Comparison and Efficacy of Soy-based Ingredients in Practical Diets for Florida Pompano (*Trachinotus carolinus*)

by

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Abstract

Three trials were performed to better understand how soy processed in different ways impacts the growth, feed conversion ratio, and health of the Florida pompano (*Trachinotus carolinus*). First two growth trials were performed to evaluate the efficacy of an open soy-based diet compared to a commercially produced, fishmeal-based diet. Trial one utilized six replicates and was terminated after 42 days, with overall biomass (p=0.026), weight gain (p=0.006), and FCR (p=0.015) being significantly different between both treatments in favor of commercially produced, fishmeal-based feed. The second trial utilized three replicates, was run over the course of 56 days, and upon termination there were no statistically significant differences due to diets. Histological samples of the distal intestine showed no statistical differences in symptoms conducive to soy-induced enteritis (lamina propria thickness, lamina propria cellularity, thickening of connective tissue, and abundancy of goblet cells) between the practical soy diet and the commercial fishmeal diet. Trial three was designed to improve the soy-based feed by evaluating nine experimental diets with varying soy sources. The basal diet contained 49.97% solvent-extracted soybean meal (SBM) which was replaced with low oligosaccharide soybean meal (LO-SBM), soy protein concentrate (SPC), and fermented soybean meal (Fer-SBM) at 50 or 100%, or expeller extruded soybean meal (EE-SBM) at 25 or 50%. Juvenile Florida pompano (4.82 ± 0.08 grams) were offered randomly assigned diets in quadruplicate for 76 days. Upon termination, there were no significant differences in weight (p=0.493), survival (p=0.925), or FCR (p=0.874) in fish offered any of the experimental diets. Histological analysis of distal intestine samples showed no statistical differences in lamina propria thickness, lamina propria cellularity, connective tissue thickening, or abundance of goblet cells.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

SBM: Soybean Meal

LO-SBM: Low Oligosaccharide Soybean Meal

SPC: Soy Protein Concentrate

Fer-SBM: Fermented Soybean Meal

EE-SBM: Expeller Extruded Soybean Meal

USD: United States Dollar

USA: United States of America

RAS: Recirculating Aquaculture System

YSI: Yellow Springs Instrument

TAN: Total Ammonia Nitrogen

TGC: Thermal Growth Coefficient

FCR: Feed Conversion Rate

DO: Dissolved Oxygen

RNA: Ribonucleic Acid

LPT: Lamina Propria Thickness

LPC: Lamina Propria Cellularity

CT: Connective Tissue

GC: Goblet Cells

ANOVA: Analysis of Variance

PWG: Percent Weight Gain

1. Introduction

Global aquaculture has grown at a steady pace of 8% since the 1970s, making it one of the fastest growing food production sector (Garlock et al., 2020; Subasinghe et al., 2009; Troell et al., 2014). As global aquaculture has been increasing for decades, so has the demand for commercial feed. Feed is the primary production cost associated with aquaculture accounting for 40-60% of all production costs (Ansari et al., 2021; O'Donncha & Grant, 2019; Sookying et al., 2013). Because of the increasing costs, there is a need to produce commercial diets that focus on using high-quality ingredients that can be found readily and at an affordable cost (Aya, 2017; Roy & Pal, 2015).

Proteins are a primary contributor to the growth of farmed aquatic organisms (Huo et al., 2014). Thus, protein characteristics are essential when formulating a diet for commercially important fish. Fishmeal has historically been the most widely used protein source in aquaculture diets due to its high protein content, amino acid profile, and is both highly palatable and digestible (Jackson, 2009). However, the use of fishmeal in diets has come under scrutiny in recent years. As demand for aquaculture feed increases, so does the demand for fishmeal, yet wild stocks are at or beyond sustainable limits resulting in an inability to meet demand (Gatlin et al., 2007; Jannathulla et al., 2019; Mukhtar et al., 2017; Olsen & Hasan, 2012). Because demand has far exceeded supply, fishmeal costs have increased substantially (Gatlin et al., 2007; Mukhtar et al., 2017; Olsen & Hasan, 2012). This has also placed serious pressure on capture fisheries that commonly source fishmeal from their catch (Jackson, 2006).Because of these factors, attention has been shifted to focus on more sustainable protein sources, with particular attention being drawn to plant-based proteins.

Soy-based protein has been an increasingly popular substitute for animal-based protein sources in aquaculture diets. This is due to its high protein content, global cultivation, and affordability (Choi et al., 2020; Watanabe, 2002). Soy-based protein has also been shown to be successfully used as a primary protein source for many commercially important species, including several species of marine finfish such as hybrid striped bass, red sea bream, cobia, Japanese flounder, red drum, and yellowtail kingfish (Biswas et al., 2007; Bowyer et al., 2013; Chou et al., 2004). This is especially noteworthy as marine finfish typically require higher-quality protein than freshwater finfish (Craig et al., 2009). Past growth trials in the past have allowed for soy-based proteins to replace fishmeal and other animal-based proteins from 20% to as high as 58% percent in some species (Alam et al., 2011, 2012; Silva-Carrillo et al., 2012).

Despite the positive qualities associated with soy-based proteins, they also come with challenges, with one of the main issues being antinutritional factors such as lectins, phytates, tannins, oligosaccharides, and trypsin inhibitors (Gu et al., 2010; Hua et al., 2019). Antinutritional factors can reduce the nutritional value of soy by interfering with protein and mineral absorption, which hinders the growth of the organisms consuming it (Adeyemo & Onilude, 2013; W. Zhang et al., 2019). In some fish species, components of soy produce an allergic response known as soybean-induced enteritis (Baeverfjord & Krogdahl, 1996). Soybean-induced enteritis has been known to affect a wide range of commercially important species, such as salmonids, cyprinids, Atlantic cod, and several species of bass, among others (Buentello et al., 2015). While some fish species can digest soy better than others, all are still affected by these antinutritional factors. Therefore, it is crucial to find ways to reduce the antinutritional factors associated with soy.

Processing soy physically, chemically, and biologically can decrease the amount of antinutritional factors found in soy-based ingredients (Barnes et al., 2014; Vielma et al., 2002; Willis, 2003). Fermentation, fractionization, heat treatment, enzymatic treatment, solvent extraction, and expeller extrusion are different methods used to alter nutritional profiles and decrease antinutritional factors. Solvent extraction is seen as the most common processing method for soybean meal, with dehulled, solvent-extracted soybean meal being the most widely used soy source in diets due to its mass of production and high protein content (O'Keefe, 2003; Powell et al., 2011; Stein et al., 2012). Although solvent-extracted soybean meal is the most widely used process variant, it is not always considered the best option for important aquaculture species. For example, Olli et al. (2009) reported that when Atlantic salmon were offered one of four soy products (solvent-extracted soybean meal, dehulled and solvent extracted soybean meal, dehulled full-fat soybean meal, and soybean concentrate), the fish responded most favorably to experimental diets composed of soybean concentrate, with results being comparable to fish offered fishmeal centered diets (Olli et al., 1994). Because of this, it is vital to determine which soy-based ingredients are best suited for the commercial diets of marine finfish.

