“Effect of varying dietary enzyme inclusion levels on the growth performance of Florida Pompano (Trachinotus Carolinus)”

by

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Florida pompano; enzyme supplementation; feed efficiency; nutrient digestibility; plant-based diets; aquaculture

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Abstract

Florida pompano (*Trachinotus carolinus*) is a marine fish that has shown a lot of promise for aquaculture because of its fast growth, high market value, and ability to grow in a wide range of salinities. With plant-based ingredients becoming more common in fish feeds, exogenous enzymes have been receiving more attention to help fish get more out of these diets. In this study, we tested two enzymes individually. Protease (AG175™) breaks down dietary proteins and helps deal with antinutritional factors, and xylanase (Econase® XT25) targets arabinoxylan, a fiber found in soybean meal and wheat that pompano cannot digest on their own. Each enzyme was added at three different levels to soybean meal-based diets, giving us seven diets in Trial 1 and five in Trial 2. We did not see any statistically significant differences in growth, FCR, or whole-body composition in either of the two trials. However, fish fed xylanase diets did numerically better in final weight and FCR in Trial 1, and in Trial 2, we started to see a trend toward better protein digestibility with xylanase. Even without statistical significance, fish on enzyme-supplemented diets generally did better than those on the basal diet. We think a big part of why we did not see stronger results has to do with how much Xylan substrate was available in the diets, and future studies should try higher inclusions of Xylan-rich ingredients to get a better sense of what these enzymes can really do for Florida pompano.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this dissertation, no Artificial Intelligence (AI) tools were used.

Digital Accessibility Use Disclosure Statement

In the preparation of this thesis, the following digital accessibility tools were used to ensure this document complies with federal requirements: Microsoft Word Tools. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

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Table of Content

List of Tables

1. Introduction

Florida pompano (Trachinotus carolinus) is a euryhaline marine fish native to the Atlantic coast of the Americas, extending from Massachusetts to Brazil (Watanabe et al., 1995; Weirich et al., 2021). Most of the wild-caught fishery commercial activity is concentrated along the Gulf of Mexico and the southeastern United States (Watanabe et al., 1995). Florida pompano commands one of the highest market prices among warm-water marine finfish in the United States, with demand consistently exceeding supply from wild commercial fisheries, making it a priority candidate for aquaculture expansion (Weirich et al., 2021). It has a high wholesale price due to its mild flavor, firm texture, and consistent consumer demand in both domestic and international markets (Weirich et al., 2021). Despite many research and development programs focusing on Florida pompano aquaculture remains in early commercial scale with less than 10 farms in the United States producing the species at a commercial scale. The most common culture systems are outdoor flow-through and indoor recirculating aquaculture systems (RAS). This species is highly suitable for intensive culture due to its rapid growth rate, tolerance of a wide range of salinities and culture conditions, acceptance of formulated diets, and notable resistance to stress. Pompano can reach market size (450–600 g) within the first 8–12 months under optimal feeding and rearing conditions (Watanabe et al., 1995). However, there are some challenges. Even though the pompano has these favorable production qualities, feed costs remain high, especially for diets containing protein-rich marine ingredients like fishmeal and fish oil, which continue to constrain the economic viability of pompano aquaculture (Weirich et al., 2021). This drives an interest in alternative feeds that include plant-derived protein sources (Novriadi & Davis, 2019).

Aquaculture has expanded globally, reaching a production volume of 223.2 million tons in 2022 with aquaculture surpassing capture fisheries as the main producer of aquatic species. Finfish

farming accounted for approximately 65% of total output. This makes for approximately 94.4 million tons of the 185.3 million tons of total aquatic animal production (FAO, 2024). To keep up with this growth rate, the industry has increasingly shifted toward plant-based feed ingredients to reduce reliance on high protein ingredients such as fish meal and fish oil derived from wild-caught marine resources. Ingredients like soybean meal, corn protein concentrate, and whole wheat are more common in commercial feeds due to their low cost, better efficiency in diets and easy access (Castillo & Gatlin, 2015; Novriadi & Davis, 2019). However, these plant-based ingredients present significant nutritional challenges for marine species like the Florida pompano, which have a limited capacity to digest non-starch polysaccharides (NSPs) including arabinoxylan, cellulose, pectin, and beta-glucans that are present in addition to starch in plant-based ingredients such as wheat and soybean meal.

Despite these limitations, carbohydrates are still practical in diets because they are naturally present in plant ingredients and contribute to feed processing, pellet stability and provide digestible energy from starch. NSP's found in soybean meal and wheat may reduce nutrient digestibility. This effect may be because NSP's can increase intestinal viscosity, interfering with digestive enzyme activity and promoting unfavorable gut microbiota (NRC, 2011). Also, plant proteins contain anti-nutritional factors (ANF's) like trypsin inhibitors and phytate that will impair protein and mineral absorption, highlighting the need for targeted feed additives to improve the digestibility of plant-based diets in pompano production (Castillo & Gatlin, 2015).

Dietary enzymes have become a promising supplement to improve the digestibility and feed efficiency in aquaculture. Enzymes like protease and xylanase act as a biological catalyst by breaking down the complex nutrients into more digestible parts. This makes it easier for nutrient absorption and reduces anti-nutritional factors in feed (NRC, 2011). The use of exogenous

enzymes is well established in non-ruminant animal production, particularly in poultry and swine diets where xylanase and protease supplementation have consistently improved nutrient digestibility and growth performance (Adeola & Cowieson, 2011). In aquaculture, similar benefits have been reported across multiple fish species, including improved growth performance and nutrient digestibility in species ranging from tilapia to salmonids (Liang et al, 2022). These enzymes oversee the hydrolysis of proteins and non- starch polysaccharides for better digestion than is typically achieved without supplements. This can lead to improved nutrient availability and reduced feed costs.

