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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*

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Abstract

This study evaluated the suitability of commercially produced fermented soybean meal (FSBM) known as PepSoyGen™, in a plant-based diet for Florida pompano, *Trachinotus carolinus* fingerlings. An 8-week growth trial was conducted to evaluate the effect of four isonitrogenous and isolipidic diets containing 0, 206, 309 and 410 g/kg FSBM, replacing approximately 0%, 50%, 75% and 100% SBM (designated as Basal, FSBM 50, FSBM 75 and FSBM 100, respectively) on growth performance, body composition, serum biochemistry and morphological condition of liver and distal intestine of Florida pompano. There were no significant differences in final mean weight, percentage weight gain, thermal unit growth coefficient and feed conversion ratio in all treatments. For serum biochemistry analysis, there were no significant differences in total protein, albumin, glucose, cholesterol, bile acids, plasma alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities in all treatments. Cellular infiltration, presence of goblet cells and the width of lamina propria in the distal intestine were partly improved in fish fed FSBM 75 and 100 compared to the fish fed FSBM 50 and basal diet. Glycogen granulation, inflammation and nuclear change condition in the liver of pompano were better as the inclusion of fermented product increased. Results of this study indicate that FSBM can be utilized as an alternative protein source and microbial fermentation process could improve the functional properties of SBM.

KEYWORDS
fermented soybean meal, growth performance, histology, serum biochemistry, *Trachinotus carolinus*

1 | INTRODUCTION

Florida pompano *Trachinotus carolinus* exhibit many favourable characteristics for aquaculture such as excellent flavour, high market value, acceptance of pelleted feeds and relatively fast growth, and they can be cultured in seawater and low-salinity environments (Lazo, Davis, & Arnold, 1998; Riche & Williams, 2010; Salze, Spangler, Cobine, Rhodes, & Davis, 2016; Weirich & Riley, 2007). Several studies have reported the effectiveness of plant-based diets for pompano culture (Lazo et al., 1998; Rossi & Davis, 2012). Moreover, Quintero, Davis, and Rhodes (2012) have indicated that fish meal (FM) can be reduced from 30% to 15% and replaced with combinations of solvent-extracted soybean meal (SBM) and soy protein concentrate (SPC) without compromising the growth performance of Florida pompano. However, reduced performance of pompano has been observed when less than 15% of animal proteins, for example FM, poultry by-product meal or meat and bone meal, included in the diets (Rossi & Davis, 2012). Nonetheless, other studies have suggested that the decreased performance in low animal meal feeds can be partially remediated through the use of amino acid supplements and to a limited degree of attractants (Gaber, 2005; Lian, Lee, & Park, 2005; Novriadi, Spangler, Rhodes, Hanson, & Davis, 2017; Rhodes, Zhou, Salze, Hanson, & Davis, 2017). The complete elimination of animal meal could, in part, be related to the nutritional characteristics of plant-based ingredients such as SBM which is used at increased levels in low animal protein diets. Hence, there is an opportunity to improve the performance and utilization by shifting the characteristics of these ingredients.

The use of microorganisms for solid-state fermentation of SBM has been proven to be an alternative method to improve the functional properties and nutritional quality of SBM, reduce the antinutrients and increase the content of soybean peptides (Bi et al., 2015; Hong, Lee, & Kim, 2004; Papagianni, Nokes, & Filer, 1999). Zhuo, Liu, and Lin (2016) indicated that fermentation process with *Lactobacillus* spp can improve the crude protein digestibility and dry matter of SBM for grouper *Epinephelus coioides*. In addition to *Lactobacillus* spp, other microorganisms such as *Aspergillus ficuum* and *Aspergillus oryzae* (Chen, Vadlani, Madl, & Gibbons, 2016), *Bacillus subtilis* (Azarm & Lee, 2014), *Lactobacillus plantarum* P8 (Wang et al., 2016) or the combination of *Bacillus* spp and *Aspergillus* spp (Barnes, Brown, Bruce, Sindelar, & Neiger, 2014) have been used to facilitate the fermentation process and decrease the antinutrients contained in SBM. Furthermore, Lee, Azarm, and Chang (2016) also indicated that fermentation process may be more cost-effective than various advanced processing strategies to improve the acceptance and utilization of soy protein for aquaculture species.