As aquaculture continues to grow, so does the interest in species for commercial propagation. Florida pompano (*Trachinotus carolinus*) is a member of the Carangidae family (Jacks), which is comprised of other highly desirable commercial food fish. Interest in the domestic culture of the Florida pompano dates back to the 1950s (Main & Rosenfeld, 1995; Weirich et al., 2021). This is due to the Florida pompano possessing many favorable qualities such as fast growth, a wide tolerance to salinity, and acceptance of pelleted feed (Riche & Williams, 2011; Weirich et al., 2021). For years, Florida pompano has been considered a desirable fish for table fare. Dockside prices have been consistently high for decades, with a modern market value ranging from six to ten dollars (USD) per pound, depending on season and size of individual fish (McMaster et al., 2006). This may increase to as much as thirty-five to forty-five dollars (USD) for individual filets, making the Florida pompano a highly profitable species for potential culture (Rossi & Davis, 2012). This is due in part to their relative abundance throughout the eastern seaboard, particularly in areas ranging from North Carolina to Florida, as well as the mild, almost sweet-like flavor of their meat (Main et al., 2007; Weirich et al., 2021; Wood, 2009). Despite these factors, the large-scale commercial culture of Florida pompano was deemed infeasible in the 1980s.

However, modern advancements in rearing techniques, spawning and larval rearing have led to a reemergence of interest in the culture of Florida pompano, with prior technological restraints no longer considered a bottleneck to production (Main et al., 2007.; Weirich et al., 2021). Currently, Florida pompano is cultured commercially with an interest in expanding production. If commercial expansion is to be successful, it is paramount to better understand Florida pompano's dietary and nutritional requirements. Over the past decade, much attention has focused on dietary formulations, with growth trials being used to assess the tolerance or acceptance of varying ingredients. To date, it has been shown that Florida pompano are able to accept soy as a primary protein source in feeds, with fish responding positively to fishmeal-free diets supplemented with low amounts of animal-based proteins such as meat and bone meal or poultry-by product meal (Rossi & Davis, 2012, 2014). While Florida pompano are very tolerant to soy-based diets, there has been little comparative research on determining which type of soy source or soy-based ingredient may be used to optimize growth and minimize deleterious health effects. Therefore, this study aimed to compare growth results on Florida pompano offered a commercial, fishmeal-based diet as opposed to a practical, soy-based diet. This was done to better understand the long terms effects a soy-based diet had on growth, feed conversion, and health. After, experimental diets with varying soy-based ingredients were offered to juvenile Florida pompano to identify nutritional response and determine optimal soy-based protein.

2. Material & Methods

Thrree growth trials were performed to evaluate the efficacy of various soy-based feeds. The first two trials compared an open, soy-based experimental diet with a commercially produced fishmeal-based one. Trial three compared nine experimental diets composed of varying soy-sourced ingredients to better determine what soy product is most suitable for Florida pompano growth and development.

2.1 Experimental Diets

All experimental diets used in trials one, two, and three were formulated and produced by the Laboratory of Aquatic Animal Nutrition, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, AL, USA. For trials one and two, the experimental, soy-based diet was formulated to be isonitrogenous and isolipidic at 40% protein and 10% lipid. The experimental soy diet was formulated to contain 55.20% solvent-extracted soybean meal (SBM) with 8% poultry by-product meal and 10% corn protein concentrate as protein sources (Table 1). For trial three, nine experimental diets with varying soy ingredients were formulated to be isonitrogenous and isolipidic at 40% protein and 8% lipid. The nine experimental diets were formulated based on a basal diet composed of 49.97% SBM with 14% poultry by-product meal and 6.75% corn protein concentrate (Table 2). Other soy protein sources used in trial 3 include a low oligosaccharide soybean meal (LO-SBM), soy protein concentrate (SPC), fermented/enzyme-treated soybean meal (Fer-SBM), and an expeller extruded soybean meal

(EE-SBM). Test diets for trial three were formulated to allow SBM to be replaced with the various soy sources on an isonitrogenous level.

Diets were formed using standard laboratory procedures. Ingredients were sourced and analyzed for proximate composition and the feed formulations were developed. The pre-ground ingredients were added to a food mixer (Globe Food Equipment Co, Moraine, OH) and mixed for 15 minutes during which oil was blended into the mash and then after mixing boiling water until an appropriate consistency was reached. Once mixed, the mash was extruded through a 3 mm die in a meat grinder and then placed in a forced air-drying oven until excess moisture was lost (<10%). Once diets had been dried to the appropriate condition, they were ground and sieved. Diets were stored at -20 C. The experimental diets were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate analysis (Table 2) and amino acid profiles (Table 3).

2.2 Growth Trials

2.2.1 Trials 1 and 2: Comparison of Soy-Based Diet to commercial fishmeal-based diet for Florida pompano

Florida pompano juveniles were obtained from Proaquatix LLC, Vero Beach, FL and were transported to Claude Peteet Mariculture Center in Gulf Shores, AL. Fish were stocked into one of two separate recirculating aquaculture systems (RAS). For trial one, fish were stocked into system A, composed of 12 circular fiberglass tanks **(**1.19 m3**)** with 15 fish stocked per tank allowing for six replicates per diet. For trial two, fish were stocked into system B, composed of six tanks (6.78 m3) with 25 fish being stocked per tank allowing three replicates per treatment.

Table 1. Dietary composition of high soy diet formulated to 40% protein and 10% lipid used in the grow out demonstration (Trials 1 and 2).

Composition	(g/100g, as is)
Poultry meal[1]	8.00
Soybean meal[2]	55.20
Corn protein concentrate[3]	10.00
Menhaden fish oil[4]	8.16
Lecithin (soy)[5]	0.50
Whole wheat	14.49
Mineral premix[6]	0.25
Vitamine premix[7]	0.50
Choline chloride [8]	0.20
Rovimix Stay-C 35%[9]	0.10
CaP-dibasic[10]	2.00
DL-Methionine[11]	0.10
Taurine[11]	0.50

[1]Darling Ingredients, Irving, TX, USA
[2]De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA
[3]Empyreal 75 TM, Cargill Corn Milling, Cargill, Inc, Blair, NE, USA
[4]Omega Protein Inc., Houston, TX, USA
[5]The Solae Company, St. Louis, USA
[6] Mineral Premix (g 100g-1 premix): cobalt chloride, 0.008; cupric sulphate pentahydrate, 0.010, ferrous sulfate heptahydrate, 20.0, manganous sulfate anhydrous, 2.90; potassium iodide, 0.024; sodium selenite, 0.048; zinc sulfate heptahydrate, 17.60, and α cellulose 59.194.
[7] Vitamin Premix (g/kg Premix): thiamin HCL, 8.0; 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 D3 (400,000 IU g-1), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.
[8]MO Biomedicals Inc., Solon, OH, USA
[9] Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA
[10]Acros Organics B.V.B.A. Thermo Fisher Scientific, Waltham, MA, USA
[11]MP Biomedicals Inc., Santa Ana, CA, USA
*Analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA)

Table 2. Composition of Florida pompano diets including various soybean meal sources.