With all these potential benefits of enzyme supplementation it is important to determine optimal levels of inclusion and types of enzymes that may benefit the Florida pompano. While some studies have demonstrated positive effects of enzyme supplementation regarding growth and nutrient digestibility, others have reported no significant difference. This highlights the need for further research to understand the specific requirements and responses of different species to enzyme supplementation in diets. Magalhães et al. (2018) showed that enzyme supplementation improved protein digestibility in European sea bass (Dicentrarchus labrax) while research on Atlantic salmon (Salmo salar) indicated that enzyme effects may vary depending on diet composition (Krogdahl et al., 2010). This research suggests that the efficacy of dietary enzyme supplementation in aquatic species may be species-specific and influenced by factors such as diet composition, enzyme type, and inclusion level.

This study aimed to evaluate the effect of varying dietary inclusion levels of protease and xylanase on growth performance, feed efficiency, and nutrient digestibility of Florida pompano. This research seeks to contribute to the development of more efficient and sustainable feeding strategies on pompano. The findings will provide understanding and valuable insight into potential

benefits of enzyme supplementation in Florida Pompano diets and optimize feed formulations to enhance production efficiency.

2. Methods and Materials

Research was conducted at the Claude Peteet Mariculture Center in Gulf Shores, AL, USA (Trial 1) and the E.W. Shell Fisheries Center in Auburn, AL, USA (Trial 2). The Claude Peteet Mariculture Center is located 3 meters above sea level, with an annual average precipitation of about 67 inches (1700 mm), where an outdoor recirculating system was used to carry out Trial 1. The E.W. Shell Fisheries Center is approximately 216 meters above sea level, with an annual average precipitation of about 52.6 inches (1336 mm), where an indoor clear-water recirculating system was used to conduct Trial 2. Florida pompano for Trial 1 was transported from Florida Atlantic University at Harbor Branch Oceanographic Institute (Fort Pierce, FL, USA). Fish from Trial 2 were brought to the E.W. Shell Fisheries Center in Auburn from Claude Peteet Mariculture Center.

2.1 Diet Formulation

The diets in this experiment were formulated in the aquatic animal nutrition laboratory of the School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, and transported to the experimental locations. The basal diet was adapted from Novriadi & Davis (2019), a plant-based formulation previously shown to support growth performance in Florida pompano comparable to fish meal-based diets, making it a nutritionally suitable baseline rather than a deficient plant-based diet. In Trial 1, a total of seven experimental diets were evaluated, including the basal diet, three diets supplemented with AG175™ protease at 0.05, 0.1, and 0.2 g kg^{-1}, and three diets supplemented with Econase® XT25 xylanase at 0.05, 0.1, and 0.2 g kg^{-1}, for a total of 7 diets. Two growth trials were conducted to evaluate the effect of enzyme supplementation on

Florida pompano performance in different culture systems and diet formulations. Trial 1 was conducted in an outdoor green water recirculating system, and Trial 2 was conducted in an indoor clear-water recirculating system. Due to a numerical response that was observed in Trial 1 regarding the xylanase performance, a second trial was conducted to evaluate the enzyme effects under diets with higher dietary Xylan substrate availability.

For Trial 2, diets were reformulated to include a 6.5% rice bran, a fiber source that contains relatively high concentrations of arabinoxylan, to increase the availability of the substrate for the xylanase enzyme. A total of five experimental diets were evaluated in this trial. These diets included basal formulation, two diets supplemented with AG175™ protease at 0.1, and 0.2 g kg^{-1}, and two diets supplemented with Econase® XT25 xylanase at 0.1 and 0.2 g kg^{-1}, for a total of 5 diets.

Both diets pre-ground dry ingredients were mixed with oil in a food mixer (Hobart, Troy, OH, USA) for 15 minutes. Boiling water (~40% by weight) was then added to the blend to reach the correct pelleting consistency. This blend was then extruded through a 3mm die to get the correct pellet size. Diets were dried at low temperature (≤45 °C) with forced air to reduce moisture while limiting enzyme denaturation; pellets were spread in a thin layer, and drying time was minimized to reach the target moisture (<10%). They were later stored in zip bags by treatments and refrigerated until served to the daily feed rations. Samples of Diets and fish body composition were analyzed for proximate by Midwest Laboratories (Omaha, NE, USA). During the trials, fish were fed experimental diets formulated to contain 40% crude protein and the inclusion of two enzymes: AG175™ protease (Jefo, Saint-Hyacinthe, Québec, Canada) and Econase® XT25 xylanase (AB Enzymes GmbH, Darmstadt, Germany).

Table 1. Formulation and proximate composition (g 100g-1, as is) of experimental diet for outdoor system (Trial 1) and with 6.5% rice bran inclusion for clear-water indoor system (Trial 2).

