Improving Plant-based Diets for Florida Pompano, *Trachinotus carolinus.*

by

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Abstract

Florida pompano aquaculture is a growing enterprise in the United States and production is increasing. Success of Florida pompano production is dependent on the supply of cost-effective diets that promote optimal growth and contain alternative plant proteins as the primary protein sources with reduced dependence on fishmeal. However, increased inclusion of plant protein sources in diets presents problems such as anti-nutritional factors, non-starch polysaccharides and imbalanced amino acid profiles. In order to increase digestion of plant proteins, supplemental carbohydrase and improvements in amino acid profiles can provide solutions. To facilitate the development of sustainable and cost-effective diets for Florida pompano, two studies evaluating dietary lysine requirement and supplementation of commercial carbohydrase enzymes *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase in plant-based diets was conducted. In the first study, an experimental growth trial was conducted where a combined eight diets (40% protein and 8% lipid) were formulated with graded levels of lysine (1.42-2.43% DM) and fed to juvenile pompano. The second research trial was conducted with iso-nitrogenous (40%) and iso-lipidic (8%) test diets containing 0, 0.015, 0.030, and 0.045% of commercial carbohydrase enzymes *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase. Results from the lysine work indicated significant differences in final weight, percent weight gain (PWG), thermal growth coefficient (TGC) and feed conversion ratio between fish reared on the basal diet and the rest of the experimental diets. Survival was variable among all treatments and significant differences existed between pompano fed the 2.12 and 1.57% lysine diets. No significant differences were observed in whole body proximate compositions of fish, but significant differences were observed in a few of the amino acid levels of whole fish including: aspartic acid, glutamic acid, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. The 4-parameter saturation kinetic model was used to model TGC

to determine the dietary lysine requirement. A quantitative lysine requirement was determined to be 1.67% of the dry diet. In the second experimental trial, no significant effects of dietary enzyme supplements were found for initial growth, final growth, PWG, FCR, feed intake, survival (%), apparent net protein retention (ANPR) and apparent net energy retention (ANER). However, TGC was significantly higher in fish fed the 0.015% carbohydrase diet compared to the 0.030% carbohydrase diet. Additionally, no significant differences were identified in mean apparent digestibility of dry matter (ADDM) and apparent digestibility of energy (ADE). The diet supplemented with 0.030% carbohydrase had significantly higher mean apparent digestibility of protein (ADP) compared to the basal diet without supplement. An additional 3.37% protein on the basis of digestible protein was available to Florida pompano fed experimental diets containing any amount of carbohydrase. Based on the results from the two growth trials, Florida pompano fed plant-based diets require a small amount of lysine (1.67%, dry weight) and commercial carbohydrase (0.015%) to optimize growth, feed utilization and protein digestibility.

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CHAPTER I

GENERAL INTRODUCTION

Florida pompano, *Trachinotus carolinus* is a sub-tropical marine fish which commercial culture is developing due to desirable characteristics such as high market prices. Retail prices of pompano fillets ranged from $35 to $45 USD/kg, depending on availability and season (Weirich 2011). Pompano broodstock and larval rearing techniques have been developed and are continuing to be improved upon for this particular species (Hoff *et al.* 1972, Hoff *et al.* 1978a, Hoff *et al.* 1978b, Main *et al.* 2007b, Weirich and Riley 2007). Moreover, Florida pompano can tolerate a wide range of salinities, showing no significant differences in growth and mortality in as low as 10 ppt (Main *et al.* 2010). Pompano can reach market size (1-1.5 pounds) relatively fast within 1-year and as short as 9 months under best conditions (McMaster *et al.* 2006, Main et al. 2007b). Florida pompano can accept feed pellets that are lower or completely void of fishmeal, with the addition of other animal protein sources in conjunction with plant-based protein sources (Rossi Jr and Davis 2012). Therefore, this species displays great mariculture potential in the United States (González-Félix *et al.* 2010, Rossi Jr and Davis 2012).