To date, relatively few studies have monitored the complementary effects of fermented products to the serum biochemical parameters and enzyme activities in fish. However, the limited resources available have led to contradictory results (Lin et al., 2012). Considering that the evaluation of these parameters was helpful in narrowing the causative factors of metabolic disorders, such as deficiency in amino acids (Xu, Wang, Zhao, & Luo, 2012) and could be used as indicators of nutritional quality (Metón et al., 1999), serum samples must be evaluated in the study of plant-based diet. In addition, determining the histomorphological change that may occur in the liver and distal intestine of fish fed with high inclusion of soy protein has been suggested to be essential for fish health and nutritional efficiency of the diet (Baeverfjord & Krogdahl, 1996; Baeza-Ariño, Martínez-Llorens, Nogales-Mérida, Jover-Cerda, & Tomás-Vidal, 2016). With regard to the use of fermented product, Yamamoto et al. (2010) found that with proper fermentation and inclusion level, FSBM could prevent various physiological abnormalities in the distal intestine of rainbow trout *Oncorhynchus mykiss*.

To our knowledge, limited studies have been reported on the use of FSBM to gradually replace SBM in Florida pompano diets. Thus, the objective of this study was to evaluate the growth performance, serum biochemical characteristics, and intestinal and liver histological condition of juvenile Florida pompano in response to several inclusion levels of FSBM to replace traditional SBM.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

This experiment was conducted using a commercial product of FSBM known as PepSoyGen (Nutrafrema, North Sioux City, SD, USA) produced via fermentation process using *Aspergillus oryzae* and *Bacillus subtilis*. Four isonitrogenous and isolipidic (400 g/kg protein and 80 g/kg lipid) diets were formulated using poultry by-product meal (PBM, Griffin Industries, Inc. Mobile, AL, USA), de-hulled solvent-extracted soybean meal (SBM, Bunge Limited, Decatur, AL, USA), fermented soybean meal (FSBM, PepSoyGen™, Nutrafrema, SD, USA) and corn protein concentrate (Empyreal 75™, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA) as the dietary protein sources. The experimental diets were formulated to contain 0, 206, 309 and 410 g/kg FSBM, with FSBM replacing approximately 0%, 50%, 75% and 100% of the solvent-extracted SBM (designated as Basal, FSBM 50, FSBM 75, and FSBM 100, respectively). 15% PBM which has been run in numerous trials for Florida pompano were included in all dietary treatments. Taurine was supplemented in all diets to match the calculated levels in basal diet and to meet the requirement of pompano. All diets were produced in the Laboratory of Aquatic Animal Nutrition, School of Fisheries, Aquaculture and Aquatic sciences, Auburn University, AL, USA, using standard procedures for Florida pompano. Briefly, diets were made by mixing preground dry ingredients and fish oil in a food mixer (Hobart, Troy, OH, USA) for approximately 15 min. The mixture was then blended with boiling water to attain appropriate condition for pelleting. The moist mash from each diet was passed through a 3-mm die in a meat grinder, and the pellets were then placed into a fan-ventilated oven (<45°C) overnight to attain a moisture content <100 g/kg. The diets were stored at −20°C, and, prior to use, each diet was ground and sieved to an appropriate size. The compositions of the experimental diets were 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 | Experimental fish and growth trial

Florida pompano fingerlings were purchased from Troutlodge Marine Farms LLC (Proaquatix), Vero Beach, Florida, USA, and nursed in an indoor recirculating system facility at Claude Peteet Mariculture Development Center (CPMC), Gulf shores, Alabama, USA. Pompano were fed with commercial diet (FF Starter, Zeigler Bros., Inc. Gardners, PA, USA) until they reached the suitable size. The trial was conducted in a semi-recirculating system consisted of twelve open top tanks equipped with a reservoir tank, biological filter, supplemental aeration (provided using a central line, regenerative blower and air diffusers) and a circulation pump. At the start of experiment, twenty uniform-sized fish (mean initial weight 17.01 ± 0.07 g) were stocked into each tank and assigned to triplicate tanks in a completely randomized design. Fish were maintained under a natural photoperiod throughout the trial. Water

TABLE 1 Composition (g/kg, as is) of diets containing various levels of fermented soybean meal (FSBM) fed to juvenile Florida pompano for 8 weeks