Ingredient	Trail 1							Trial 2				
	Basal (%)	Jefo-0.5x (%)	Jefo-1x (%)	Jefo-2x (%)	Eco-0.5x (%)	Eco-1x (%)	Eco-2x (%)	Basal (%)	Jefo-1x (%)	Jefo-2x (%)	Eco-1x (%)	Eco-2x (%)
Poultry meal[1]	14	14	14	14	14	14	14	14	14	14	14	14
Soybean meal[2]	50	50	50	50	50	50	50	50	50	50	50	50
Corn Protein Concentrate[3]	6.77	6.77	6.77	6.77	6.77	6.77	6.77	6.8	6.8	6.8	6.8	6.8
Jefo AG175™ [4]	0	0.005	0.01	0.02	0	0	0	0	0.01	0.02	0	0
Econase® XT25[5]	0	0	0	0	0.005	0.01	0.02	0	0	0	0.01	0.02
Corn Starch[6]	0.09	0.09	0.08	0.07	0.09	0.08	0.07	0.05	0.04	0.03	0.04	0.03
Whole wheat[7]	20	20	20	20	20	20	20	13.9	13.9	13.9	13.9	13.9
Rice Bran	--	--	--	--	--	--	--	6.5	6.5	6.5	6.5	6.5
Menhaden fish oil[8]	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.03	3.03	3.03	3.03	3.03
Soy oil	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82	1.82
Lecithin (soy)[9]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral premix[10]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[11]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride[12]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Rovimix Stay-C 35%[13]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
CaP-dibasic[14]	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Methionine [15]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Taurine[15]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate Analysis* (g 100g-1 as is)												
Crude Protein	41.6	41.4	41.1	42	42.3	41.7	42.1	42.7	42.4	43.1	43.4	42.2
Moisture	9.22	9.22	10.43	8.76	8.04	9.2	8.7	8.15	7.43	7.49	7.56	7.49
Crude Fat	9.18	9.14	8.28	9.22	8.97	9.15	9.38	8.44	8.76	8.88	8.75	8.62
Crude Fiber	3.0	3.9	3.6	3.2	4.4	3.8	3.8	6.4	6.4	6.4	6.4	6.0
Ash	6.51	6.68	6.43	6.65	6.63	6.5	6.73	7.63	7.71	7.52	8.24	7.58

1 River Valley Ingredients, Hanceville, AL, 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 Jefo Nutrition Inc., 5020 Av. Jefo, Saint-Hyacinthe, QC J2R 2E7, Canada.

5 AB Vista, Woodstock Court, Blenheim Road, Business Park, Marlborough, Wiltshire, SN8 4AN.

6 Ingredi Company, 501 Chesapeake Park Plaza Baltimore, MD, USA

7ADM, 4666 E Faries Pkwy, Decatur, IL, USA

8 Omega Protein Inc., Reedville, VA, USA.

9 The Solae Company, St. Louis, USA

10 ASA Premix (g 100g-1 premix): cobalt chloride, 0.004; cupric sulphate 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

11 ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU g-1), 2.40; vitamin D3 (400,000 IU g-1), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258

12 MP Biomedicals Inc., Solon, OH, USA.

13 Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA

14 BeanTown Chemical, 9 Sagamore Park Road Hudson, NH, USA

15 Tokyo Chemical Industry, Porland, OR, USA

*Analyzed at Midwest Laboratories® (Omaha, NE, USA).

2.2 Experimental systems

Trial 1 was conducted in an outdoor recirculating system consisting of 36 circular culture tanks (0.8 m3 of culture water), a common drain to a sump tank, a circulation pump, aeration (utilizing a regenerative blower, central airline, and air diffusers), and mechanical and biological filtration placed under a greenhouse. Fish were size sorted, placed in each tank, with 5 replicates randomly assigned to each treatment (7 experimental diets). They were stocked at an initial weight of 8.30 ± 0.27 g (mean ± SD) at a density of 25 fish m^{-3}, for a total of 20 fish per tank. An initial sample of fish was euthanized, collected, and stored frozen (-40°C) at the beginning of the trial to analyze whole-body composition. Feeding for Trial 1 lasted 10 weeks. Fish were hand-fed four times daily according to body weight (%). Feeding rates averaged approximately 5.6% of body weight per day (range 4.5-7%). Feed rations were adjusted weekly based on biomass of tank weights, feed conversion ratio (FCR), and visual response to feed. The system was run with saltwater at a mean salinity of 28.9 ppt. Water temperature was not actively controlled as Trial 1 was conducted in an outdoor system subject to ambient conditions. Mean water temperature was 29.22 ± 1.86°C, with a range of 24-35°C throughout the trial period (Table 2). System maintenance included removal of solids and water by siphoning on an as-needed basis, bi-weekly water exchanges, and backwashing of bead filters.

Trial 2 was conducted in an indoor recirculating system with a total of 20 polypropylene tanks (0.156 m3 of culture water), a circulation pump, aeration, and mechanical and biological filtration. Ten fish were stocked in each tank, with 4 replicates randomly assigned to each treatment (5 experimental diets). Fish were stocked at an initial weight of 8.90 ±0.77 g (mean ± SD) with 10 fish per tank, for a total stocking density of 64.1 fish m^{-3}. Trial 2 had a similar husbandry, and the feeding protocol was followed in the same manner. However, this trial was conducted in an indoor 4,140 L RAS system. Trial 2 lasted 12 weeks. Feeding protocols consisted of four feedings a day,

hand-fed. Feed rations were adjusted weekly based on tank biomass and observed response to feed. Feeding rates averaged approximately 4.2% (ranged from 3.5-7%) of body weight per day. The system was run in clear water. Artificial seawater was prepared by dissolving commercial sea salt (Instant Ocean ®, Blacksburg, VA) into freshwater. Salinity was kept at 15.7 ppt at 28.1°C. The system was siphoned and backwashed once a week to maintain optimal water quality parameters.

2.3 Growth performance and sampling

For both trials, Fish in each tank were group-weighed every two weeks during the culture period to monitor growth, survival, and adjust feeding rates. Observable mortalities were recorded and removed from tanks and feed inputs adjusted.