Composition (g/100g, as is)	SBM	LO-SBM 50%	LO-SBM 100%	SPC 50%	SPC 100%	Fer SBM 50%	Fer SBM 100%	EE SBM 25%	EE SBM 50%
Poultry by-product meal[1]	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Soybean meal[2]	49.97	24.99	0.00	24.99	0.00	24.99	0.00	36.49	24.99
Bright Dayl[3]	0.00	21.00	42.00	0.00	0.00	0.00	0.00	0.00	0.00
Soycomil PE[4]	0.00	0.00	0.00	18.55	36.95	0.00	0.00	0.00	0.00
HP 300[5]	0.00	0.00	0.00	0.00	0.00	20.55	40.90	0.00	0.00
Expeller-extruded SBM[6]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.18	24.36
Corn protein concentrate[7]	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75	6.75
Menhaden fish oil[8]	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.42
Soy oil	1.82	1.89	1.95	1.89	1.95	1.61	1.40	1.01	0.19
Lecithin (soy)[9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Corn Starch	0.13	4.05	7.98	6.60	13.13	4.92	9.89	2.23	2.37
Whole wheat	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Mineral premix[10]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[11]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[12]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Rovimix Stay-C 35%[13]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[14]	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
DL-Methionine[15]	0.11	0.10	0.10	0.01	0.00	0.00	0.00	0.12	0.13
Taurine [15]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Proximate Analyses (as is)									
Crude protein	42.67	42.02	41.76	36.88	41.72	40.59	40.37	39.42	39.86
Moisture	5.65	5.95	7.03	7.2	6.07	5.99	6.56	6.2	8.42
Crude Fat	7.22	7.19	6.65	6.26	5.95	6.38	6.25	6.8	6.34
Crude Fiber	3.12	3.04	2.45	3.45	3.66	3.13	2.85	3.5	3.53
Ash	7.1	6.77	6.35	6.22	5.61	6.52	6.38	6.72	6.77

[1]Darling Ingredients, Irving, TX, USA

[2]De-hulled solvent-extracted soybean meal, Bunge Limited, Decatur, AL, USA
[3]Benson Hill, St. Louis, MO, USA
[4]ADM Animal Nutrition, Quincy, IL, USA
[5]Hamlet Protein, Findlay, OH, USA
[6]All Sustained LLC, Boston, MA, USA
[7]Empyreal 75 TM, Cargill Corn Milling, Cargill, Inc, Blair, NE, USA
[8]Omega Protein Inc., Houston, TX, USA
[9]The Solae Company, St. Louis, USA
[10]Mineral Premix (g 100g-1 premix): cobalt chloride, 0.008; cupric sulphate pentahydrate, 0.010, ferrous sulfate heptahydrate, 20.0, manganous sulfate anhydrous, 2.90; potassium iodide, 0.024; sodium selenite, 0.048; zinc sulfate heptahydrate, 17.60, and α cellulose 59.194.
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[14]Acros Organics B.V.B.A. Thermo Fisher Scientific, Waltham, MA, USA
[15]MP Biomedicals Inc., Santa Ana, CA, USA
*Analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA)

Table 3. Amino acid analysis of Florida pompano diets including various soybean meal sources analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Composition	SBM	LO-SBM 50%	LO-SBM 100%	SPC 50%	SPC 100%	Fer SBM 50%	Fer SBM 100%	EE SBM 25%	EE SBM 50%
Alanine	2.19	2.14	2.15	2.12	2.16	2.05	2.08	2.12	2.12
Arginine	2.57	2.61	2.56	2.38	2.51	2.42	2.48	2.36	2.35
Aspartic Acid	3.79	3.86	3.84	3.62	3.80	3.59	3.60	3.57	3.52
Cysteine	0.60	0.60	0.60	0.57	0.59	0.59	0.58	0.58	0.57
Glutamic Acid	7.62	7.63	7.69	7.28	7.59	7.20	7.27	7.22	7.17
Glycine	2.08	2.01	2.07	2.02	2.08	1.95	2.03	2.00	2.02
Histidine	0.99	0.99	0.98	0.97	0.97	0.93	0.95	0.98	0.96
Hydroxylysine	0.03	0.03	0.03	0.02	0.03	0.01	0.02	0.02	0.03
Hydroxyproline	0.28	0.24	0.26	0.34	0.30	0.25	0.31	0.29	0.39
Isoleucine	1.93	1.87	1.89	1.74	1.90	1.81	1.82	1.73	1.69
Lanthionine §	0.06	0.06	0.06	0.04	0.05	0.06	0.06	0.02	0.01
Leucine	3.63	3.59	3.58	3.40	3.56	3.43	3.45	3.37	3.33
Lysine	2.28	2.24	2.22	2.12	2.17	2.00	2.17	2.12	2.04
Methionine	0.80	0.81	0.80	0.69	0.80	0.74	0.79	0.81	0.79
Ornithine §	0.02	0.02	0.02	0.03	0.04	0.04	0.02	0.04	0.04
Phenylalanine	2.09	2.08	2.07	1.92	2.06	1.97	1.97	1.91	1.88
Proline	2.66	2.69	2.69	2.35	2.67	2.55	2.58	2.31	2.33
Serine	1.65	1.72	1.69	1.77	1.67	1.56	1.55	1.75	1.75
Taurine §	0.71	0.76	0.73	0.62	0.71	1.18	0.76	0.66	0.67
Threonine	1.47	1.50	1.46	1.46	1.47	1.39	1.39	1.45	1.43
Tryptophan	0.44	0.46	0.45	0.41	0.45	0.44	0.43	0.41	0.41
Tyrosine	1.44	1.59	1.47	1.32	1.41	1.43	1.42	1.32	1.35
Valine	2.04	1.97	2.00	1.90	2.00	1.92	1.94	1.87	1.85

*Expressed as an as-is basis

Both systems were comprised of a series of culture tanks, a reservoir, a biological filter, and water pumps. Supplemental aeration was provided using a central line, a regenerative blower, and air diffusers.

Water quality parameters including temperature, dissolved oxygen, and salinity were monitored twice daily using a multi-probe YSI ProPlus meter (YSI ProPlus, Yellow Spring Instruments Co., Yellow Springs, OH, USA). At the same time, pH was measured twice weekly using a handheld pH meter (EcoSense pH 10A, YSI, Yellow Springs, OH) and a WaterLink SpinTouchFF meter (LaMotte Company, Chestertown, MD, USA). Total ammonia nitrogen (TAN) and nitrite were measured twice per week using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA) as well as a WaterLink SpinTouchFF meter (LaMotte Company, Chestertown, MD, USA). System A was terminated after 42 days and system B was terminated after 56 days. Survival, final weight, weight gain, thermal growth coefficient (TGC) and feed conversion ratio (FCR) were calculated upon experimental termination. The equation used for the calculation of TGC is as follows:

$$(\text{final weight}^{1/3} - \text{initial weight}^{1/3}) / (\text{temperature} \times \text{day}) \times 100$$

2.2.2 Trial 3: Evaluation of soy sources

Florida pompano juveniles were obtained from Proaquatix LLC, Vero Beach, FL and were transported to EW Shell Fisheries Center, Auburn, AL. Fish were then acclimated and maintained in a RAS system consisting of a series of culture tanks, reservoir tanks, a bead filter, mechanical and biological filtration and circulation pumps Fish were fed a commercial diet consisting of 50% protein and 15% lipid levels (FF Starter, Ziegler Bros., Inc., Gardners, PA, USA) until experimental stocking.