Production of Florida pompano in the United States started back in the early 1960s with commercial farming and research in ponds (Berry and Iversen 1967). In recent years, intensive recirculating systems and cages have been in use to produce pompano to any size and market-size year-round (Rossi Jr and Davis 2012, Weirich *et al.* 2006, Weirich *et al.* 2009). As the aquaculture industry expands, so does the need of feed production to keep up with the ever-increasing demand of aquaculture production. Feed production is expected to grow to 73.15 million tonnes by 2025. Marine feed production is expected to increase from 4.32 to 5.90 million tonnes during the timespan of 2017-2025 (Tacon 2019). Within the feed matrix, protein is the most expensive

component of aquaculture diets; hence, the dietary protein levels and type affect production cost. Marine fish diets often require high levels of dietary protein as compared to many of the freshwater species (Wilson 1989). Generally, Florida pompano require about 40-45% crude protein to optimize their growth and feed utilization (Lazo *et al.* 1998, Rossi Jr and Davis 2012).

Marine aquaculture feed production has been largely dependent on fishmeal to supply the essential nutrients needed and utilized by marine fish species. Aquaculture has been the largest consumer of fishmeal for over a decade (Naylor *et al.* 2009, Tacon and Metian 2008), consuming 68% of the global fishmeal production in 2011 and 2012 (Mallison 2013, Tacon and Metian 2015). Fishmeal possesses a nutritional profile that sufficiently provides the nutritional requirements of most farmed aquatic species (National Research Council 2011). Moreover, fishmeal is an excellent protein source that contains all the essential amino acids and is also a good source of essential fatty acids, nucleotides, phospholipids, minerals, and vitamins (Tacon *et al.* 2009). In marine fish feeds, fishmeal often consists of 30% to 60% of the formulations (Wang *et al.* 2006). Historically, cost-effective practical diets to enhance pompano growth have contained 30% fishmeal (Lazo et al. 1998). Previous research has indicated that fishmeal can be reduced from 30% to 15% and replaced with combinations of soybean meal with soy and corn protein concentrates without compromising growth performance of Florida pompano (Quintero *et al.* 2012). In addition, other animal protein sources such as poultry by-product meal and meat and bone meal, can completely replace fishmeal in Florida pompano diets without hindering growth performance. However, supplemental taurine is needed when fishmeal is completely replaced with alternative animal protein sources (Rossi Jr and Davis 2012, Rossi Jr and Davis 2014). Due to cost and availability, fishmeal is no longer the primary protein source in many fish feeds including the Florida pompano.

Plant-based protein sources are garnering much needed attention as partial or complete substitutions for fishmeal in aquaculture feeds. Feed manufacturers and fish farmers prefer to use alternative protein sources to produce cost-effective feeds (Sookying *et al.* 2013). Plant-based protein sources are an economical alternative protein source to fishmeal (Castillo and Gatlin III 2015). Among plant-protein sources, soybean meal has received considerable attention in the replacement of fishmeal and other animal protein sources because of its balanced amino acid profile, consistent composition, lower price and worldwide availability (Akiyama *et al.* 1991, Amaya *et al.* 2007, Colvin and Brand 1977, Divakaran *et al.* 2000, Hardy 1999, Lim and Dominy 1990, Lim *et al.* 1998, Samocha *et al.* 2004, Swick *et al.* 1995, Tacon 2000). Soybean meal has a reported apparent energy and protein digestibility of 67.4% and 84.3%, respectively, for Florida pompano (Gothreaux *et al.* 2010). Soy proteins have been used as major plant protein sources in many marine aquaculture species, such as red drum *Scieanops ocellatus* (Rossi *et al.* 2017) California yellow-tail *Seriola dorsalis* (Buentello *et al.* 2015), cobia *Rachycentron canadum (Suarez et al. 2013)* and Florida pompano (Lech and Reigh 2012). Alternative protein sources such as soybean meal (Rhodes *et al.* 2013), soy protein concentrate (Quintero et al. 2012), corn protein concentrate (Novriadi *et al.* 2019b), dry fermented biomass (Rhodes *et al.* 2015), and cotton seed meal (Cook *et al.* 2016), have been evaluated and used to partially or completely replace fish meal and other animal protein sources.

Despite considerable research, decreased growth performance occurs when no fishmeal or animal protein sources are supplemented in the diet, indicating possible nutritional issues (Quintero et al. 2012, Rossi Jr and Davis 2012, Rossi Jr and Davis 2014). Anti-nutritional factors such as lectins, phytic acid, saponins, phytosterols, and allergens are found in many plant meals. These anti-nutritional factors may impair fish health and growth performance (Francis *et al.* 2001,

National Research Council 2011). Furthermore, plant-based ingredients may not be as palatable and do not contain all the nutrients found in fishmeal. Thus, a more complete knowledge of nutritional requirements is needed to balance the diets (Gatlin III *et al.* 2007, Glencross *et al.* 2007a).