	Diet code			
	Basal	FSBM 50	FSBM 75	FSBM 100
Ingredient (g/kg, as is)				
Poultry by-product meal[a]	150.0	150.0	150.0	150.0
Soybean Meal[b]	472.1	235.4	116.8	0.0
Fermented Soybean Meal[c]	0.0	206.0	309.0	410.7
Corn protein concentrate[d]	63.0	63.0	63.0	63.0
Menhaden Fish Oil[e]	47.4	49.0	49.7	50.5
Corn Starch[f]	7.0	37.2	52.4	67.0
Whole wheat[f]	220.0	220.0	220.0	220.0
Trace Mineral premix[g]	2.5	2.5	2.5	2.5
ASA Vitamin premix w/o choline[h]	5.0	5.0	5.0	5.0
Choline chloride[f]	2.0	2.0	2.0	2.0
Stay C 35%[i]	1.0	1.0	1.0	1.0
CaP-dibasic[f]	20.0	19.0	18.7	18.5
Lecithin (soy commercial)[j]	5.0	5.0	5.0	5.0
Taurine[f]	5.0	4.9	4.9	4.8
Proximate analyses (g/kg, as is)[k]				
Crude Protein	423.5	416.0	416.9	418.9
Moisture	47.5	60.9	64.3	65.1
Crude Fat	99.6	93.2	95.9	95.8
Crude Fibre	26.2	32.3	30.1	27.9
Ash	67.4	65.7	64.9	64.0

[a]Griffin Industries, Inc., Mobile, AL, USA.
[b]De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA.
[c]PepSoyGen™, Nutraferma, Protein and Biotech Products, Sioux City, IA, USA.
[d]Empyreal 75™, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.
[e]Omega Protein Inc., Houston, TX, USA.
[f]MO Biomedicals Inc., Solon, OH, USA.
[g]ASA Premix (g per 100 g 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 sulphate heptahydrate, 13.193; and α cellulose 81.826.
[h]ASA Premix (g/kg Premix): thiamine 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.
[i]Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.
[j]The Solae Company, St. Louis, MO, USA.
[k]Analyses conducted by the University of Missouri-Columbia, Agricultural Experiment Station Chemical Laboratory, MO, USA.

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

AA (g/kg, dry matter)	Diet code			
	Basal	FSBM 50	FSBM 75	FSBM 100
Taurine	7.3	6.9	6.7	6.6
Hydroxyproline	3.1	3.5	3.0	3.8
Aspartic Acid	37.1	37.1	37.2	38.0
Threonine	14.7	14.8	14.9	15.1
Serine	17.8	18.3	18.0	18.8
Glutamic Acid	75.3	75.3	74.7	76.4
Proline	24.7	23.7	23.8	23.9
Lanthionine	0.3	0.4	0.5	0.4
Glycine	21.4	21.7	21.3	21.9
Alanine	21.5	21.7	21.7	22.0
Cysteine	5.9	5.7	5.7	5.7
Valine	19.7	19.6	19.8	20.2
Methionine	7.4	7.4	7.3	7.2
Isoleucine	17.8	17.7	18.0	18.1
Leucine	35.1	34.9	35.3	35.6
Tyrosine	15.2	14.8	12.8	15.3
Phenylalanine	20.1	19.9	20.0	20.2
Hydroxylysine	0.9	0.8	0.8	0.7
Ornithine	0.00	0.1	0.1	0.00
Lysine	22.0	20.9	20.8	21.3
Histidine	9.5	9.4	9.4	9.5
Arginine	25.7	25.1	24.1	25.2
Tryptophan	4.7	4.6	5.0	4.5

temperature (28.64 ± 1.59°C), salinity (31.17 ± 3.92 ppt), pH (8.05 ± 0.38) and dissolved oxygen (5.89 ± 0.49 mg/L) were measured two times daily with a water quality multiparameter (ProPlus, Yellow Spring Instruments Co., Yellow Springs, OH, USA). In addition, total ammonia nitrogen (0.13 ± 0.23 mg/L) was measured two times per week using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA) and nitrate (7.10 ± 5.90 mg/L) was measured once a week using colorimetric test kits (La Motte Chemicals, Chestertown, MD, USA). Fish were fed four times per day, and the daily ration was adjusted to apparent satiation weekly throughout the trials. Additionally, feed inputs were calculated on a two-week basis after each sampling to adjust for growth and mortalities. The growth trial lasted for 8 weeks. At the end of growth trials, fish were grouped and individually weighed to obtain the final biomass, final weight, percentage weight gain (PWG), feed conversion ratio (FCR), percentage survival (SR) and thermal unit growth coefficient (TGC), calculated as follows:

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

$$\text{FCR} = \frac{\text{average individual dry matter feed intake}}{\text{average individual weight gain}}$$

TABLE 3 Growth performance of juvenile Florida pompano (mean initial weight 17.01 ± 0.07 g) offered fermented soybean meal at varying levels for 8 weeks

	Diets	Final mean weight (g)	PWG (%)	TGC	Feed Intake (g/fish)	FCR	Survival (%)
1	Basal	72.44	324.81	0.1016	94.99	1.72	93.3[a]
2	FSBM 50	68.25	301.68	0.0960	89.75	1.78	78.3[b]
3	FSBM 75	73.48	335.18	0.1025	85.78	1.54	80.0[b]
4	FSBM 100	69.10	305.95	0.0979	82.33	1.58	73.3[b]
	p-value	.7531	.7857	.7788	.0791	.3119	.0027
	PSE	3.98	26.43	0.01	3.00	0.09	2.50

FCR, Feed conversion ratio; PSE, Pooled standard error.