At the beginning of the trial, 360 fish with a mean initial weight of 4.82 g (± 0.08) were moved to a separate system comprised of 36 identical glass aquaria (80 L), with ten fish being stocked per tank. The system was comprised of culture tanks, reservoir tank, a bead filter (Aquadyne, Crane Enterprise, Hartwell, Georgia, USA), fluidized bed biological filter and circulation pumps. Each tank was randomly assigned a dietary treatment (n=4). Fish were weighed bi-weekly, during which the fish were treated with chloroquine diphosphate and given a freshwater dip for disease prevention. Feed rations were adjusted after each weighing to determine the appropriate amount of feed to offer fish.

Water quality parameters including temperature, salinity, and dissolved oxygen (DO) were measured twice per day with a water quality multi-parameter meter (YSI ProPlus, Yellow Spring Instruments Co., Yellow Springs, OH, USA), while pH was measured twice a week using a Waterproof Pocket pH Tester with 0.1 Resolution (pHep, Hanna Instruments, Smithfield, RI, USA). TAN and nitrite were measured twice per week using a YSI 9500 economical photometer (YSI Inc., YellowSprings, OH, USA). Additives such as salt and calcium bicarbonate were added to the system routinely to maintain water quality parameters at appropriate levels. The system was routinely treated for parasites using copper sulphate pentahydrate at 0.15-0.2 mg/L Cu^2 weekly (Reed & Francis-Floyd, 1994; Yanong, 2010). Copper levels present in the water were tested the same day using a Chemetric copper testing kit (CHEMetrics, AquaPhoenix Scientific, Midland VA, USA).

The experiment was terminated after 76 days. Upon trial termination, fish were group-weighed and counted, with data being collected for final biomass, final mean weight, weight gain, FCR, and survival. Four fish from each tank were euthanized and stored at -20°C for later proximate analysis. Additionally, four more fish were euthanized and distal gut samples were removed and placed into Bouins solution (picric acid-formalin-acetic acid mixture, Ricca Chemical, Arlington, TX, USA) for histological evaluation. An additional separate section of the distal gut sample was preserved in RNA Shield (Zymo Research, Irvine, CA, USA) for inflammatory gene expression response.

2.3 Histological Analysis

Histological samples of the distal intestines were standardized and collected upon each trial's termination for discerning intestinal inflammation due to soy diets. Collected samples were first stored in Bouins Solution for 20 hours before being transferred to a 70% ethanol solution. Samples were processed by Auburn University's SSRC Histopathological Laboratory (College of Veterinary Medicine, Auburn University, Auburn, AL). Sectioned and stained samples distal intestine samples were then observed under a Nikon TS2R-FL microscope (Nikon Instruments Inc., Melville, NY) equipped with a Nikon DSRi2 at 100× and 400× magnification. Grading of gut samples was performed in accordance with methods used in previous Florida pompano studies (Novriadi et al., 2018), but an additional parameter, connective tissue beneath the folds (Barnes et al., 2014) was implemented. Pictures of distal intestine samples at 100× and 400× were taken and then evaluated by three double-blind individuals on a 1 (normal) to 5 (severe) grading scale. Grading was based on lamina propria thickness (LPT), lamina propria cellularity (LPC), widening of connective tissue beneath the folds (CT), and prevalence of goblet cells within the folds (GC).

2.4 Proximate Analysis

Whole body proximate and mineral analysis were performed at Mid-West Labs (Omaha, NE, USA) on initial samples and samples collected post-trial for the third trial. The samples taken were stored in -20 °C and were ground down using a food processor before being sent to the laboratory. Mineral analysis and proximate analysis of crude protein, ash determination, crude fat, crude fiber, and moisture were performed in accordance with the Association of Official Analytical Chemists, International (AOAC) Official Methods. Moisture follows methods MWL FD 016 based on AOAC 930.15. Crude Protein follows MWL FD 070 based on AOAC 990.03. Crude Fat follows MWL FD 026 based on AOAC 2003.05. Ash follows MWL FD 019 based on AOAC 942.05.

2.5 Statistical Analysis

Data was analyzed statistically using Statistical Analysis Software for Windows (V9.4 SAS Institute, Cary, NC) and reported as mean values of the replicates. Data regarding survival, final biomass, final mean weight, weight gain, FCR, and TGC were analyzed using one-way analysis of variance (ANOVA). Differences were considered significant at $p<0.05$. Student-Neuman Keuls' multiple range test was run to determine significant differences among treatments. Histological data for Trial Two was analyzed using the Mann-Whitney Nonparametric Test, while Trial Three was analyzed using a Kruskal-Wallis Test, followed by Tukey's HSD tests to determine significant treatment differences.

3. Results

All water quality parameters measured (Mean ± Standard Deviation) for the duration of all 3 trials were sustained within the desirable range for culture of Florida pompano (Table 4).

3.1 Trials 1 and 2: Comparison of soy-based diet to commercial fishmeal-based diet for Florida pompano

For both trials, final biomass, final individual weight, overall weight gain, PWG, TGC, and survival were all higher for tanks offered the commercial diet than the soy-based experimental diet (Table 5). FCR was also lower in the commercial diet for both trials, implying either a preference for an animal-based protein source over a soy-based one, or an easier uptake and utilization of nutrients within the diet. In trial 1, significant differences were observed in terms of final biomass, PWG, TGC, and FCR. However, for trial two, there were no significant differences between fish offered the commercial diet than those offered the experimental diet.

Histological samples from Trial 2 showed no morphological differences in exposure to soybean-induced enteritis, between individuals offered a practical, soy-based diet as opposed to the commercial fishmeal-based diet. Both treatment samples showed mild inflammatory symptoms, but not to a serious or noteworthy degree that it would impair fish growth or compromise survival.

3.2 Trial 3: Evaluation of soy sources

Growth results showed mean final biomass ranging from 306.93g to 413.65g and mean final individual weight ranging from 32.39g to 45.97g among all treatments (Table 6). Overall, fish fed every treatment saw an average growth of over 500% for the duration of the growth trial. Statistical analysis of final biomass, final individual weight, weight gain, PWG, FCR, TGC and survival showed no significant difference ($P>0.05$) among treatments (Table 6). However, it is important to note that of all dietary treatments, fish offered the basal diet (composed of solvent-extracted soy) had the lowest final biomass, the lowest final individual weight, weight gain, and PWG. Fish offered EE-SBM 25% had the highest final biomass, highest final individual weight, weight gain, and PWG. Fish offered EE-SBM 25% also had the lowest FCR among all nine experimental diets.