The most obvious issue that occurs when replacing fishmeal with other protein sources is a need to meet essential amino acid requirements. Lysine, an essential amino acid, is found in low concentrations in plant-based ingredients such as soybean meal. Plant proteins, such as soybean meal and peanut meal, contain 48.5 and 45.6% protein, respectively, and 3.08 and 1.54% lysine, respectively. On the contrary, fishmeal on average contains 72% protein and 5.57% lysine (Batal *et al.* 2005, NRC 1993). Lysine is often one of the first limiting amino acid in diets formulated with high levels of plant proteins used to prepare commercial fish feeds (Harris 1980, National Research Council 2011). Lysine serves as a precursor of carnitine, which acts as a carrier of long-chain fatty acids into the mitochondria for beta-oxidation, thus metabolizing lipids (Walton *et al.* 1984). Moreover, lysine takes part in a singular metabolic pathway that is targeted for muscle growth (Valente *et al.* 2013). A deficiency in lysine fed to fish will result in loss of appetite, growth reduction, fin erosion, and increased mortality (Borlongan and Benitez 1990, Ketola 1983, Mai *et al.* 2006). Deficient amino acids are commonly supplemented when plant protein sources are used in fish feeds (Fournier *et al.* 2003, National Research Council 2011). Therefore, indispensable amino acids, such as lysine, need to be balanced in feed formulations.

As feed formulations move towards increased levels of plant-based ingredients, non-starch polysaccharides (NSPs) also increase. These can be problematic as most fish lack the enzymes to hydrolyze the beta-glycosidic bonds in NSPs (Krogdahl *et al.* 2005, Yigit and Olmez 2011). Plant protein sources such as soybean meal and rapeseed meal have been reported to contain 27.6 and

15.5% soluble NSP (% of DM), respectively (Knudsen 2014). Thus, the NSPs are indigestible and negatively affect nutrient digestibility of the feed (Sinha *et al.* 2011). The use of exogenous enzymes, such as carbohydrases, can be utilized as an agent to digest the NSPs and can improve the nutritional value of the diets. Carbohydrases are used to breakdown carbohydrates found in plants to simple sugars. Supplementation of carbohydrases increases digestibility of energy-yielding nutrients, such as starch and fat, because NSPs reduce the capacity for nutrient absorption by reducing enzyme accessibility to substrates (Adeola and Bedford 2004). Moreover, carbohydrases improve nitrogen and amino acid utilization by increasing the access to protein for digestive proteases (Tahir *et al.* 2008). Additionally, carbohydrases reduce the digestive viscosity caused by NSPs to improve gut health, increase enzyme accessibility to substrates to improve nutrient absorption, and increase the amount of minerals in the diet (Vahjen *et al.* 2007, Castillo and Gatlin III 2015). More than 80% of the global carbohydrase market is accounted for by two dominant enzymes, xylanase and glucanase. Other commercially-available carbohydrases include amylase, mannanase, galactosidase and pectinase. However, the usage of carbohydrases in aquaculture has only increased in recent years (Castillo and Gatlin, 2015). Carbohydrases have been shown to improve apparent digestibility coefficients in rainbow trout (Dalsgaard *et al.* 2016). In addition, Magalhaes *et al.* (2016) found that supplementation of 0.04% Natugrain commercial carbohydrase in 5% fishmeal diets greatly increased feed, protein and energy utilization in white seabream, demonstrating their application in fish. Presently, there is limited information in exogenous carbohydrase enzyme usage in plant-based diets fed to Florida pompano.

To advance feed formulations for the Florida pompano, critical amino acids need defined and the effects of NSPs determined on performance as these are going up as feeds move towards

higher inclusions of plant-based ingredients. Hence, the current study was conducted with two major objectives:

1. To determine the dietary lysine requirement for juvenile Florida pompano.
2. To investigate the efficacy of using a commercial carbohydrase enzyme complex composed of *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase on growth performance, feed utilization, and digestibility in diets fed to juvenile Florida pompano.

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CHAPTER II

QUANTITATIVE LYSINE REQUIREMENT FOR JUVENILE FLORIDA POMPANO, *Trachinotus carolinus* FED PLANT-BASED DIETS.

