejia, 2008), lower levels of ANFs (Lim et al., 2010; Mukherjee, Chakraborty, & Dutta, 2016), and improvement of the nutritional quality and fibrinolytic enzyme activity of the commercial SBM (Bi et al., 2015). Likewise, the combination of a nonalcohol extraction process and enzymatic treatment to produce ESBM has been shown to improve the protein level and reduce the level of ANFs and oligosaccharide contained in SBM (Amezquita & Arana, 2015). Our recent study with Florida pompano showed that the combination of 47.2 g/kg SBM and 102 g/kg ESBM supplemented with 40 g/kg squid hydrolysates was effective to reduce dietary animal meal and partly prevented the alteration in the distal intestine of pompano (Novriadi et al., 2017). Meanwhile, even though the inclusion of 206, 309, and 410 g/kg FSBM to incrementally replace 50, 75, and 100% of solvent-extracted SBM did not improve the growth, higher amounts of FSBM were able to partially prevent the histological alteration in the liver and distal intestine of pompano (Novriadi et al., 2018). As 150 g/kg PBM was still included in all diets, the cause for insignificant growth effects and better liver and distal intestine condition found in the FSBM study remain unclear (Novriadi et al., 2018). In this context, it was of interest to further explore the efficacy of advanced soy product to completely replace animal meal on the growth and distal intestine morphology of fish.

Other than soy sources, corn protein concentrate (CPC), which is the dried protein fraction obtained after removal of the majority of the nonprotein components of the corn by using enzymatic solubilization, is often utilized in plant-based diets for fish due to the ability to balance the AA profile of SBM, resulting in diets that are highly digestible and rich in methionine and cysteine (Gatlin et al., 2007; Hardy, 2010; Khalifa, Belal, El-Tarabily, Tariq, & Kassab, 2018; Phillips & Sternberg, 1979; Robinson & Li, 2008). Recently, CPC at the level of 80 g/kg has been frequently used to improve the nutritional quality of soy-based diets for pompano (Cook et al., 2016; Novriadi et al., 2017; Rhodes et al., 2017). Interestingly, in juvenile white seabass, *Atractoscion nobilis*, higher inclusion of CPC at the range of 117.9–119.9 g/kg to complement the use of 150 g/kg SBM or FSBM in combination with 175–186 g/kg soy protein concentrate (SPC) was effective to reduce the inclusion of fishmeal (FM) and yielded similar performance with fish fed 480 g/kg FM (Trushenski, Rombenso, Page, Jirsa, & Drawbridge, 2014). Hence, these results suggest that it may be possible to increase the inclusion level of CPC to more than 80 g/kg in the development of practical diets for pompano.

From a nutritive value standpoint, porcine meal (PM) derived from fat trimmings of fresh pork carcasses is a good source of protein for fish and shrimp (Hernández et al., 2010; Hernández, Olvera-Novoa, Aguilar-Vejar, González-Rodríguez, & de la Parra, 2008; Wang et al., 2012), due to the balanced AA profile with considerable levels of hydroxyproline (Hyp), which is limited in plant protein sources (Aksnes, Mundheim, Toppe, & Albrektsen, 2008; Wu et al., 2011). Although described as a nonessential AA that can be derived from the posttranslational hydroxylation of proline (Aksnes et al., 2008), a sufficient level of Hyp may play a role in inducing the gustatory response (Hara, 2012; Marui, Evans, Zielinski, & Hara, 1983), maintaining structure and function of cells (Wu et al., 2011), and in the biological performance of some species of fish (Aksnes, Hope, Høstmark, & Albrektsen, 2006). However, to the best of our knowledge, the potential value of PM as a supplement in Florida pompano diets has not been adequately studied. Therefore, based on all the background information, the objective of the present study was to evaluate the nutritive value of two commercially available advanced SBM (ESBM and FSBM) or CPC fortified with PM on growth performance, body composition, and distal intestine morphology of Florida pompano compared to the reference diet.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Four diets were formulated to be isonitrogenous and isolipidic to contain approximately 400 g/kg protein and 80 g/kg lipid as a combination of two or more protein sources: dehulled solvent extracted SBM (Bunge Limited, Decatur, AL), ESBM (NutriVance, Midwest Ag Enterprises, Marshall, MN), FSBM (PepSoyGen, Nutrafrema, Protein and Biotech Products, Sioux City, IA), poultry byproduct meal (PBM; Griffin Industries, Inc., Mobile, AL), CPC (Empyreal 75, Cargill Corn Milling, Cargill, Inc., Blair, NE), and PM (Innomax MPI, Sonac LLC, Maquoketa, IA) (Table 1). A reference diet (PBM diet [PBMD]) that has been run in numerous trials was produced by utilizing 150, 495, and 70 g/kg of PBM, SBM, and CPC, respectively. Three experimental diets were produced by completely replacing PBM with 150 g/kg CPC (designed as CPC diet [CPCD]) or replacing PBM and SBM with 535 g/kg fermented soy (FSBM diet [FSBMD]) or 451.3 g/kg enzyme-treated soy (ESBM diet [ESBMD]). All experimental diets were supplemented with 38 g/kg of PM and three AAs: L-lysine (Lys), DL-methionine (Met), and taurine (Tau) (MP Biomedicals Inc., Santa Ana, CA) to match the calculated levels in PBM as the reference diet. All diets were produced at the Laboratory of Aquatic Animal Nutrition, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, AL, using standard procedures for pompano. Briefly, diets were prepared by mixing preground dry ingredients along with menhaden fish oil in a food mixer (Hobart Corporation, Troy, OH) for approximately 15 min. Hot water was then blended into the mixture to attain an appropriate consistency for pelleting. Diets were pressure pelleted using a meat grinder with a 3-mm die,