Analysis of whole-body proximate fish sampled upon trial termination showed no statistical differences among the nine experimental diets for crude protein, moisture, fat, ash, crude and protein retention (Table 7). The crude protein of sampled fish ranged from 57.00 to

64.73 %, with protein retention ranging from 18.30 to 25.10 %. Moisture of sampled fish ranged from 69.83 to 73.35 %. Fat of samples was between 23.10 to 27.48 %. Ash ranged from 9.52 to 13.10 %.

Histological gut samples from trial three showed no significant differences in lamina propria thickness, lamina propria cellularity, or increased vacuolization between the experimental diets. However, the thickness of the connective tissue was shown to be significantly different (p=0.003) specifically between SPC at 100% replacement (1.92) and Fer-SBM at 50% replacement (1.27) (Table 8). All diets displayed varying degrees of intestinal inflammation similar to the samples collected in the second growth trial, with none being severe enough to impair growth or survival.

4. Discussion

4.1.1 Soy growout

Soy-based proteins have successfully replaced fishmeal as the primary protein source in Florida pompano diets (Lech & Reigh, 2012; Rhodes et al., 2013; Rossi & Davis, 2012)). This experiment sought to compare the growth of Florida pompano utilizing a commercially produced, fishmeal-based diet alongside a practical, soy-based diet. Results from trials one and two demonstrate that fish offered the commercial diet performed better overall than fish offered the soy-based diet. However, fish offered the commercial diet only showed marginally better growth results. Therefore, while the practical soy diet needs further improvement, growth results are similar to that of a commercial fishmeal diet.

Table 4. Results (mean ± standard deviation) of water quality parameters observed across trials.

Parameter	Trial 1	Trial 2	Trial 3
Temperature (°C)	29.51 ± 2.06	29.22 ± 2.43	27.76 ± 0.45
Salinity (g/L)	22.41 ± 3.31	20.98 ± 3.18	11.23 ± 0.76
DO (mg/L)	6.38 ± 0.56	6.46 ± 0.64	6.58 ± 0.49
pH	7.77 ± 0.40	7.41 ± 0.45	7.14 ± 0.27
TAN (mg/L)	0.09 ± 0.17	0.12 ± 0.16	0.27 ± 0.20
Nitrite (mg/L)	0.21 ± 0.16	0.47 ± 0.42	0.22 ± 0.15

Table 5. Growth performance of juvenile Florida pompano (Trial 1 23.2 g and Trial 1 38.1 g mean initial weight) reared over a 42-day or 56-day grow-out period while being offered either a soy-based practical diet or a commercial fishmeal diet (n=6, n=3).

Trial 1 (n=6)	Biomass (g)	Mean weight (g)	Weight Gain (%)	TGC[1]	FCR[2]	Survival (%)
Comm. Diet	886.92[a]	60.45	160.02[a]	0.087[a]	1.86[a]	97.78
Exp. Diet	780.92[b]	55.17	138.83[b]	0.078[b]	2.05[b]	94.44
P-value	***0.03***	0.08	***0.01***	***0.002***	***0.02***	0.09
PSE	28.71	1.89	4.28	0.0017	0.05	1.27
Trial 2 (n=3)						
Comm. Diet	3357.3	137.95	269.20	0.11	1.89	97.3
Exp. Diet	2852.7	125.56	226.10	0.10	2.05	90.7
P-value	0.19	0.33	0.28	0.44	0.58	0.25
PSE	227.55	7.94	0.18	0.01	24.63	3.53

[1]Thermal Growth Coefficient
[2]Feed Conversion Ratio

Table 6. Growth response of juvenile (4.82 g mean initial weight) of Florida pompano offered diets containing various soybean meal sources over a 76-day growth trial (n=4, Trial 3).

Diets	Biomass (g)	Mean weight (g)	Weight gain (g)	Weight gain (%)	TGC	FCR	Survival (%)
SBM	306.93	32.39	27.67	587.67	0.071	2.12	95.00
LO-SBM 50%	315.40	34.58	29.79	631.35	0.074	2.19	92.50
LO-SBM 100%	367.95	39.87	34.93	713.68	0.080	2.12	92.50
SPC 50%	329.83	37.68	33.22	743.46	0.069	2.33	86.67
SPC 100%	357.33	38.60	33.67	686.43	0.079	2.12	92.50
Fer-SBM 50%	389.78	39.98	35.19	742.80	0.081	2.09	97.50
Fer-SBM 100%	351.35	36.89	32.07	672.67	0.077	2.08	95.00
EE SBM 25%	413.65	45.97	41.06	840.07	0.089	1.88	90.00
EE SBM 50%	383.95	39.52	34.73	727.41	0.081	2.12	97.50
P-value	0.46	0.49	0.52	0.72	0.46	0.87	0.92
PSE	36.31	3.47	4.50	84.26	3.68	0.13	6.04

Table 7. Proximate composition of juvenile (4.82 g mean initial weight) Florida pompano offered diets containing various soybean meal sources over a 76-day growth trial (n=4, Trial 3). Proximate composition was performed at Mid-West Labs (Omaha, NE, USA).

	Moisture*	Protein	Lipid	Ash	Apparent net protein retention
Basal	72.77	63.87	25.82	10.97	19.01
LO-SBM 50%	71.77	62.05	27.05	9.55	21.59
LO-SBM 100%	71.77	60.25	27.47	12.75	21.63
SPC 50%	70.92	63.17	24.92	9.52	21.79
SPC 100%	72.35	62.77	27.42	12.24	22.36
Fer-SBM 50%	71.90	64.72	24.70	11.81	24.93
Fer-SBM 100%	69.82	57.00	27.32	9.82	23.90
EE SBM 25%	73.35	62.95	23.10	13.10	25.54
EE SBM 50%	71.82	64.17	23.92	10.55	25.06
P-value	0.47	0.71	0.77	0.17	0.33
PSE	1.01	2.92	2.14	1.10	1.95

*Expressed as an as-is basis

Table 8. Histological gradings of distal intestine samples based on the thickness of the lamina propria (LPT), lamina propria cellularity (LPC), widening of the connective tissue beneath the folds (CT), and abundance of goblet cells (GC). Symptoms were graded on a 1-5 scale with one showing no symptoms and five showing severe symptoms.