Abstract

Florida pompano, *Trachninotus carolinus* is positioned to be an important fish species in US mariculture. If commercial production is to be developed sustainably and with reduced feed costs, it is critical to maximize the level of plant-based proteins in the diet. Thus, good information is needed first on limiting amino acids, which would include lysine. This work was conducted using eight diets formulated with graded levels of lysine (1.42-2.42% dry weight) and fed to juvenile pompano (mean initial weight 13.07 ± 0.46). Dose responses and significant effects were observed in final weight (FW), percent weight gain (PWG), thermal growth coefficient (TGC) and feed conversion ratio (FCR). Significant differences in mean fish whole body amino acids were observed in aspartic acid, glutamic acid, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Regression models, such as broken-quadratic model (BQM), 4-parameter saturation kinetic model (4-SKM), 5-parameter saturation kinetic model (5-SKM), one-slope broken line model (BKL.1) and two-slope broken line model (BKL.2) from R were fitted against different parameters to determine the dietary lysine requirement. Thermal growth coefficient was the parameter utilized to fit a 4-SKM model and determine the quantitative lysine requirement. Based on these results, a minimum lysine requirement of 1.67% DM is recommended for practical feed formulations.

KEYWORDS: pompano, lysine, requirement, model selection

1. Introduction

Florida pompano, *Trachinotus carolinus*, is a marine fish species that is garnering more attention in the United States due to its excellent potential for mariculture (González-Félix et al. 2010, Rossi Jr and Davis 2012). This species contains several characteristics that make it a viable candidate for commercial aquaculture, such as high market prices, tolerance of a wide range of salinities, captive larval and broodstock protocols, fast growth, and acceptance of pelleted feeds that are high in plant-based proteins and low in animal protein sources (McMaster et al. 2006, Main *et al.* 2007a, Weirich and Riley 2007, Main et al. 2010, Weirich 2011, Rossi Jr and Davis 2012). A closely related species, the Golden pompano *T. ovatus* is an important economic aquaculture species in southeast Asia (Lin *et al.* 2012). In addition, the Golden pompano is commercially distributed in China, Japan, Australia, and other countries (Yang 2006). In an effort for commercial production of Florida pompano to grow and be profitable in the United States, alternative protein sources and refinements in aquaculture feeds are needed.

Animal protein sources are often replaced or complemented with plant-based protein sources to decrease costs in aquaculture feeds. Numerous plant protein sources, such as those from soybeans, corn and cotton, have been evaluated and used in Florida pompano diets to partially or completely replace fishmeal and other animal protein sources (Cook et al. 2016, Lech and Reigh 2012, Novriadi *et al.* 2019a, Quintero et al. 2012, Rhodes et al. 2013, Rhodes et al. 2015). However, reducing animal protein sources from 15% to 0% in Florida pompano diets shows a dramatic decrease in fish growth performances (Rhodes *et al.* 2017, Rossi Jr and Davis 2012). Plant-based protein sources can be implemented in Florida pompano diets, but a supplemental animal protein source is presently needed to support optimal growth and feed utilization (Novriadi *et al.* 2017, Quintero et al. 2012, Rhodes et al. 2017, Rossi Jr and Davis 2012, Rossi Jr and Davis

2014). Protein source and quality in amino acid profiles in diets are essential for maximum growth in fish (Ahmed and Khan 2004).

Fish require the same ten indispensable amino acids (IAA) as other animals for growth (Wilson 1985). Indispensable amino acid deficiency will result in poor growth and diet utilization (Wilson and Halver 1986) as well as economic inefficiencies. Lysine is often one of the most limiting amino acid in ingredients used to prepare commercial fish feeds (Harris 1980). Lysine serves an important role in the structure of collagen since collagen contains hydroxylysine, which is derived from lysine by the enzyme lysyl hydroxylase (National Research Council 2011). Lysine regulates carnitine synthesis in skeletal muscle cells and in the liver, which then transports long chain fatty acids into the mitochondria for beta oxidation of lipids. During fasting, these fatty acids are oxidized to fulfill energy needs (Walton et al. 1984). In addition, lysine is involved in maintaining acid-base concentrations and balances the osmotic pressure in the body (Chiu *et al.* 1988). Dietary lysine supplementation in fish feeds decreases mortality and inhibits fin rotting (Li *et al.* 2009). Moreover, lysine enhances protein deposition in the body and fillet content. Given the importance of IAA requirements and their need for proper feed formulations, it is critical that we define the requirements.