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

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

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

Twenty fish from the initial stock population were sampled, and four fish from each tank (twelve fish per treatment) were randomly sampled at the end of the trial and stored at −80°C for body composition analysis. Prior to proximate analysis, dried whole fish were rigorously blended and chopped in a mixer according to methods described by Association of Official Analytical Chemists (AOAC, 1990). All parameters were analysed at Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO, USA), and the mean of each treatment was taken.

2.4 | Serum biochemical analysis

At the end of growth trial, twelve fish per treatment (four fish per replicate) were immediately euthanized with Tricaine-S (MS-222, tricaine methanesulfonate salt, Western Chemical, Inc., Ferndale, WA, USA), and blood samples were taken from the caudal vein after 12-hr fasting. Serum was obtained by centrifugation at 1200 *g* for 10 min and stored at −80°C until serum biochemical analysis. Biochemical parameters in the serum samples were analysed for total protein, albumin, glucose, cholesterol, bile acid concentration, activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using an automated chemistry analyser (Cobas C311, Roche Diagnostics, IN, USA) following protocol described by Salze et al. (2016).

2.5 | Histopathology analysis

Fish samples for histopathological studies were selected randomly and individually anesthetized in a solution of Tricaine-S (MS-222, tricaine methanesulfonate salt, Western Chemical, Inc., Ferndale, WA, USA). Liver and distal intestine of fish were preserved in Bouin's solution for 20 hr at room temperature and then transferred to 70% ethanol solution until processed by standard histological techniques. The blocks of designed sample, dehydrated through a standard ethanol series to 100%, were embedded in paraffin wax and sectioned at 4-μm intervals for staining with haematoxylin–eosin (H&E) stain (Merck, Darmstadt, Germany). For estimations, double-blinded evaluation with a grading scale of 1–5 was used. Score 1 was considered as the normal condition, and subsequent scores accounted for increasing levels of histopathological alteration compared to the normal condition. The following parameters were taken into account for liver sections: glycogen granulation, inflammation and nuclear change. While intestinal samples were evaluated for cellular infiltration, presence of goblet cells and widening of the lamina propria within the intestinal folds, images were acquired using a digital imaging microscope (ECLIPSE 80i, Nikon, Japan).

2.6 | Statistical analysis

Statistical analyses were conducted using SAS system (V9.3, SAS Institute, Cary, NC, USA). All data except for histopathological analysis were analysed using one-way analysis of variance to determine the significant difference ($p < .05$) among the treatment means followed by the Tukey's multiple comparison test to determine difference between treatments means in each trial. The pooled standard errors are presented across growth trials, proximate composition, serum levels and enzyme activities, as the variance of each treatment is the same. Histopathological scores were treated as categorical data, tested for normality and homoscedasticity and subsequently analysed using Welch's one-way analysis of variance followed by Games–Howell *post hoc* tests to determine significant differences between treatments. The results for histopathological evaluation are shown as mean ± standard deviation.

3 | RESULTS

3.1 | Growth performance

Nutrient composition of the experimental diets and fish growth performance are presented in Tables 2 and 3. No significant differences were observed in final mean weight, percentage weight gain, TGC,

TABLE 4 Proximate composition (g/kg) of whole body of pompano fed experimental diets for 8 weeks

	Diets	Crude protein	Moisture	Fat	Crude Fibre	Ash	Phosphorus
1	Basal	184.4	713.3	69.5	0.6[ab]	31.5	5.8
2	FSBM 50	189.8	711.2	69.7	0.9[b]	32.9	6.1
3	FSBM 75	186.5	707.9	72.3	0.8[ab]	32.8	5.8
4	FSBM 100	184.8	713.7	68.5	0.5[a]	34.0	6.3
	p-value	.3772	.8030	.9480	.0335	.5356	.2198
	PSE	0.22	0.46	0.47	0.01	0.12	0.02

feed intake and FCR across all dietary treatments (Table 3). However, fish fed with basal diet without any inclusion of FSBM had significantly higher survival rates as compared to fish fed with various inclusion levels of FSBM (Table 3).