	Treatment	LPT	LPC	CT	GC
Trial 2	Exp. Diet	1.93	2.00	1.55	2.56
	Comm. Diet	2.30	2.48	1.92	2.59
	P-value	0.365	0.093	0.343	0.416
	PSE	0.404	0.373	0.279	0.452
Trial 3	SBM	1.89	1.98	1.80[a]	1.65
	LO-SBM 50%	1.69	1.93	1.42[ab]	1.76
	LO-SBM 100%	1.84	2.07	1.69[ab]	1.58
	SPC 50%	1.60	2.20	1.83[ab]	1.74
	SPC 100%	1.88	2.14	1.92[a]	1.71
	Fer-SBM 50%	1.78	2.07	1.27[b]	1.60
	Fer-SBM 100%	1.83	2.05	1.57[ab]	1.71
	EESBM 25%	1.89	1.98	1.67[ab]	1.87
	EESBM 50%	2.00	2.13	1.80[ab]	1.77
	P-value	0.783	0.927	***0.003***	0.494
	PSE	0.302	0.310	0.206	0.562

Statistical differences were observed in biomass, weight gain, TGC, and FCR in trial one while not being observed in trial two. This is likely due to the increased number of replicates in trial one as compared to trial two. Florida pompano have a tendency to grow nonuniformly among cohorts (Ma et al., 2017; Weirich et al., 2021). Thus, it is important to have a high number of replicates to increase the accuracy and precision of the data. This can also be further corroborated by comparison pooled standard error (PSE) across the experiments which were in general much lower for trial one which had more replicates. Despite the differences in statistical significance, both trials showed similar results in terms of trends in growth and feed conversion. The results of these trials are in partial agreement with Rhodes et al., (2013) where a series of Florida pompano growout trials compared Florida pompano growth performances against a fishmeal-centered diet and a fishmeal-free diet utilizing solvent-extracted SBM as the primary protein source. Their study showed no difference in survival, final individual weights, or percent weight gain of Florida pompano (Rhodes et al., 2013).

4.1.2 Soy Comparison

To further improve upon a soy-based diet formulated for Florida pompano, nine experimental diets containing differently sourced, soy-based products at different levels of inclusion. Solvent-extracted SBM was chosen for the basal diet due to it being the most widely used SBM in animal feed and the most common SBM in terms of market supply (Powell et al., 2011; Stein et al., 2008). The remaining soy sources were selected based on nutritional benefits associated with growth while potentially reducing the risk of symptoms conducive to soybean-induced enteritis. For example, a soy product with reduced amounts of oligosaccharides in could positively impact the growth and development of Florida pompano. Oligosaccharides bind to bile salts and inhibit digestive enzymes, which reduces the bioavailability of key nutrients found within soy (Tibaldi et al., 2006). Likewise, soy protein concentrate has a very high protein level when compared to solvent-extracted SBM, at 65% or more, as opposed to 44-49% (Banaszkiewicz, 2011; USSEC, 2023b). SPC is also highly digestible and low in antinutritional factors (Li et al., 2015). Fermented soy has also been shown to offer several benefits when offered in diets, such as reduced amounts of ANF as well as improved digestion (USSEC, 2023a). Lastly, expeller-extruded soybean meal usually contains less crude protein than solvent-extracted SBM, at around 42%. However, levels of residual oil are typically higher in extruded soybean meals as opposed to ones using a solvent, such as hexane, for oil extraction (Blomme et al., 2022; Stein et al., 2012). The increased levels of residual oils found in the remaining grain after extraction, as well as the changing of protein and starch structures through the extrusion process, allow for easier digestibility and energy utilization (Cao et al., 2023; Grieshop et al., 2003; Vidal et al., 2017).

Among the nine experimental diets containing varying soy sources, there were no significant differences ($P>0.05$) in any growth parameters among treatments. Fish offered the experimental diets (apart from LO-SBM 50% and EE-SBM 25%) grew between 10% and 20% more than individuals offered the basal diet, demonstrating that individuals offered solvent-extracted soy proteins as the only SBM in their respective experimental diets grew the least amount across the duration of the trial. It is also of note that fish offered EE-SBM 25% grew almost 30% larger than fish offered the basal diet composed of just solvent-extracted SBM (45.92g and 32.39g, respectively). One benefit to culturing Florida pompano is a short rearing time, with the duration of commercial growout typically lasting until individuals reach between 450 to 680 grams (Weirich et al., 2021). This culture period has the potential to be further reduced by adding different soy sources to commercially produced diets.

Our results corroborate a study performed by Refstie, Storebakken, and Roem (1998) that compared the growth of Atlantic salmon (*Salmo salar*) offered diets comprised of fishmeal, soybean meal, and a soybean meal with significantly reduced levels of oligosaccharides and other antinutrients (Refstie et al., 1998). Salmon offered either the fishmeal diet or the low oligosaccharide soybean meal diet exhibited similar growth, feed conversion, and digestibility of lipids and proteins. In contrast, fish offered a traditional solvent-extracted soy fared far worse. The primary reason for the reduced growth with conventional soybean meal was attributed to heat-resistant antinutritional factors still present in the solvent-extracted soy, but not in low oligosaccharide soy.

Conversely, both Colburn et al. (2012) as well as Salze et al. (2010) discuss that lower inclusions of soy protein concentrate are shown to be more advantageous than higher inclusions, showing no significant differences in feed intake, survival, or overall growth (Colburn et al., 2012; Salze et al., 2010). With respect to Colburn et al. (2012), fish offered diets with 100% replacement of solvent extracted SBM with soy protein concentrate showed the lowest growth of all treatments. Within this study, results showed that Florida pompano offered 100% replacement of solvent-extracted SBM with SPC grew larger than those offered only a 50% replacement. Additionally, fish offered 100% SPC also had a lower FCR and higher survival overall than fish offered 50% SPC. This is also in disagreement with previous, unpublished data from our laboratory. Nonuniform growth among cohorts could be the reason for these confounding results, but further investigation on the subject matter is required.

Regarding fermented soy, Novriadi (2017) investigated the use of fermented soybean meal as an effective protein substitute in diets for Florida pompano and concluded no statistical differences in fish growth. However, fish offered diets composed of fermented soybean meal had improved FCR and showed reduced inflammation in the liver and gut. Our results corroborate the results obtained by Novriadi (2017), as fish offered FerSBM 50% grew to the second highest overall biomass as well as one of the highest individual weights as well, while fish offered FerSBM 100%, however, did not grow as well (Novriadi, 2017). Within this study, the fermented soy product was also supplemented with phytase. Soy enzymatically treated with phytase has been shown to have many positive benefits in the use of diets, specifically to improve the digestibility of minerals, specifically phosphorus (Biswas et al., 2007).