TABLE 1 Composition (g/kg as is) of diets fed to juvenile Florida pompano for 8 weeks

	Diet code			
	PBMD	CPCD	FSBMD	ESBMD
Ingredient (g/kg, as is)				
Poultry byproduct meal[1]	150.0	0.0	0.0	0.0
Soybean meal[2]	495.0	495.0	0.0	0.0
Fermented soybean meal[3]	0.0	0.0	535.0	0.0
Enzyme treated soy[4]	0.0	0.0	0.0	451.3
Corn protein concentrate[5]	70.0	150.9	70.0	70.0
Porcine meal[6]	0.0	38.0	38.0	38.0
Menhaden fish oil[7]	49.0	70.0	70.0	70.0
Corn starch[8]	14.5	20.1	61.0	148.7
Whole wheat[8]	160.0	160.0	160.0	160.0
Trace mineral premix[9]	2.5	2.5	2.5	2.5
ASA vitamin premix w/o choline[10]	5.0	5.0	5.0	5.0
Choline chloride[8]	2.0	2.0	2.0	2.0
Rovimix stay C 35%[11]	1.0	1.0	1.0	1.0
CaP-dibasic[8]	35.0	35.0	35.0	35.0
Lecithin (soy commercial)[12]	5.0	5.0	5.0	5.0
L-lysine[8]	1.0	5.5	5.5	1.0
DL-methionine[8]	5.0	5.0	5.0	5.0
Taurine[8]	5.0	5.0	5.0	5.0
Proximate analyses (g/kg, as is)[13]				
Crude protein	423.4	421.9	405.6	409.0
Moisture	71.5	86.1	128.9	78.7
Crude fat	94.7	87.1	60.5	84.7
Crude fiber	28.4	29.9	30.0	32.7
Ash	78.4	61.4	65.2	59.8

Note. CPCD = corn protein concentrate diet; ESBMD = enzyme-treated soybean meal diet; FSBMD = fermented soybean meal diet; PBMD = poultry byproduct meal diet.

[1]Griffin Industries, Inc., Mobile, AL. [2]Dehulled solvent extracted soybean meal (Bunge Limited, Decatur, AL. [3]PepSoyGen, Nutraferma, Protein and Biotech Products, Sioux City, IA. [4]NutriVance, Midwest Ag Enterprises, Marshall, MN. [5]Empyreal75 Cargill Corn Milling, Blair, NE. [6]Innomax MPI, Sonac USA LLC, Maquoketa, IA. [7]Omega Protein Inc., Houston, TX. [8]MP Biomedicals Inc., Santa Ana, CA. [9]ASA premix (g 100/g 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. [10]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. [11]Stay C (L-ascorbyl-2-polyphosphate 35% active C); Roche Vitamins, Inc., Parsippany, NJ. [12]The Solae Company, St. Louis, MO. [13]Analyses conducted by the University of Missouri-Columbia, Agricultural Experiment Station Chemical Laboratory, MO.

and air dried (<45 °C) overnight to attain a moisture content less than 10%. Pellets were crumbled, packed in sealed plastic bags and stored at −20°C until use. The proximate composition and AA profile of the diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories and summarized in Tables 1 and 2, respectively.