At the conclusion of each trial, fish in each tank were counted, and group weighed to determine final body weight, biomass, feed conversion ratio (FCR), and survival. Trial 1, after the final weight day, 5 fish from each tank were euthanized, bagged, labeled, and stored at -40°C for subsequent analyze. Frozen fish were thawed, homogenized, and ground to a uniform consistency and then submitted to Midwest Laboratories (Omaha, NE, USA) for proximate composition and mineral analysis. These data were used to measure protein retention.

$$Protein\ Retention\ Efficiency\ (\%) = \frac{Protein\ gain\ per\ fish}{Protein\ Intake\ per\ fish} \times 100$$

$$Protein\ gain = (Final\ mean\ weight \times Final\ protein\ \%) - (Initial\ mean\ weight \times Initial\ protein\ \%)$$

$$Protein\ intake = Total\ feed\ intake \times Dietary\ protein\ \%$$

For Trial 2, after the final weight day, all remaining fish not euthanized for selected treatments were returned to their tanks to proceed with acclimation to digestibility diets.

2.4 Digestibility

Fish from the basal and the highest enzyme level treatments, which were not euthanized, were returned to the system and fed digestibility diets marked with yttrium oxide (Y_2O_3), an indigestible feed marker. By comparing the concentration of yttrium in the diet to its concentration in the feces, along with the concentration of the nutrient of interest, apparent digestibility coefficients can be calculated without requiring total fecal collection (Liang et al., 2022). Before fecal collection, fish were acclimated to the digestibility diets for 3 days to ensure that the marker was present during excretion for sample collection. On collection day, Fish were fed until apparent satiation 4 hours before fecal collection occurred.

For fecal collection, fish were individually netted and lightly anesthetized to minimize stress and better handling. Gentle pressure in the abdomen was applied toward the ventral surface to manually extract fecal material. To minimize contamination of intestinal tissue or urine, the first portion of feces was discarded, and the remaining material was collected into sterile tubes.

Fecal samples from fish within the same tank were pooled to obtain sufficient material for analysis. Before analysis, samples were dried to constant weight, ground into fine powder, and analyzed for nutrient composition and yttrium concentration. Apparent digestibility coefficients of dry matter (ADDM), energy (ADE), and protein (ADCP) were calculated based on the ratio of yttrium and nutrient concentrations in the dry diet and fecal samples.

$$\text{ADDM}(\%) = 100 - \left[100 \times \left(\frac{Yttrium\ in\ diet}{Yttrium\ in\ feces}\right)\right]$$

$$ADE = 100 - \left(100 \times \frac{Yttrium\ in\ diet}{Yttrium\ in\ feces} \times \frac{Gross\ Energy\ in\ feces}{Gross\ Energy\ in\ diet}\right)$$

$$ADC_{protein} = 100 - \left(100 \times \frac{Yttrium\ in\ diet}{Yttrium\ in\ feces} \times \frac{nutrient\ in\ feces}{nutrient\ in\ diet}\right)$$

2.5 Water Quality

Parameters of water quality such as dissolved oxygen (mg/L−1), oxygen saturation (%), pH, temperature (ºC), and salinity (mg/L−1) were monitored using YSI ProQuatro Multiparameter Meter (Yellow Springs Instrument Co., Yellow Spring, OH, USA) two times per day. Total ammonia nitrogen was monitored once a week using a Thermo Scientific™ Orion™ 4-Star Plus pH/ISE Benchtop Multiparameter Meter (Thermo Fisher Scientific., Waltham, MA, USA). While pH, alkalinity, ammonia, phosphate, nitrate, nitrite, hardness, magnesium, and calcium were analyzed using a Water Link Spin Touch photometer (LaMotte®., Chestertown, MD, USA) once per week.

2.6 Statistical Analysis

Data was analyzed using a two-way analysis of variance to determine whether significant variance existed between the treatment means. To determine the significant difference between treatment means we used the Tukey's Honest significant difference test. Statistical analysis was conducted using SAS (V9.4, SAS Institute, Cary, NC).

3. Results

3.1 Water quality

Water quality parameters in Trial 1 (summarized in Table 2) show suitable ranges for Florida pompano culture (Wills et al., 2023; Weirich et al., 2021). Dissolved oxygen, temperature, salinity, and pH ranged from 4.40–9.95 mg L^{-1}, 24–35 °C, 26.40–38.50 g L^{-1}, and 6.72–9.60, respectively. Total ammonia nitrogen (0.20 ± 0.14 mg L^{-1}) and nitrite nitrogen (0.28 ± 0.12 mg L^{-1}) remained low throughout the trial. In Trial 2 (Table 2), Dissolved oxygen, temperature, salinity, and pH ranged from 6.03–8.92 mg L^{-1}, 24.50–30.70 °C, 14.10–18.03 g L^{-1}, and 6.40–8.26, respectively. Total ammonia nitrogen (0.58 ± 0.60 mg L^{-1}) and nitrite nitrogen (0.04 ± 0.05

mg L^{-1}) were within suitable ranges for the culture of this species (Wills et al., 2023; Weirich et al., 2021). Recommended water quality thresholds for Florida pompano culture include dissolved oxygen above 5.0 mg L^{-1}, pH between 7.5–8.5, and temperature between 24–30°C, with salinity optimally maintained between 30–35 ppt for marine systems and as low as 12 g L^{-1} for low-salinity culture (Wills et al., 2023; Weirich et al., 2021).