3.2 | Proximate composition of whole fish

Body composition of the fish fed with diets containing various levels of FSBM is shown in Table 4. No significant differences were identified in the crude protein, moisture, fat, ash and phosphorus content among the treatments ($p > .05$). Crude fibre content of fish fed diets FSBM 100 was lower compared to FSBM 50 ($p = .0335$).

3.3 | Serum levels and enzyme activities

There were no significant differences in plasma total protein, albumin, glucose and bile acid levels among the treatments (Table 5). Plasma ALP, ALT and AST activities did not differ among the fish groups fed the experimental diets.

3.4 | Liver and intestine histopathology

The histopathological examination of fish liver (Figure 1a and Table 6) indicated that fish fed with basal diet showed a higher score for glycogen granulation, inflammation and nuclear change compared to other dietary treatments (Table 5 and Figure 1a). As the inclusion level of FSBM to substitute SBM increases, liver condition of pompano was better with lower numerical value for glycogen granulation, inflammation and nuclear change. At the end of the growth trial, no incidence of green liver was observed in fish across all dietary treatments. The administration of experimental diet significantly affects the morphology of intestines. In distal tract of intestine (Figure 2), fish fed with basal diet and FSBM 50 showed an abundance number of goblet cells (GC) compared to FSBM 75 and 100. The width of the lamina propria (LP) and cellular infiltration in the distal intestine of fish fed basal diet and FSBM 50 are wider compared to FSBM 75 and FSBM 100.

4 | DISCUSSION

A previous study indicated that the basal diet consisting of 50% SBM and 15% PBM supplemented with taurine can be used as a good alternative ingredients for animal meal without any negative impact on growth performance of juvenile Florida pompano (Rossi & Davis, 2012). To improve the efficacy of soy-based protein, we utilized FSBM to incrementally (0, 50, 75, and 100%) replace SBM on an isonitrogenous basis. Our results indicated that the use of commercially produced fermented product, PepSoyGen, to replace the traditional SBM had no effect on growth performance and the proximate composition of Florida pompano. Our results corroborate the study of Choi, Rahman, Lee, Chang, and Lee (2016) which reported that the use of various level of FSBM processed with *Phaffia rhodozyma* to replace traditional SBM had no effect on growth performance, feed utilization and proximate composition of juvenile rainbow trout. Similarly, other studies have reported no significant effect on the growth

TABLE 5 Effect of different diets on serum levels and enzyme activities in Florida pompano

	Diets	Total protein (g/dl)	Albumin (g/dl)	ALP (U/L)	ALT (U/L)	AST (U/L)	Glucose (mg/dl)	Cholesterol (mg/dl)	Bile acid (mg/dl)
1	Basal	4.06	1.30	35.50	17.33	222.0	251.67	173.67	9.80
2	FSBM 50	4.29	1.42	42.93	10.67	100.67	237.67	184.00	8.93
3	FSBM 75	4.05	1.33	38.30	18.00	161.33	240.00	179.67	5.80
4	FSBM 100	4.17	1.41	44.23	11.50	96.33	246.67	176.67	3.83
	p-value	.3927	.1079	.0540	.2778	.2515	.9227	.5686	.5623
	PSE	0.11	0.03	2.05	3.69	45.97	16.13	5.20	3.24

ALP, Alkaline phosphatase; ALT, Alanine transaminase; AST, Aspartate transaminase; PSE, Pooled standard error.