2.2 | Fish and experimental design

The growth trial was carried out at the Claude Peteet Mariculture Development Center (CPMC), Gulf Shores, AL. Florida pompano fingerlings were obtained from a commercial hatchery (Proaquatix, Vero Beach, FL), and

TABLE 2 Amino acid (AA) composition (g/kg, dry matter basis) of experimental diets utilized in the trial

	Diet code			
AA (g/kg, dry matter)	PBMD	CPCD	FSBMD	ESBMD
Taurine	7.0	6.3	5.5	6.8
Hydroxyproline	2.4	2.8	3.0	3.2
Aspartic acid	37.8	35.4	37.6	37.0
Threonine	15.2	14.3	14.4	14.3
Serine	17.8	18.3	17.7	17.9
Glutamic acid	73.5	77.3	71.3	74.3
Proline	22.6	28.9	22.2	25.0
Glycine	20.5	19.8	19.7	20.9
Alanine	21.9	23.7	20.1	21.3
Cysteine	5.9	6.1	5.5	5.6
Valine	19.6	18.5	18.5	18.2
Methionine	12.2	11.3	10.3	9.7
Isoleucine	18.4	17.6	17.6	17.4
Leucine	35.4	40.1	33.5	34.9
Tyrosine	15.4	15.9	14.4	14.6
Phenylalanine	20.7	21.5	20.2	20.5
Hydroxylysine	1.0	1.1	0.9	1.2
Ornithine	0.2	0.2	0.2	0.2
Lysine	23.9	23.5	24.3	21.1
Histidine	9.7	9.3	9.2	9.4
Arginine	26.3	23.6	24.3	25.4
Tryptophan	5.0	4.6	5.6	5.3

Note. CPCD = corn protein concentrate diet; ESBMD = enzyme-treated soybean meal diet; FSBMD = fermented soybean meal diet; PBMD = poultry byproduct meal diet.

acclimatized for 3 weeks to the experimental facilities and fed with a commercial diet (FF Starter, Zeigler Bros., Inc. Gardners, PA) until reaching a suitable size. At the start of the trial, 20 fish (mean individual weight = 8.06 ± 0.22 g) were randomly stocked into each tank with three replicates per treatment. An 8-week feeding trial was carried out in a semi-recirculating system consisting of 12 culture open-top 800-L tanks equipped with reservoir tank, biological filter, supplemental aeration (provided using a central line, regenerative blower, and air diffusers), and circulation pump. During the trial, fish were fed four times per day and the daily ration was adjusted based on a percentage of bodyweight after sampling the fish every 2 weeks. The culture systems were located in a greenhouse that provided a natural photoperiod throughout the trial. Mean water-quality parameters ± *SD* of water temperature (27.91 ± 1.52 °C), salinity (25.36 ± 3.16 ppt), pH (7.81 ± 0.19), and dissolved oxygen (6.03 ± 0.61 mg/L) were monitored two times daily with a multiparameter (ProPlus, YSI Inc., Yellow Springs, OH), total ammonia nitrogen (0.03 ± 0.05 mg/L) was measured two times per week using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA), and nitrate-nitrogen (24.93 ± 23.51 mg/L) and nitrite-nitrogen (0.15 ± 0.09 mg/L) were measured once a week using colorimetric test kits (La Motte Chemicals, Chestertown, MD). A subsample of fish from the initial stocking was frozen for body composition analysis. At the end of the feeding period, all fish were grouped and individually weighed to calculate the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), survival, voluntary feed intake (VFI), protein retention efficiency (PRE), and thermal unit growth coefficient (TGC) as follows:

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

$$\mathrm{FCR} = \frac{\text{feed given (g) in dry weight basis}}{\text{alive weigh gain (g)}}$$

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

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

$$\mathrm{PRE} = \frac{(\text{final total body protein} - \text{initial total body protein})}{\text{total dietary protein fed}} \times 100$$

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

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 the trial, four fish from each tank or 12 fish per dietary treatment were randomly sampled and stored at −60°C for body-composition analysis. Prior to proximate and 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 (1990). Proximate composition and mineral contents of whole pompano body was analyzed by Midwest Laboratories (Omaha, NE).

2.4 | Histological section

At the termination of the feeding trial, three fish per each tank with a total of nine fish for each dietary treatment were randomly sampled after an overnight fast for histological analysis. Fish were individually euthanized in a solution of Tricaine-S (MS-222, tricaine methanesulfonate salt; Western Chemical, Inc., Ferndale, WA) and dissected to collect the distal intestine tissue. Distal intestine samples of approximately 0.5 cm were immediately fixed in Bouin's solution for 20 h at room temperature and then transferred to 70% ethanol solution 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 stain (Merck, Darmstadt, Germany). Histopathological evaluation for the distal intestine was performed following the scoring system described in Novriadi et al. (2018). The following parameters were taken into account for distal intestine analysis: serous layer (SL), muscular layer (ML), submucosal layer (SML), the appearance of goblet cells (GCs), cellular infiltration (CI), and widening of the lamina propria (LP) within the intestinal folds (Figure 1). In total, 108 distal intestine sections were evaluated with three sections per fish. Histomorphological images were acquired by using a microscope (Olympus BX41, Olympus Optical Co., Ltd., Tokyo, Japan).