3.2 Growth Performance

Following growth trials in which Florida pompano were offered diets with varying levels of protease and xylanase products, no significant differences were observed in final weight, weight gain, or feed conversion ratio (FCR) compared to the basal diet ($p > 0.05$) (Table 3). Fish in all treatments grew from 8.30 g initial average weight to final weights that ranged from 55.72 g (basal) to 62.95 g (Eco-2x) after 10 weeks of culture. Two-way ANOVA revealed no significant effects of enzyme level, enzyme type, or their interaction on any growth parameter ($p > 0.05$).

Even though endpoints were not statistically different among treatments, fish that were fed with Xylanase-supplemented diets had numerically higher final weights and percent weight gain in comparison to those fed basal and protease diets. This can be seen in Eco-2x, where fish reached 62.85 g with a 657% weight gain in comparison to the other experimental diets that showed numerically less performance. Mortality remained low through all treatments, showing a 90-95% survival rate with no specific treatment effect on survival. Feed efficiency ratio (FCR) from 1.68 to 1.80 across all treatments, with no significant differences detected ($p > 0.05$). It is worth noticing that the lowest values observed in FCR resulted by inclusion of xylanase-based groups (Eco-0.5x:1.68; Eco-2x: 1.69), suggesting there was a slight numerical improvement in feed efficiency with xylanase inclusion.

Table 2. Water quality summary for Florida Pompano (*Trachinotus carolinus*) in a saltwater recirculating system for ten weeks (Trial 1) and twelve weeks (Trial 2) fed with different inclusion levels of enzyme (Protease & Xylanase).

Parameters	**Values trial 1**	**Values trial 2**
Dissolved Oxygen (mg L-1)	6.08 ± 0.64	7.29 ± 0.57
Temperature (°C)	29.22 ± 1.86	28.16 ± 1.06
Salinity (g L-1)	32.99 ± 3.75	15.70 ± 0.81
pH	8.00 ± 0.55	7.65 ± 0.50
Total Ammonia nitrogen (mg L-1)	0.20 ± 0.14	0.58 ± 0.60
Nitrite nitrogen (mg L-1)	0.28 ± 0.12	0.04 ± 0.05

Apparent net protein retention efficiency was similar among treatments, which ranged from 24.2% (Jefo-2x) to 25.9% (Eco-0.5x) (Table 5). Although there is no statistical difference, the highest PRE values were also shown by xylanase-supplemented diets. This suggests a possible trend toward improved protein utilization with xylanase inclusion.

In Trial 2, no statistically significant differences in final weight, weight gain (g), or FCR were noticed throughout the trial when Florida pompano were offered experimental diets supplemented with xylanase or protease compared to a basal diet ($p > 0.05$; Table 4). Final weights ranged from 35.01 g (Jefo-2x) to 39.13 g (Basal). Percent weight gain for the xylanase-2x group (299.27 ± 2.01%) was significantly lower than both the basal (339.13 ± 2.01%) and xylanase-1x (336.91 ± 2.01%) groups ($p = 0.0118$), indicating a significant negative effect of xylanase supplementation at the highest inclusion level on growth performance. This result suggests that excessive xylanase activity at the 2x dose may have had a detrimental effect on nutrient absorption, possibly through over-hydrolysis of arabinoxylan producing fermentable oligosaccharides that disrupted intestinal function in a species with a short gastrointestinal tract and fast gut transit time. The means were highlighted using letter superscript to show the differences. Survival rate was high across all treatments, ranging from 92.50% to 100.00%, with no significant differences among diets. FCR values ranged from 1.86 to 2.06, with no significant difference detected ($p > 0.05$). Two-way ANOVA reported no significant effects of enzyme level, type, or interaction on any growth performance parameter.

Table 3. Response of Florida Pompano (*Trachinotus carolinus*) (mean initial weight 8.30 ±0.27 g) to the different levels of inclusion of different enzymes (Protease & Xylanase) through ten weeks in a green water outdoor system (Trial 1).

Diet	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Survival rate (%)	FCR	ANPR
Basal	55.72	47.50	577	90.83	1.74	24.96
Jefo-0.5x	59.28	50.89	608	92.00	1.75	24.98
Jefo-1x	59.81	51.57	626	94.00	1.71	25.41
Jefo-2x	56.27	47.91	573	91.25	1.80	24.15
PSE	2.034	2.0093	24.29	3.426	0.078	1.7760
p-value	0.389	0.387	0.399	0.913	0.887	0.5681
Basal	55.72	47.50	577	90.83	1.74	24.96
Eco- 0.5x	60.93	52.68	639	95.00	1.68	25.86
Eco-1x	59.61	51.33	621	95.00	1.72	25.27
Eco-2x	62.85	54.55	657	95.00	1.69	25.71
PSE	2.224	2.177	24.48	2.475	0.075	1.8559
p-value	0.145	0.138	0.128	0.506	0.933	0.8624
Two-way ANOVA						
Level	0.965	0.965	0.941	0.912	0.731	0.7260
Type	0.136	0.124	0.094	0.350	0.099	0.7191
Level × Type	0.287	0.272	0.247	0.912	0.345	0.5290

[1] Weight gain= (Final weight - Initial weight)/ Initial weight × 100%

[2] FCR = Feed conversion ratio = Feed offered/ (Final weight - Initial weight).

Table 4. Response of Florida Pompano (*Trachinotus carolinus*) (mean initial weight 8.90 ±0.77 g) to the different levels of inclusion on different enzymes (Protease & Xylanase) through twelve weeks in clear water indoor system (Trial 2).