Lysine requirements have been quantified and reported for other pompano species. For instance, Du *et al.* (2011) found that juvenile golden pompano require 2.94% of lysine in their diets, on the basis of dry diet. Moreover, silver pompano, *T. blochii*, require a lysine content of 2.40-2.45% of dry diet to optimize growth performance (Ebeneezar *et al.* 2019). The need to quantify a dietary lysine requirement for Florida pompano is essential. Therefore, the objective of the present study was to determine the dietary lysine requirement for Florida pompano.

2. Materials and Methods

2.1 Experimental Diets

The experimental diets were prepared at the Aquatic Animal Nutrition Laboratory, School of Fisheries, Aquaculture, and Aquatic Sciences (SFAAS), Auburn University (Auburn, AL, USA) using standard protocols. Diets were made by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH, USA) for 15 min. Boiling water was then added and blended into the mixture to attain a consistency appropriate for pelleting. The moist mash from each diet was compressed and passed through a 3 mm die in a meat grinder, and the subsequent pellets were dried in a forced air-drying oven (35°C) to attain moisture content of <10%. Diets were stored at -20°C, and, prior to use, each diet was ground and sieved to an appropriate size. Practical diets were designed to be iso-nitrogenous and isolipidic (40% intact protein and 8% lipid). In the trial, eight diets were prepared with increasing lysine inclusion level from 1.42-2.43% of the dry weight (n = 4 tanks per diet, Table 1). The experimental diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate analysis and amino acid profile (Table 2).

2.2 Experimental Fish and Growth Trial

Florida pompano fingerlings (~7 g mean weight) were obtained from Aquaco Farms, Vero Beach, FL, USA. Fish were loaded into a hauling tank equipped with a supplemental oxygen supply system and transported to Claude Peteet Mariculture Center (CPMC) in Gulf Shores, AL, USA. Fish were then transferred and acclimated to a semi-closed recirculating system comprising of 36 circular tanks (750 L) located at CPMC. The recirculating system consisted of a reservoir tank, bead filters and circulation pumps. Supplemental aeration was provided by a regenerative blower and air diffusers. The culture systems were located in a greenhouse that provided a natural

light cycle, approximately 14-hr light day and 10-hr dark day. Fish were fed to apparent satiation with a 55% crude protein and 15% crude fat commercial diet (FF Starter, Ziegler Bros., Inc., Gardners, PA, USA) until appropriate size was reached for the growth trial.

At the start of the trial, twenty fish (mean initial weight 13.07 ± 0.46) were stocked into each tank and assigned to quadruplicate tanks in a completely randomized design. Water temperature, salinity, pH and dissolved oxygen (DO) were measured twice daily with a water quality multi-parameter meter (ProPlus, Yellow Spring Instruments Co., Yellow Springs, OH, USA). Total ammonia nitrogen (TAN) was measured twice per week using an ion-selective electrode (Orion 4-Star Plus pH/ISE, Thermo Fisher Scientific, Waltham, MA, USA). In addition, nitrite and nitrate were measured once per week using a WaterLink SpinTouchFF meter (LaMotte Company, Chestertown, MD, USA). Water quality data are presented in Table 3.

2.3 Sample Collection and Body Composition Analysis

Fish from each tank were group-weighed and counted to determine the number of fish, biomass and mean weight. Fish were weighed at the beginning of the experiment and every other week thereafter. At the conclusion of the growth trial, mean final biomass, mean final weight (FW), feed conversion ratio (FCR), percent weight gain (PWG) and percentage survival were calculated. Thermal unit growth coefficient, TGC, (Iwama and Tautz 1981) was calculated as: $100 * [FBW^{1/3} - IBW^{1/3} / \sum D*T]$, where FBW and IBW are the final and initial body weights in grams, D is number of days and T is the overall average water temperature degrees Celsius. Upon termination of the trial, four fish per replicate were randomly selected and stored at -70 °C for whole body proximate and amino acid analysis. Prior to body composition analyses, whole fish from each tank were chopped and blended in a food processor. Proximate analysis and amino acid analysis of wet fish were performed by the Agricultural Experiment Station Chemical Laboratories

of the University of Missouri (Columbia, MO, USA). Protein retention efficiency (PRE) and lysine retention efficiency (LRE) were calculated after the analyses were completed and finalized. PRE was calculated as: (protein gained (g) / protein fed)*100. LRE was calculated as: (lysine gained (g) / lysine fed)*100.