2.5 | Statistical analysis

Growth, body composition, PRE, and distal intestine histological scores were analyzed using one-way ANOVA to determine the significant differences among treatments followed by Tukey's multiple comparison test to determine the difference between treatment means. Histological scores for distal intestine were treated as categorical data, tested for normality and homoskedasticity and subsequently analyzed using Welch's one-way ANOVA followed by

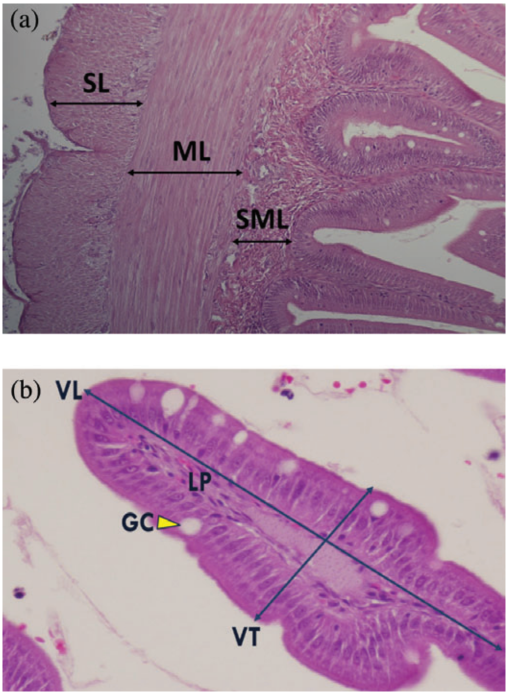

FIGURE 1 Representative histopathological image of Florida pompano distal intestine. (a) Measurement of the serous layer (SL), muscular layer (ML), and submucosal layer (SML) with 40× magnification. (b) Measurement of the villi length (VL), villi thickness (VT), goblet cells (GC), and lamina propia (LP) with 40× magnification measurements

Games-Howell post hoc tests to determine significant differences between treatments. All statistical analyses were conducted using SAS system (V9.4. SAS Institute, Cary, NC).

3 | RESULTS

3.1 | Experimental diets

The crude protein values ranged from 405.6 to 423.4 g/kg and were fairly constant among the experimental diets. Despite being designed to be isolipidic, FSBMD showed the lowest fat content at the level of 60.5 g/kg compared to PBMD, CPCD, and ESBMD, which showed a comparable value at the level of 94.7, 87.1, and 84.7 g/kg diet, respectively (Table 1). Tau level was lower in FSBMD (5.5 g/kg) compared to those in PBMD (7.0 g/kg), ESBMD (6.8 g/kg), and CPCD (6.3 g/kg). Supplementation of Lys induced a higher level in FSBMD (24.3 g/kg) compared to PBMD (23.9 g/kg), CPCD (23.5 g/kg), and ESBMD (21.1 g/kg). The inclusion of 150.9 g/kg CPC into the soy-based diet increased the level of cysteine in the CPCD (6.1 g/kg) compared to cysteine in PBMD (5.9 g/kg) and advanced soy product diets (5.5–5.6 g/kg). The inclusion of PM (38 g/kg) increased the level of Hyp in all test diets (2.8–3.2 g/kg) and slightly higher than PBMD (2.4 g/kg) (Table 2).

3.2 | Growth performance

The dietary treatments affected the growth performance of the fish (Table 3). Fish fed CPCD and ESBMD performed equally well in terms of final body weight, thermal growth coefficient (TGC), and PWG in comparison to fish fed PBMD as the reference diet ($p > .05$). Feeding FSBMD resulted in poor feed conversion and lower feed intake

TABLE 3 Growth performance of juvenile Florida pompano (mean initial weight 8.06 ± 0.22 g) fed experimental diets for 8 weeks[1]

Items	Experimental diets					
	PBMD	CPCD	FSBMD	ESBMD	*p* value	PSE
FBW (g)	51.49[a]	49.45[a]	32.39[b]	51.55[a]	.0151	3.6238
TGC	0.1036[a]	0.0922[a]	0.0601[b]	0.0857[a]	.0018	0.0050
PWG (%)	538.97[a]	517.78[a]	300.02[b]	535.72[a]	.0115	43.0379
FCR	1.46[ab]	1.42[ab]	2.24[a]	1.25[b]	.0403	0.2092
Survival (%)	100.00[a]	96.67[a]	93.33[a]	80.00[b]	.0043	2.7638
VFI (g/g)	69.85[a]	62.76[ab]	49.79[c]	58.472[b]	.0020	2.3328
PRE	34.17	29.23	34.35	47.84	.0589	4.1091

Note. CPCD = corn protein concentrate diet; ESBMD = enzyme-treated soybean meal diet; FBW = final body weight; FCR = feed conversion ratio; FSBMD = fermented soybean meal diet; PBMD = poultry byproduct meal diet; PRE = protein retention efficiency; PSE = pooled standard error; PWG = percentage weight gain; VFI = voluntary feed intake; TGC = thermal growth coefficient.