Diet	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Survival rate (%)	FCR
Basal	39.13	30.23	339.13	92.50	1.87
Protease-1x	35.48	26.55	295.18	95.00	2.02
Protease-2x	35.01	26.14	297.94	97.50	2.06
PSE	1.1316	1.0451	3.0678	1.3647	0.2056
p-value	0.4558	0.3505	0.2063	0.6224	0.2839
Basal	39.13	30.23	339.13a	92.50	1.87
Xylanase-1x	38.97	30.04	336.91a	93.33	1.86
Xylanase-2x	35.49	26.61	299.27b	100.00	1.99
PSE	1.0577	0.9537	2.0141	1.3103	0.1590
p-value	0.4438	0.3146	0.0118	0.2729	0.1908
Two-way ANOVA					
Level	0.3798	0.3224	0.3387	0.0870	0.3269
Type	0.3792	0.3088	0.2426	0.8675	0.1804
Level × Type	0.5006	0.4329	0.2712	0.4113	0.5666

3.3 Whole-Body Proximate Composition

Whole body proximate composition (as-is basis) was only performed for fish in Trial 1; pompano was not significantly affected by enzyme supplementation (Table 5). Crude protein ranged from 17.33% (Jefo-2x) to 18.24% (Jefo-1x), and crude fat from 4.92% to 6.25%. Ash values averaged around 3% across all treatments. Iron (Fe) concentration showed a significant difference through protease-supplemented diets ($p = 0.001$) and a significant effect of enzyme level in the two-way ANOVA ($p = 0.045$). However, no consistent biological pattern was noticed for enzyme type or inclusion level. A significant interaction effect (Level x Type, $p = 0.036$) was observed in magnesium, and sodium showed a significant effect of enzyme level ($p = 0.0003$). Although this data could indicate shifts in mineral availability, these mineral differences were not considered biologically meaningful given the absence of consistent trends and overall similarity in proximate composition across treatments.

3.4 Digestibility

In Trial 1, apparent digestibility coefficients for dry matter (ADDM), energy (ADE), and protein (ADCP) were summarized for the basal, protease-2x and xylanase-2x treatment only (Table 6). ADDM ranged from 45.42%- 49.84%, ADE ranged from 64.19% -66.75%, and ADCP 74.44% -77.05%. Statistical analysis could not be conducted for Trial 1 digestibility data due to insufficient fecal sample collection from the replicates. Enzyme-supplemented diets showed numerically lower values across all three digestibility coefficients in comparison to the basal diet. The observation is reported only as descriptive data and should be interpreted with caution due to the absence of a statistical analysis.

In Trial 2, an adequate replicate sample of feces was obtained; however, apparent digestibility coefficients were not significantly different among treatments ($p > 0.05$; Table 6). ADDM ranged from 43.43%- 45.98%, ADE ranged from 60.72% -62.96%, and ADCP 72.54% -74.56%. All enzyme-supplemented diets showed numerically higher ADE values compared to the basal; ADDM was slightly lower. Treatment with xylanase-2x showed the highest ADCP among all Trial 2 diets, slightly surpassing even the basal diet. Despite the reformulation of diets to include 6.5% rice bran to increase Xylan substrate availability, there was no statistically significant difference in nutrient digestibility observed.

Table 5. Proximate analysis of whole-body composition of Florida pompano reared in an outdoor system (Trial 1).

Diet	Moisture(%)	Dry Matter (%)	Crude Protein (%)	Fat (%)	Ash (%)	Sulfur (%)	P (%)	K (%)	Mg (%)	Ca (%)	Na (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Basal	74.18	25.82	18.08	5.6	3.2	0.25	0.57	0.33	0.05	0.79	0.13	14.2	3.22	16.23
Jefo-0.5x	73	27	17.78	5.33	3.24	0.25	0.52	0.34	0.04	0.7	0.11	10.94	2.58	14.28
Jefo-1x	73.14	26.86	18.24	5.83	3.12	0.25	0.57	0.34	0.04	0.79	0.12	12.4	2.96	15.34
Jefo-2x	73.83	26.18	17.33	5.38	2.93	0.26	0.65	0.34	0.05	0.94	0.15	13.28	3.55	15.75
PSE	0.858	0.858	0.49	0.676	0.186	0.005	0.059	0.007	0.003	0.119	0.009	0.432	0.495	0.825
p-value	0.715	0.715	0.621	0.951	0.704	0.683	0.594	0.85	0.07	0.642	0.066	0.001	0.607	0.39
Eco- 0.5x	72.76	27.24	17.9	6.25	3.16	0.25	0.66	0.35	0.04	0.96	0.12	13.1	3.72	16.3
Eco-1x	73.2	26.8	17.96	4.92	2.62	0.25	0.48	0.35	0.03	0.61	0.11	12.56	2.52	14.42
Eco-2x	72.62	27.38	18.02	5.4	3.39	0.24	0.54	0.33	0.04	0.73	0.14	13.46	3.02	15.76
PSE	0.806	0.806	0.665	0.597	0.281	0.006	0.055	0.01	0.003	0.111	0.009	0.454	0.436	0.78
p-value	0.48	0.48	0.997	0.486	0.286	0.724	0.173	0.229	0.031	0.203	0.175	0.091	0.307	0.313
Two-way ANOVA														
Level	0.931	0.931	0.807	0.786	0.442	0.807	0.421	0.339	0.061	0.448	0.001	0.045	0.5	0.549
Type	0.569	0.569	0.741	0.983	0.862	0.539	0.65	0.649	0.266	0.655	0.723	0.055	0.885	0.568
Level × Type	0.804	0.804	0.762	0.404	0.263	0.385	0.08	0.412	0.036	0.108	0.176	0.093	0.16	0.169

Table 6. Apparent digestibility coefficients of dry matter (ADDM), energy (ADE), and protein (ADCP) of the various diets offered to Florida pompano (*Trachinotus carolinus*) fed experimental diets containing enzyme supplementation for 10 weeks (Trial1) and 12 weeks (Trial2).