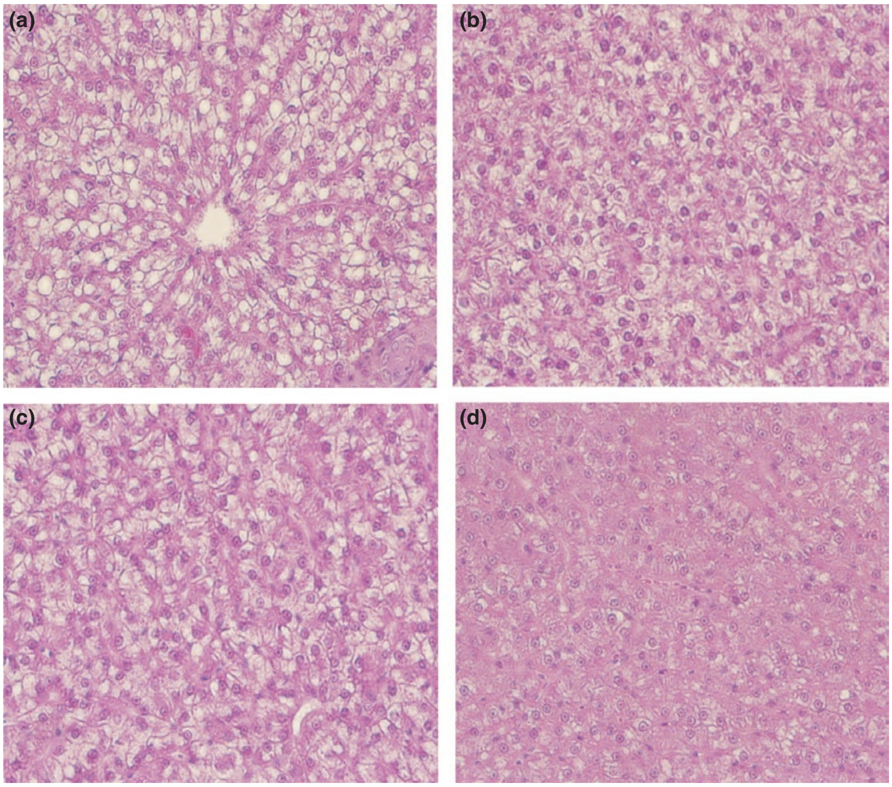

FIGURE 1 Representative histopathological images of haematoxylin- and eosin-stained sections of liver from Florida pompano after 8 weeks of being fed with (a) basal diet, (b) FSBM 50, (c) FSBM 75 and (d) FSBM 100

performance of Nile tilapia, *Oreochromis niloticus* (Lim & Lee, 2011) and white shrimp, *Litopenaeus vannamei* (Lin & Mui, 2017) when 50% or 100% of SBM was replaced by FSBM.

Fermentation process enhances the bioavailability of nutritious components (Chi & Cho, 2016) and reduces the immunoreactivity and allergic reactions caused by soy products (Frias, Song, Martínez-Villaluenga, De Mejia, & Vidal-Valverde, 2007). However, depending on the type of fermentation and environmental condition, the use of different microorganisms might significantly decrease some amino acids (Song, Frías, Martinez-Villaluenga, Vidal-Valdeverde, & de Mejia, 2008). According to Lim and Lee (2011), microbial fermentation with *A. oryzae* decreased lysine and threonine concentration in SBM by 19% and 16%, respectively, while *Bifidobacterium lactis* or *Lactobacillus plantarum* significantly decreased the level of methionine and cysteine in SBM (Song et al., 2008). In the present study, taurine was supplemented in the basal diet at the level of 5 g/kg and into the FSBM diet at a range of 4.8–4.9 g/kg to meet the estimated nutrient requirements for Florida pompano. However, fermentation process seems to have an effect to the taurine profile in the final fermented product, as indicated by the low level of taurine in the FSBM diet (6.6–6.9 g/kg) compared to basal diet (7.3 g/kg). There is growing evidence that taurine is essential for certain life stages in Florida pompano and reduced growth performance was observed in this fish when taurine requirement level is not met (Rossi & Davis, 2012). In addition, lysine level in the presence of fermented product (20.8–21.3 g/kg) was also lower compared to basal diet (22 g/kg). Albeit lysine level remains in the predicted requirement (Riche & Williams, 2011), deficiency of this amino acid (AA) has been shown to cause a reduction in growth performance, feed efficiency and health issues (Ketola, 1983; NRC, 2011). As a result, it could be said that the degradations of AA influence the growth performance of pompano. Hence, to improve the efficacy of soy-source protein, high inclusion of fermented product in the diet formulation will either need to be balanced with proper level of AA in its purified form or supplemented with other protein sources rich in AA, especially taurine and lysine.

Fermentation process provides several benefits, such as a reduction in soy-allergic immunoreactivity and an enhancement of the bioavailability of nutritious components to improve the physiological status of the fish (Chi & Cho, 2016; Frias et al., 2007). The results of the present study showed that the total protein, albumin, glucose, cholesterol, bile acid, ALP, ALT and AST were not significantly affected, indicating that the inclusion of FSBM did not cause any serious alteration in all clinical variables compared to traditional SBM. Several studies have been carried out to determine the effect of fermented product on the serum and blood condition of fish. Study from Lim and Lee (2011) reported that plasma glucose, cholesterol and plasma total protein of Nile tilapia were not affected using FSBM to replace the traditional SBM. Moreover, Murashita et al. (2013) indicated that in comparison with traditional SBM, the use of fermented soy as the primary protein source did not significantly improve the total protein, cholesterol, glucose and total bile acid concentration of rainbow trout. On the contrary, Kader et al. (2012) found that the total serum protein of Japanese flounder (*Paralichthys olivaceus*) tended to increase with the increasing of FSBM inclusion levels in the diets. Moreover, Yamamoto et al. (2012) reported that the haemoglobin content of rainbow trout *Oncorhynchus mykiss* fed diet FSBM was significantly higher than in fish fed diet SBM. Thus, based on these findings, collection of blood samples at different time points after the last feeding