[1]Values represent the mean of three replicates. Results in a row with different superscript letter are significantly different ($p < .05$) based on one-way ANOVA followed by the Tukey's multiple comparison test.

compared to other treatments. Fish fed ESBMD had the lowest ($p = .0043$) percentage survival and no mortality was observed in fish fed PBMD. Results of Tukey's multiple comparison test indicate that there was no significant difference in terms of PRE among dietary treatments ($p = .0589$).

3.3 | Body composition

Proximate composition and mineral content of whole pompano body on a wet-weight basis are presented in Table 4. No significant differences were observed in the moisture, dry matter, protein, fat, and ash content in the whole body of pompano across all treatments. Likewise, no significant effect was observed in phosphorus, sulfur, potassium, magnesium, calcium, sodium, and zinc contents of whole-pompano body across all the treatments. However, manganese level of fish fed with CPCD was significantly higher compared to other dietary treatments ($p = .0006$).

3.4 | Distal intestine histology

Representative histopathological images of Florida pompano distal intestine are shown in Figures 2 and 3. There were no significant differences in the thickness of SL, ML, and SML among the dietary treatments. The sections in fish fed CPCD showed a significant widening of LP with an increase of CI and higher presence of GCs compared to other dietary treatments, while no significant difference in these histological features was seen in fish fed PBMD, FSBMD, and ESBMD.

4 | DISCUSSION

In previous studies, complete removal or lowering the reference level of animal meal in the diet resulted with significant reduction in growth performance (Rhodes et al., 2017; Rossi & Davis, 2012). A recent study also showed that the average final weight, biomass, TGC, and feed intake were significantly lower when PBM in the reference level was completely replaced with 150 g/kg ESBM (Novriadi et al., 2017). However, in the present study, fish fed both a high protein content of enzyme-treated soy (451.3 g/kg) as the primary protein sources and a moderate level of corn (150.9 g/kg) supplemented with 38 g/kg of PM yielded similar growth performance in comparison to the group of fish fed with PBMD as the reference diet. A study using a moderate level of CPC has been carried out in red drum,

TABLE 4 Proximate composition and mineral contents of whole Florida pompano body in wet weight basis offered experimental diets for 8 weeks

Compositions	Treatments PBMD	CPCD	FSBMD	ESBMD	*p* value	PSE
Proximate composition (g/kg)						
Moisture	694.7	689.0	703.0	708.3	.1989	0.6142
Dry matter	305.3	311.0	297.0	291.7	.1989	0.6142
Protein	195.7	188.7	180.3	184.3	.1937	0.4649
Fat	91.9	89.6	85.3	91.5	.8796	0.6435
Ash	30.2	30.9	30.7	27.4	.7653	0.2614
Macro-elements (g/kg)						
Sulfur	2.7	2.5	2.6	2.5	.3475	0.0078
Phosphorus	6.0	6.0	6.5	6.6	.5773	0.0394
Potassium	3.5	3.4	3.4	3.3	.1954	0.0070
Magnesium	0.4	0.4	0.4	0.4	.5957	0.0024
Calcium	8.3	8.2	9.4	9.7	.4725	0.0798
Sodium	1.0	0.9	0.9	0.9	.8592	0.0033
Micro-elements (mg/kg)						
Iron	12.4	11.2	12.6	10.8	.3166	0.7439
Manganese	2.7^b	6.1^a	3.4^b	3.8^b	.0006	0.3439
Zinc	15.8	15.7	18.1	15.2	.1122	0.7699

Note. CPCD = corn protein concentrate diet; ESBMD = enzyme-treated soybean meal diet; FSBMD = fermented soybean meal diet; PBMD = poultry byproduct meal diet; PSE = pooled standard error.