Treatments1	ADDM (%) Trial 1	ADE (%) Trial 1	ADCP (%) Trial 1	ADDM (%) Trial 2	ADE (%) Trial 2	ADCP (%) Trial 2
Basal	49.84	66.75	77.05	45.98	60.72	73.90
Protease 2x	45.42	64.19	74.44	44.79	62.96	72.54
Xylanase 2x	46.79	65.54	74.52	43.43	62.13	74.56
p-value2	-	-	-	0.3629	0.5543	0.1599
PSE3	-	-	-	1.1607	1.3954	0.6502

[a] Trial 1: N = 2 replicates per treatment; statistical analysis could not be performed due to insufficient replication. Values are reported as descriptive observations only. Trial 2: N = 3 replicates per treatment.

4. Discussion

As the industry seeks sustainable alternatives to marine-derived proteins, plant-based ingredients such as soybean meal and wheat, are increasingly being added to fish feeds. However, these ingredients bring non-starch polysaccharides (NSPs) and antinutritional factors that may reduce nutrient availability, particularly in marine fish (NRC,2011). Supplementation of exogenous enzymes, such as proteases and xylanases, is often suggested to enhance nutrient digestibility and feed efficiency in the animal industry including swine, poultry, and fish culture. It is important to note that AG175™ is a single alkaline serine endopeptidase with no carbohydrase activity, and Econase® XT25 is a single xylanase with no protease activity. Each enzyme was tested on its own, so any effects we saw can be linked specifically to either protein breakdown or arabinoxylan degradation, with no overlap between the two treatments. Soybean meal diets with supplementation of protease (AG175™) or xylanase (Econase® XT25) for juvenile Florida pompano did not result in statistically significant differences in growth performance, FCR, ANPR, or whole-body composition in either trial.

The lack of a significant growth response to enzyme supplementation demonstrates consistency with other studies involving marine fish fed plant-based diets. Stites et al. (2024) evaluated a carbohydrase complex (xylanase and glucanase) in Florida pompano diets and found no major changes in growth or FCR when testing carbohydrase in soy-based diets, even though protein digestibility improved. Diógenes et al. (2019) also reported that the addition of NSP-degrading enzymes to gilthead seabream (Sparus aurata) diets improved feed efficiency and nutrient retention but did not significantly change growth rates. These outcomes may reflect a ceiling effect, meaning better digestibility does not always lead to better growth when the base diet already meets nutritional needs. It is also possible that the level of natural variation between fish

within each tank made it difficult to detect small treatment effects statistically, even if a biological response was present (NRC, 2011)

In contrast, studies that have documented significant enzyme-related growth responses in fish often involve diets with higher plant ingredient inclusions and greater NSP substrate concentrations than those used in the present study, where whole wheat comprised 20% in Trial 1 and 13.9% in Trial 2, and soybean meal was held constant at 50% across both trials. Mohammady et al. (2025) showed enhanced growth performance in European seabass fed soybean meal-based diets supplemented with papain-based protease, particularly in diets with higher soybean meal inclusion that provide higher protease activity. Goda et al. (2020) also reported improved growth performance and feed utilization in European seabass when diets had inclusion of protease in diets for which distillers' dried grains (DDGS) replaced part of soybean meal. These findings highlight that the efficiency of enzyme supplementation is highly dependent on diet composition, ingredient processing methods, and the availability of target substrates.

The small numerical improvement observed with xylanase in trial 1, specifically in the final weigh and FCR may reflect partial hydrolysis of NSPs in the wheat and soybean meal fractions of the diet. This could reduce intestinal viscosity and improve nutrient absorption (Castillo & Gatlin, 2015). This interpretation is supported by work conducted at Auburn University, where Roe et al. (2019) showed that carbohydrase supplementation in high soy diets for Florida pompano improved feed efficiency and nutrient utilization, especially in plant-based diets. However, the NSP content of the Trial 1 basal diet, which contained 20% whole wheat and 50% soybean meal with a crude fiber content of approximately 3.0 g $100g^{-1}$, may have been insufficient to allow xylanase to produce a measurable effect on nutrient release. This is notably lower than the substrate levels used in studies reporting positive enzyme responses, such as

Mohammady et al. (2025) and Goda et al. (2020), where higher soybean meal inclusions or additional fiber sources provided greater arabinoxylan substrate availability for enzyme activity These effects were mentioned by Dalsgaard et al. (2016), who demonstrated that carbohydrase improved the digestibility of specific non-starch polysaccharides in soybean diets for trout. However, the combination of enzymes did not produce growth effects, suggesting that nutrient digestibility was not the primary limiting factor for growth under these dietary conditions. This is consistent with the idea that when a basal diet already meets the nutritional needs of the fish, improvements in digestibility through enzyme supplementation may not translate into measurable growth responses; a pattern also observed in the present study where the basal diet supported strong growth performance across both trials.