TABLE 6 Diagnostic features of liver and distal intestine of fish based on comparison of dietary treatments

Feature	Experimental diet Basal	FSBM 50	FSBM 75	FSBM 100	*p*- value
Intestine					
Goblet cells	4.2 ± 0.4[a]	3.8 ± 0.5[a]	2.4 ± 0.5[b]	2.3 ± 0.5[b]	<.0001
Cellular infiltration	3.8 ± 0.4[a]	3.7 ± 0.5[a]	2.4 ± 0.5[b]	2.0 ± 0.4[c]	<.0001
Lamina Propria width	3.8 ± 0.5[a]	3.8 ± 0.4[a]	2.4 ± 0.5[b]	2.1 ± 0.4[b]	<.0001
Liver					
Glycogen granulation	4.1 ± 0.4[a]	3.6 ± 0.5[b]	2.4 ± 0.5[c]	2.0 ± 0.0[d]	<.0001
Inflammation	3.9 ± 0.3[a]	3.3 ± 0.5[b]	2.4 ± 0.4[c]	1.8 ± 0.2[d]	<.0001
Nuclear change	3.8 ± 0.1[a]	3.2 ± 0.4[b]	2.3 ± 0.5[c]	1.8 ± 0.3[d]	<.0001

Results presented as mean ± standard deviation (n = 12). Results in the same row with different superscript letter are significantly different (p < .05) based on Welch's one-way analysis of variance followed by Games–Howell post hoc tests to determine significant differences between treatments.

is needed to clarify whether the inclusion of fermented soy might influence the haematological conditions and other enzyme activities in Florida pompano.

In some species, SBM causes several physiological abnormalities in liver (Murashita et al., 2013) and distal intestinal of cultured fish, such as the abnormal vacuolization, widening of central stroma and inflammatory cells infiltration in lamina propria (LP) (Baeverfjord & Krogdahl, 1996; Burrells, Williams, Southgate, & Crampton, 1999). Study from Yamamoto et al. (2012) showed a morphological changes in the distal intestine of rainbow trout, including an increase in connective tissue in the submucosa and few vacuoles in the cytoplasm of hepatocytes in fish fed SBM. In the present study, the distal intestine of Florida pompano seems to be mildly sensitive to the dietary SBM. The LP with cells infiltration in fish fed with basal diet is significantly wider compared to FSBM 75 and FSBM 100. It has been reported that the use of high inclusion level of SBM might induces the cellular infiltration of the submucosa and LP in the Egyptian sole *Solea aegyptiaca* and Gilthead sea bream *Sparus aurata* (Bobadilla et al., 2005; Bonaldo, Roem, Pecchini, Grilli, & Gatta, 2006). However, Yamamoto et al. (2010) found that the inclusion of fermented product produced via fermentation process