Sciaenops ocellatus, in which fish fed a soy-based diet containing 150 g/kg CPC had comparable growth performance as those fed a FM-based diet (Rossi, Tomasso, & Gatlin, 2015). In addition, a comparative study performed by Minjarez-Osorio et al. (2016) demonstrated that up to 50% FM can be replaced using CPC supplemented with AA without any negative impact to the growth performance of the red drum, *S. ocellatus*, and shortfin corvina, *Cynoscion parvipinnis*. Referring to the nutritional content of ESBM and beneficial complementarity in terms of limiting AA between corn and soy (Hertrampf & Piedad-Pascual, 2012), comparable growth of fish fed ESBMD or CPCD with PBMD would be due to the availability of required nutrient and synergistic interactions with PM as well as with other AA supplementation.

In contrast, even when fortified with CPC and PM, fish fed with FSBMD showed the lowest final individual body weight, TGC, and PWG compared to other dietary treatments. Based on visual observations, pompano do not respond well to high levels of fermented products, which results in lower feeding activity compared to other dietary treatments. This is in line with our previous findings (Novriadi et al., 2018) that with an increasing dietary inclusion level of FSBM to replace traditional SBM, feed intake and growth performance of pompano tended to decrease. The reduction in VFI with a high inclusion level of FSBM has also been reported in rainbow trout, *Oncorhynchus mykiss* (Yamamoto et al., 2010) as well as in Chinese sucker, *Myxocyprinus asiaticus* (Yuan et al., 2013). According to Kader et al. (2011), growth depression and decreased feed efficiency with high inclusion levels of FSBM are most likely due to the small quantitative contribution of the fermentation process to completely eliminate the ANFs within the final product. Furthermore, the low levels of fat in FSBMD might also influence the outcome. However, as the level was still within the optimal range, the reported differences may not be a major concern. In addition, protein and carbohydrate present in the diet could also serve as the lipid sources through lipogenesis with AAs and pyruvate (National Research Council, 2011). Thus, based on the results of this study and previous studies, feeding pompano with diets containing high inclusion levels of fermented product should be carefully considered and proper supplementation with limiting AA are required to support an optimal growth for Florida pompano.

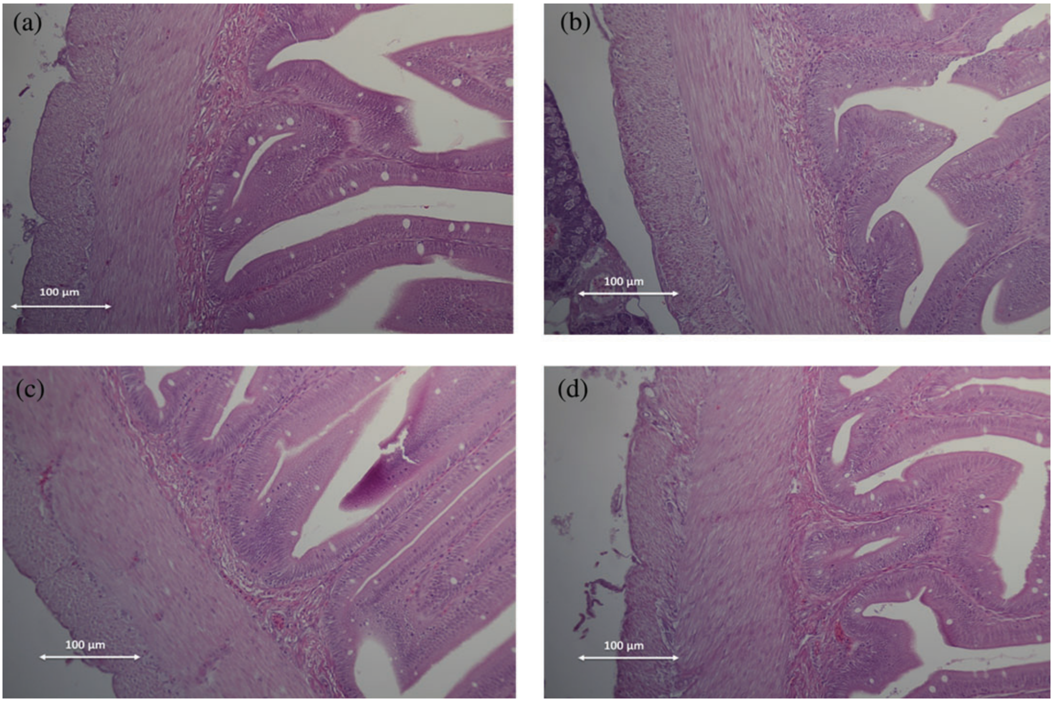

FIGURE 2 Representative histopathological images of hematoxylin and eosin-stained sections for detail of thickness on distal intestine layers (20× magnification) from Florida pompano after 56 days of being fed with (a) PBMD, (b) CPCD, (c) FSBMD, and (d) ESBMD. *Note.* CPCD = corn protein concentrate diet; ESBMD = enzyme-treated soybean meal diet; FSBMD = fermented soybean meal diet; PBMD = poultry byproduct meal diet