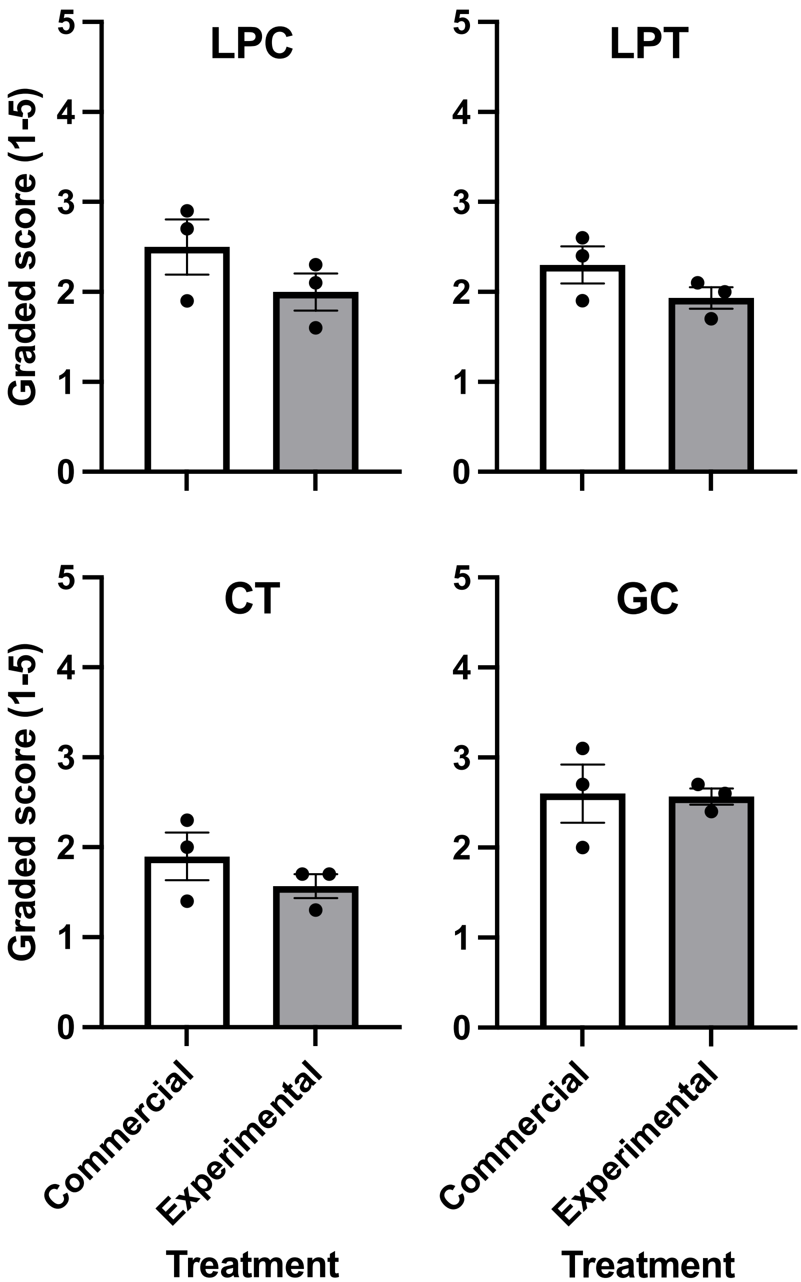
While there has been considerable work regarding SPC and fermented soy, there has been noticeably less research performed on the effects of expeller-extruded soy in aquaculture diets. Previous growth and digestibility trials consisting of diets composed of expeller extruded soy for Turbot, Japanese Flounder, Yellow Perch, and Nile Tilapia demonstrate that lower inclusion levels of EE-SBM can be utilized with no ill effects ((Kasper et al., 2007; Saitoh et al., 2003; Vidal et al., 2017, p. 20; Y. Zhang et al., 2023)). However, growth studies involving EE-SBM have been studied more closely within the sectors of swine, broiler and dairy cattle production (Crowe et al., 2001; Mohammad et al., 2023; Reese & Bitney, 2000). A study performed by Mohammad et al. (2023) concluded that supplementing broiler diets with 10%, 15%, and 20% EE-SBM resulted in positive growth results with no ill effects. They also reported that diets supplemented with these levels of EE-SBM resulted in greater economic efficiency (Mohammad et al., 2023). If this were to be utilized in the diets of fish, this may be seen as both advantageous economically and for the well-being of the fish. Commercial feed is usually seen as one of the costliest expenditures on farms (O'Donncha & Grant, 2019). Expeller-extruded soy has the potential to be more cost-effective than traditional solvent-extracted soy in terms of production

(Crowe et al., 2001). This could in turn reduce production costs in feed manufacturing and result in a more affordable product.

4.2 Histology

Histological analyses of the distal intestine have been used in several studies to assess gut degradation from soybean-induced enteritis in several key species (Barnes et al., 2014, 2015; Bonaldo et al., 2006; Horn et al., 2023; Seibel et al., 2022; C. Zhang et al., 2018; W. Zhang et al., 2019), including the Florida pompano (Novriadi et al., 2019). Histological samples for trial two showed no statistical difference between treatments offered the commercial diet as opposed to treatments offered a practical soy diet. Both diets only showed mild symptoms of inflammation (Figure 1). Because there is little difference in intestinal inflammation to individual Florida pompano offered a soybean meal-based diet as opposed to a fishmeal-based diet, it can be inferred that a commercial diet could safely take advantage of using more soybean meal and less fishmeal in a formulation without risking the fish developing enteritis. Because fishmeal is in such high demand for different commercial diets and is also expensive, this would lead to a commercially formulated Florida pompano that is much more cost-effective than other diets for commercially important species such as salmonids, which require a high amount of fishmeal and tolerate plant alternatives poorly (Booman et al., 2018; Kononova et al., 2019). A diet that is much higher in soybean meal than in fishmeal could potentially be manufactured more quickly and easier due to soy products being much more accessible than fishmeal products (Liu et al., 2020).

Figure 1: Comparison of histological gradings of Trial 2 between commercially produced fishmeal diet and experimental, soy-based diet. Bars represent the average score per diet, error bars represent the standard error of the mean. Symptoms were graded on a 1-5 scale based on Lamina Propria thickness (LPT), Lamina Propria cellularity (LPC), widening of the connective tissue beneath the base of the folds (CT), and abundance of goblet cells (GC). A score of one shows no symptoms of enteritis, while five shows severe symptoms.



Histological samples of Florida pompano from trial three showed no statistical differences between lamina propria thickness, increased lamina propria cellularity, or presence of goblet cells in the distal intestines among treatments. However, there was a significant difference ($p=0.002$) in the widening of central stroma/connective tissue among treatments. The treatment with the lowest amount of widening of the connective tissue was the fermented soybean meal at 50% replacement, whereas the treatment with the highest amount of widening was soy protein concentrate at 100% replacement. All nine diets displayed varying degrees of mild inflammation commonly associated with offering fish a diet comprised of soybean meal. Soy source did not seem to make a difference in the severity of the symptoms, except for the widening of the connective tissue.

Results are in line with previous research, as it is well known that Florida pompano is more accepting and tolerant to soy-based diets than other species of marine finfish (Novriadi & Davis, 2018; Quintero et al., 2012; Riche & Williams, 2011; Roe, 2016). However, some individuals sampled within replicate tanks showed moderate to severe inflammation in one of the four categories. In Figure 2, the individual offered the assigned experimental diet demonstrated mild symptoms of inflammation within the intestine. On average, most of the individual fish sampled showed similar levels of severity for enteritis-based symptoms, regardless of assigned dietary treatment. Figure 3 shows symptoms of increased severity, with the widening of the central stroma and increased number of goblet cells being symptoms of moderate to severe inflammation. These results could explain that varying levels of inflammation among cohorts could be based more on the genetics of individual fish as opposed to the entire species. Overall, the present experiment demonstrates that Florida pompano offered soy as the primary protein source in diets, regardless of treatment or processing, and experience less severe symptoms associated with soy-induced enteritis than other comparable aquaculture species.