4.1 Digestibility and Nutrient Utilization

In Trial 2, we were able to run statistical analysis, but no significant differences in digestibility were found among treatments ($p > 0.05$). However, when you compare both trials together, there is an interesting pattern. In Trial 1, the basal diet had no rice bran and relied mainly on whole wheat and soybean meal as fiber sources. Under those conditions, both Protease-2x and Xylanase-2x groups showed numerically lower ADDM, ADE, and ADCP than the basal diet. In Trial 2, we added 6.5% rice bran to give the xylanase more substrate to act on, and that is when we started to see a shift in both enzyme-supplemented diets, which began to numerically exceed the basal in ADE. Xylanase-2x showed the highest ADCP of all Trial 2 treatments ($74.56 \pm 0.65\%$), marginally above the basal ($73.90 \pm 0.65\%$), though this difference of less than 1 percentage point was not statistically significant ($p = 0.1599$) and is unlikely to be biologically meaningful. Similarly, while enzyme-supplemented diets numerically exceeded the basal in ADE, these differences were small in magnitude and did not reach statistical significance, suggesting that the

addition of 6.5% rice bran in Trial 2 was insufficient to produce a digestibility response of biological relevance. This pattern is not new. Castillo & Gatlin (2015) looked at carbohydrase supplementation across different fish species and found that enzymes tended to work better when the diet had more fiber to begin with, which suggests that substrate availability plays a big role in whether you see a response or not. Dalsgaard et al. (2016) found something along the same lines in rainbow trout, where carbohydrases only made a difference in NSP digestibility when the right substrate was there in enough quantity. Work done by Roe et al. (2019) also showed that carbohydrase supplementation improved feed efficiency in Florida pompano when soybean meal inclusion was high enough to give the enzyme something meaningful to act on.

The fact that we did not see significant digestibility improvements in either trial is different from what some other marine fish studies have reported. Diógenes et al. (2018) found better nutrient digestibility and higher digestive enzyme activity in turbot when exogenous enzymes were added to plant-based diets. Magalhães et al. (2018) saw a similar result in European seabass fed diets with Natugrain, where nutrient digestibility went up quite a bit. It may be that the fiber content of the diets used in the present experiment were not high enough, especially in Trial 1, to give the enzymes enough substrate to make a difference.

We did not see a significant digestibility response in either trial, and part of that may be because the fiber content of our diets was not high enough to give the enzymes enough to work with. That said, just adding more wheat bran or fiber to the diet to create more substrate would likely hurt growth performance, which defeats the purpose of trying to develop a better diet for pompano in the first place. A more practical direction for future research would be to test enzyme supplementation in diets that are already using high plant ingredient levels for cost reduction reasons. In those situations, where fish meal is being replaced by soybean meal and other plant

ingredients to cut feed costs, there would naturally be more fiber and ANFs in the diet, giving enzymes a real problem to solve rather than one we created just for the experiment. It is also worth keeping in mind that Florida pompano does not digest complex carbohydrates the same way omnivorous species like Nile tilapia do. Tilapias have a more diverse gut microbiota and naturally produce more carbohydrate-digesting enzymes, which gives them a clear advantage when it comes to responding to carbohydrase supplementation. de Brito et al. (2022) found big improvements in growth, gut health, and microbiome diversity in tilapia fed diets with xylanase and β-glucanase, and de Macêdo et al. (2023) saw very similar results in tilapia on carbohydrase-supplemented diets with soybean meal and DDGS. Those kinds of responses make a lot of sense for a species that is built to handle plant-based diets. Pompanos are different; they get most of their energy from protein and lipid, and their responses to changes in plant-based diet composition tend to be smaller and harder to pick up statistically (Ngo et al., 2025; Novriadi & Davis, 2019). So even if we put more Xylan-rich ingredients in the diet, we should not expect pompano to respond the same way as tilapia or other omnivorous species would.

5.Conclusion

Adding protease (AG175™) or xylanase (Econase® XT25) to soybean meal-based diets did not significantly improve growth performance, FCR, ANPR, or whole-body composition in juvenile Florida pompano across two trials run under different culture systems and dietary conditions. From a practical standpoint, what we found suggests that enzyme supplementation at the levels we tested does not reliably improve growth in Florida pompano under these dietary conditions, and producers should not count on seeing consistent results without first making sure the diet has enough substrate for the enzyme to work with. That said, the numerical trends we saw

with xylanase in Trial 2 tell us this enzyme is worth looking into further, especially under conditions where Xylan substrate is higher.

Other studies in marine fish species with limited carbohydrate digestion capacity have reported something similar; enzymes tend to show up more in digestibility numbers than in actual growth, especially when the base diet is already doing a good job meeting the fish's nutritional needs. Looking ahead, as the industry keeps adding more plant-based ingredients to bring feed costs down, xylanase may naturally become more relevant because there will simply be more substrate in the diet for it to act on.

Future studies should use ingredients with higher Xylan content, like wheat bran or DDGS to make sure the enzyme has enough substrate to work with in the diet. Having enough fecal samples and replicates from each treatment is also something that needs to be prioritized to make sure digestibility data can be properly analyzed. Future studies should also evaluate the combined supplementation of protease and xylanase in the same diet. In the present study each enzyme was tested independently, so we could only measure the effect of protein hydrolysis or arabinoxylan degradation separately. Combining both enzymes would allow us to test whether targeting multiple anti-nutritional factors simultaneously produces a greater response than either enzyme alone. This is relevant because soybean meal-based diets contain both plant proteins that limit amino acid availability and arabinoxylan that increases gut viscosity, and addressing only one of these constraints at a time may not be enough to produce a measurable growth response. A combined enzyme treatment would tell us whether the lack of effect we observed was due to the enzymes being insufficient individually or whether pompano simply do not respond to exogenous enzyme supplementation regardless of how many ANFs are targeted.

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