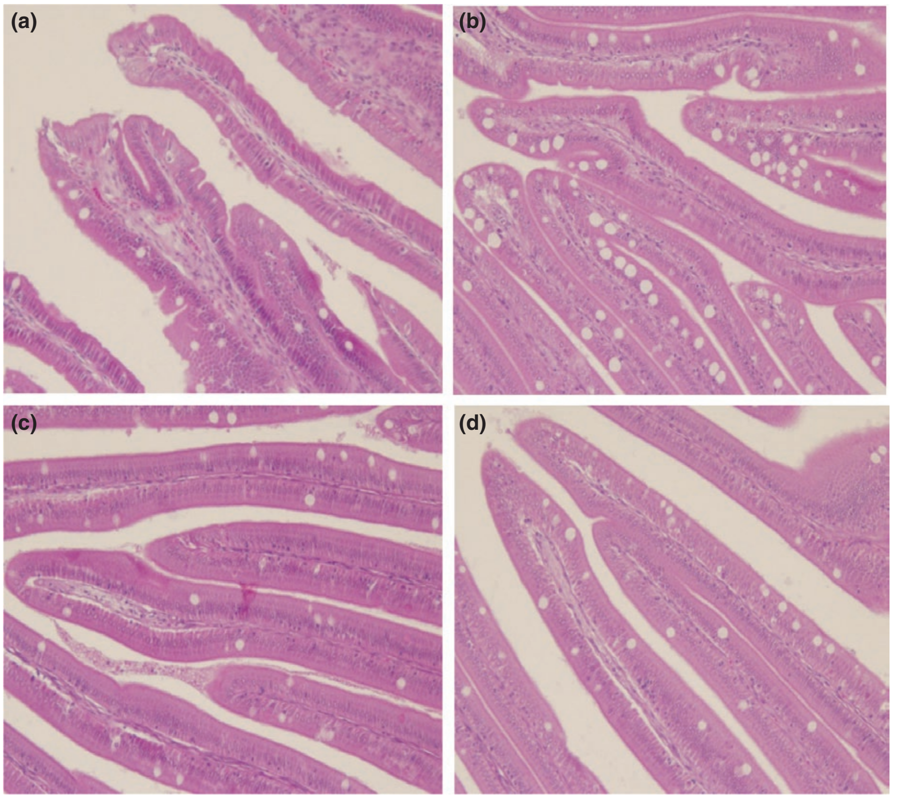

FIGURE 2 Representative histopathological images of haematoxylin- and eosin-stained sections of distal intestines from Florida pompano after 8 weeks of being fed with (a) basal diet, (b) FSBM 50, (c) FSBM 75 and (d) FSBM 100

using as compound bacteria predominantly *Bacillus* spp for 10 hr could prevent the abnormalities in the LP of mucosal folds caused by SBM and showed normal conditions as shown in fish fed with FM-based diet. With regard to the presence of goblet cells (GC), the lowest number was found in fish fed with FSBM 75 and 100 than in fish fed FSBM 50 and basal diet. According to Marchetti et al. (2006), the emergence of GC associated with the production of mucus to protect the mucous membrane from chemical and mechanical damage. In addition, Baeza-Ariño et al. (2016) suggest that the increased presence of GC could also be related to the unsatisfactory of protein digestion process. Thus, based on these findings, the use of high inclusion level of fermented soy may serve as a promising ingredient to partly prevent various physiological abnormalities that occur in the distal intestine of pompano fed with plant-based diet.

In this study, histopathological examination of the liver in fish fed with basal diet showed a modest amount of glycogen vacuolization, inflammation and nuclear change. Martínez-Llorens, Baeza-Ariño, Nogales-Mérida, Jover-Cerdá, and Tomás-Vidal (2012) suggest that providing plant-based diet for certain period of time will cause a metabolic dysfunction, indicated with glycogen deposition in the liver. The use of FSBM 50 showed better condition than basal diet in terms of glycogen granulation, inflammation and nuclear change. In addition, liver condition of fish fed with FSBM 75 presented less morphological change than FSBM 50, and liver condition of fish fed with FSBM 100 appeared to be much better than other dietary treatments. However, it remains unclear whether the better liver condition of fish fed with FSBM 100 may be attributable to the reduction in ANFs found in the fermented product or other beneficial factors obtained from microbial fermentation process. Several authors reported that microbial fermentation of SBM could enhance the bioavailability of potential antioxidants, production of enzymes, carbohydrase and proteinase, leading to improved digestibility and health condition of fish (Aidoo, Smith, & Wood, 1994; Chou, 1995; Kim, Pham, Kim, Son, & Lee, 2010; Kim et al., 2009; Lim & Lee, 2011; Pham & Lee, 2007). Although the fermentation process could be an important factor for the improvement of physiological condition in the liver and distal intestine of pompano, other ANFs that still remain in the final fermented product and responsible for the morphological changes and growth performance of fish need to be carefully evaluated.

The observation made in the present study suggests that FSBM produced via fermentation process using *Aspergillus oryzae* and *Bacillus subtilis* may serve as a good source of protein, and the inclusion of 309 and 410 g/kg FSBM to replace approximately 75% and 100% SBM was able to partially prevent the development of marked histological alteration in the liver and distal intestine of Florida pompano compared to basal and the inclusion of 206 g/kg FSBM to replace approximately 50% traditional SBM. Based on the results of the growth trial, further research is required to clarify whether the supplementation of essential amino acid to satisfy the nutrient requirement of fish fed with high inclusion level of FSBM may have beneficial effects on growth performance and physiological conditions of Florida pompano.

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