5. Conclusions

The results of this study demonstrate Florida pompano offered an experimental diet composed of soybean meal as the primary protein source performed similarly well compared to individuals offered a commercially produced fishmeal-based diet. When offered diets composed of differently sourced soy at different inclusion levels, Florida pompano grew to a larger size when offered diets composed of solvent-extracted SBM supplemented with one of the other soy-based protein sources (LO-SBM, SPC, Fer-SBM, EE-SBM). Histological results revealed no increased risk of soybean-induced enteritis among individuals offered any soy-based product over fishmeal as the primary protein source in the diet. These results further show that Florida pompano may be provided a fishmeal-free diet with soy as the primary protein source.

Figure 2: Distal intestine sample showing long, finger-like villi. Noted also is a minimal number of goblet cells and thin layer of connective tissue beneath the base of the folds.

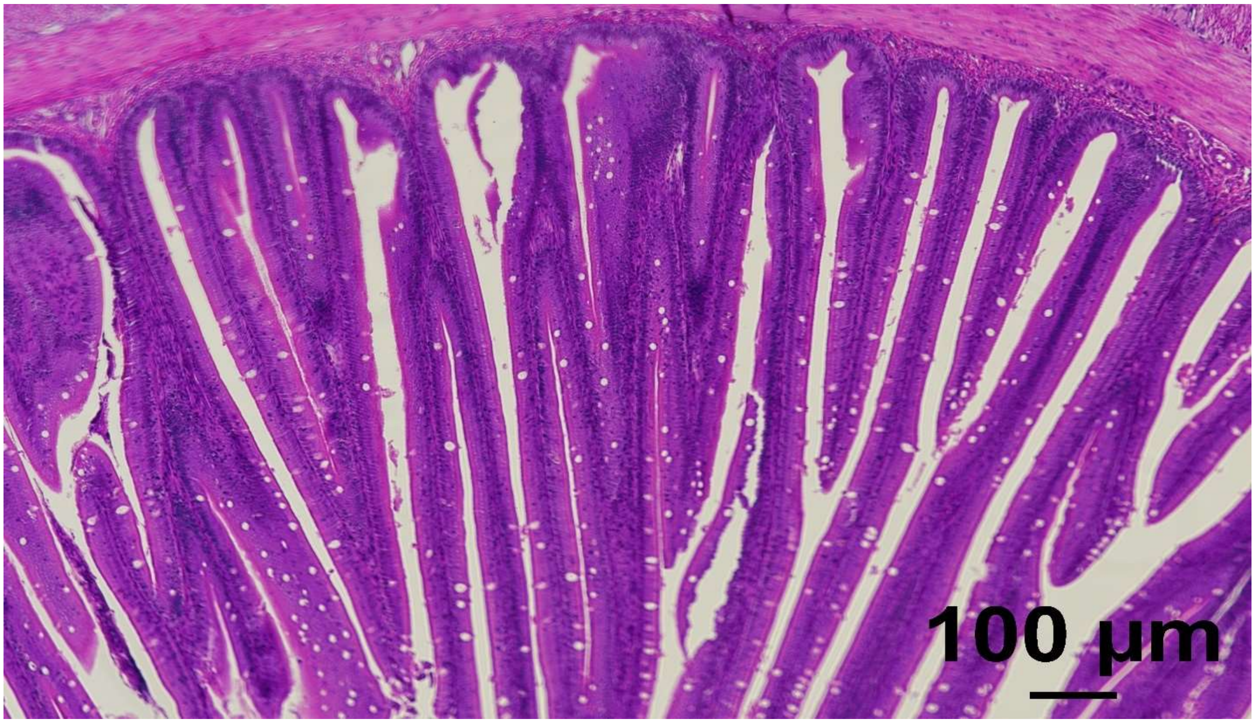
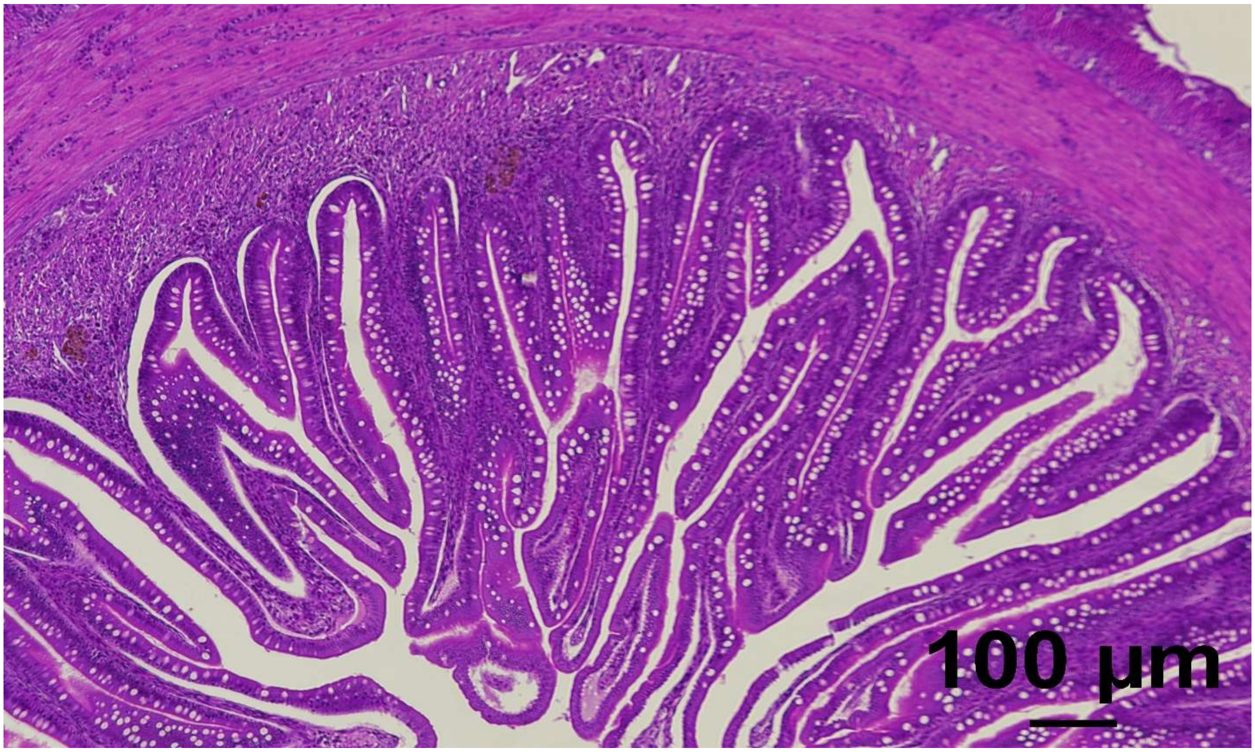


Figure 3: Distal intestine sample showing increased clubbing of the villi, a widening of the connective tissue beneath the folds, increased cellularity within lamina propria, and an abundance of goblet cells.



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