In the present study, no significant differences were observed in the moisture, protein, fat, dry matter, and ash content (on a wet-weight basis) in the whole body of pompano across all treatments. This is parallel with our previous findings in which the proximate composition of pompano fed with ESBMD supplemented with various levels of squid products did not show any significant differences with the reference diet (Novriadi et al., 2017). Moreover, pompano fed with diets containing various levels of FSBM to replace traditional SBM did not show any significant differences in the level of crude protein, moisture, fat, ash, and phosphorus (Novriadi et al., 2018). With regard to the use of other advanced soy products, Rhodes et al. (2017) reported that a plant-based diet containing 350 g/kg SBM and 300 g/kg SPC supplemented with valine, glycine, and histidine also did not result in any differences in terms of crude protein, crude fat, moisture, fiber, and ash content in the whole body of pompano compared to fish fed with 294 g/kg PBM.

A number of studies acknowledge that high inclusion levels of low-processed SBM induce intestinal inflammation in the hindgut of some species of fish (Baeverfjord & Krogdahl, 1996; Bakke-McKellep, Press, Baeverfjord, Krogdahl, & Landsverk, 2000; Ingh, Olli, & Krogdahl, 1996; Krogdahl, Bakke-McKellep, & Baeverfjord, 2003). Gut inflammation or soy-induced enteritis have been described as widening of LP of mucosal folds, infiltration of inflammatory cells in the LP, reduced number or even absence of supranuclear vacuoles in the absorptive epithelium, elevated numbers of GCs and levels of lysozyme in the gut mucosa (Merrifield, Olsen, Myklebust, & Ringø, 2011; Trushenski, 2015). According to Sanden, Berntssen, Krogdahl, Hemre, and Bakke-McKellep (2005) trypsin inhibitors and lectins might be responsible for the morphological change in the distal intestine of Atlantic salmon *Salmo salar* L. However, Knudsen, Urán, Arnous, Koppe, and Frøkiær (2007) argued that gut inflammation in Atlantic salmon might be induced by saponins alone or in combination with other factors, such as the intestinal microflora or

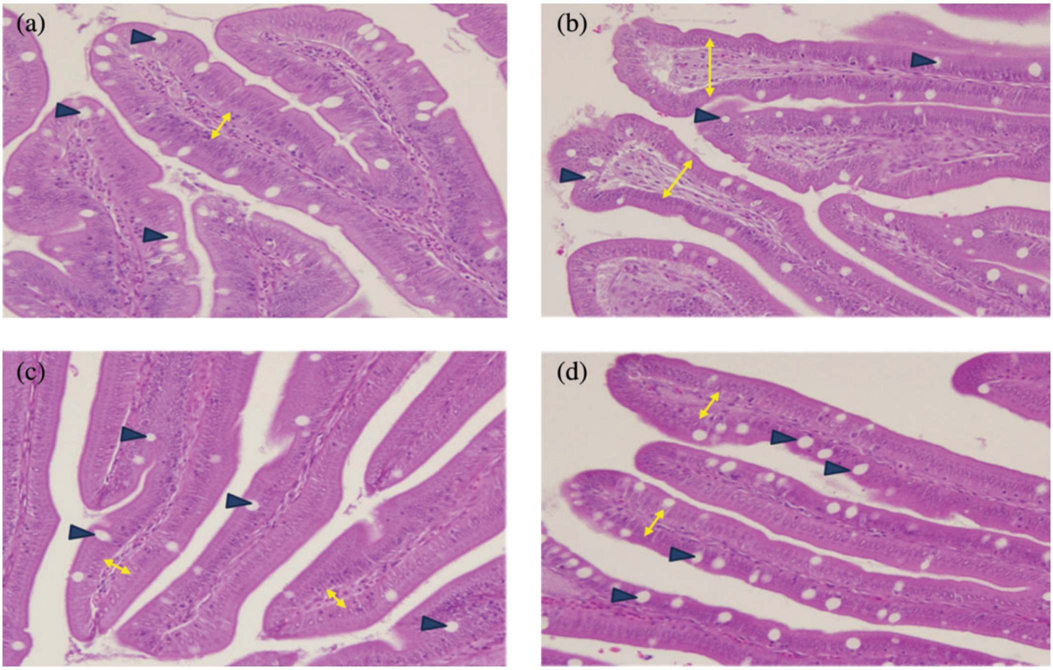

FIGURE 3 Representative histopathological images of hematoxylin and eosin-stained sections of distal intestines (20× magnification) from Florida pompano after 56 days of being fed with (a) PBMD, (b) CPCD, (c) FSBMD, and (d) ESBMD. (▶) goblet cells; (↔) lamina propria thickness. *Note.* CPCD = corn protein concentrate diet; ESBMD = enzyme-treated soybean meal diet; FSBMD = fermented soybean meal diet; PBMD = poultry byproduct meal diet

antigenic soybean proteins. Because soybean-derivative products can contain a variety of ANFs, the primary causative agent for the enteritis problem remains unclear.

Advanced processing techniques may effectively remove some ANFs, but not all (Trushenski, 2015). Fermented product, for example, still contains low levels of tannin, phytate, trypsin inhibitor, and protease inhibitor (Adeyemo & Onilude, 2013). In our previous study, inclusion of a low level of ESBM to replace PBM in combination with a high percentage of SBM still produced gut inflammation characteristics, such as widening of LP with heavy infiltration of inflammatory cells as well as a high number of GCs. However, this inflammation was partly prevented with the addition of 40 g/kg squid hydrolysate into the diet, and fish fed the experimental diet maintained growth and physiological condition similar to a group of fish fed with 150 g/kg PBM (Novriadi et al., 2017). In addition, the morphology of LP and number of GCs become more normal as the inclusion level of FSBM to replace traditional SBM increased, indicating the more favorable outcome with high inclusion levels of fermented products (Novriadi et al., 2018).

In the present study, fish fed CPCD displayed increased infiltration of inflammatory cells in the LP and a greater number of GCs, as well as widening of LP compared to fish fed FSBMD, ESBMD, and PBMD. The elevated number of GCs, especially in the distal intestine, suggests an unsatisfactory process of protein digestion (Baeza-Ariño, Martínez-Llorens, Nogales-Mérida, Jover-Cerda, & Tomás-Vidal, 2016), while the cell infiltration into LP indicates the presence of inflammation (Kiron, Kulkarni, Dahle, Lokesh, & Kitani, 2015). Meanwhile, morphological features of the distal intestine in fish fed FSBMD and ESBMD were partly improved similarly to the fish fed PBMD. Considering all information obtained from Novriadi et al. (2017) and Novriadi et al. (2018), the efficacy of ESBM and FSBM in combination with PM to partly prevent histomorphological alteration could be caused by the synergistic effect of these ingredients to improve the functional nutrition and health of the diet. Regarding the inferior outcome in fish fed CPCD, minor morphological change could be attributed to the high inclusion level of traditional SBM without considerable amounts of animal protein in the diet. Despite having changes in the distal intestine morphology, fish fed CPCD have equal growth performance with the reference diet and ESBMD, suggesting that these minor changes did not significantly influence the growth of pompano.

Regarding the distal intestine layers that are responsible for nutrient circulation in the epithelium through the blood vessels (Al-Hussaini, 1949; Baeza-Ariño et al., 2016), individual variations led to nonsignificant differences in the thickness of the SL, ML, and SML among dietary treatments. Baeza-Ariño et al. (2016) also reported that the different inclusion levels of pea protein concentrates and rice protein did not significantly affect the ML and SML thickness in the distal intestine of sea bream, *Sparus aurata*. In addition, Martínez-Llorens, Baeza-Ariño, Nogales-Mérida, Jover-Cerdá, and Tomás-Vidal (2012) only found differences in SL of fish fed 339 g/kg of carob seed meal (CSM) and greater thickness in ML on fish fed 339 and 518 g/kg of CSM, while no significant differences were detected in SML of fish among the dietary treatments.

5 | CONCLUSION

Under the experimental conditions, enzyme-treated soy (ESBM) at inclusion levels up to 451 g/kg or inclusion of CPC at 150 g/kg in a soy-based diet formulation supplemented with 38 g/kg of PM can be utilized in the development of practical diets for Florida pompano without any adverse effects on growth and nutritive parameters. The histological study of the distal intestine provides useful information regarding the effects of a high inclusion level of advanced soy products or a moderate level of CPC fortified with PM.

ACKNOWLEDGMENTS

We express our gratitude and appreciation to those who have taken the time to critically review this manuscript as well as those who helped support this research at the CPMC in Gulf Shores, AL. Special thanks to students and staff who help maintain the daily management during the trial and sampling program. This work was supported in part by the Hatch Funding Program of Alabama Agriculture Experiment Station and the Fulbright Scholar Program with IIE grantee ID #15150910. The mention of trademarks or proprietary products does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable.

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How to cite this article: Novriadi R, Spangler E, Allen Davis D. 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*. *J World Aquacult Soc*. 2018;1–15. https://doi.org/10.1111/jwas.12547