2.4 Dietary Estimate Requirements

Mean final weight, TGC, PWG, PRE and LRE were fitted against analyzed dietary lysine levels (% DM) using five models: broken-quadratic model (BQM), 4-parameter saturation kinetic model (4-SKM), 5-parameter saturation kinetic model (5-SKM), one-slope broken line model (BKL.1) and two-slope broken line model (BKL.2) in order to estimate the quantitative lysine requirement. The BQM fits a second-degree polynomial for values of x below the requirement and a linear regression for values of x above the requirement. The abscissa of the breaking point (x_{bp}) defines the requirement. SKM models are non-linear and therefore, the requirement is defined as 95% of the maximum fitted value (Mercer 1992, Pesti *et al.* 2009). In addition, the SKM models do not possess breaking points. The 4-SKM in particular is characterized by a sigmoid curve with an asymptotic plateau; whereas, the 5-SKM produces an asymmetrical curve with three areas: ascending, maximum response and decreasing areas, which define the deficient, replete and toxic zones. The 4-SKM produces a single point and a range of dietary concentration for the 5-SKM. The BKL models are fitted by the method of least squares and yields an estimate of the level of the dietary nutrient requirement. Any response that approaches an asymptote can be fitted by a broken line for a narrow range of doses and a large error variance (Robbins *et al.* 1979). A BKL consists of a straight line that is either increasing or decreasing and a horizontal line. The breakpoint is at the point of interaction between the two lines. A BKL.1 model is often used for fitting growth data over a narrow range of tested levels. A BKL.2 model describes two intersecting

straight lines, both with non-zero slopes.

In mathematical terms, the models are described as: (1) BQM: $y = a + U(x_{bp}-x)^2 + V(x-x_{bp})$, where $(x_{bp}-x)$ equals 0 when $x>x_{bp}$, a is an asymptote of the quadratic ascending segment of the model, x_{bp} is the abscissa of the models' breaking point, and U and V are model parameters. Together, a, U and V determine the polynomial coefficients of each segment of the model. (2) 4-SKM: $y = (i * k_{0.5} + y_{max} * x^n) / (k_{0.5} + x^n)$, where i is the model's intercept, y_{max} is the maximum theoretical response, $k_{0.5}$ is the level for half of $(y_{max} + i)$, and n is the apparent kinetic order. (3) 5-SKM: $y = (i * k^n_{0.5} + y_{max} * x^n +)(i * x^{2n}/k^n_s) / (k^n_{0.5} + x^n + x^{2n}/ k^n_s)$, where i, Y_i, $k_{0.5}$ and n are as described for the 4-SKM, and k_s is the inhibition constant. (4) BKL.1: $Y = L + U(R-X_{LR})$ and (5) BKL.2: $Y = L + U(R-X_{LR}) + V(X_{GR}-R)$, where L is the ordinate and R is the abscissa of the breakpoint in the curve. U is the slope of the line for X<R, and for the two-slope model, V is the slope of the line at X>R. By definition, $(R-X_{LR})$ is equal to zero when X>R, and $(X_{GR}-R)$ is zero when X<R (Robbins 1986).

Corrected Akaike Information Criterion (AICc), R^2 and Akaike weights were used to make informed decisions regarding model selection. Akaike weights are the probability of a given model to be truly the best among those tested (Anderson *et al.* 2000, Arnold 2010). The 95% confidence intervals (CI) of the BQM was calculated based on the standard error of the breaking point multiplied by 1.96, then added or subtracted from the breaking point to get the CI ranges. The value of 1.96 is based on the fact that 95% of the area of a normal distribution is within 1.96 standard deviations of the mean. The 95% CI for the requirements derived from 4-SKM were calculated by bootstrapping from the "boot" package in R. The 95% CI used for 4-SKM were percentile CIs, which the bootstrap estimates the lowest and highest value, then taking the 2.5^{th} and 97.5^{th} percentile to obtain the CIs. It is not possible to solve the 5-SKM model for values of

X; so, a range of lysine requirements are presented instead. Typically, 95% of y_{max} is used; however all of the models solved here did not yield values that were greater than 95% of the y_{max}; so, a range of lysine requirement levels greater than 55% of y_{max} was used for PRE and greater than 90% of y_{max} for FW, TGC, PWG and LRE, respectively. The 95% CI for BKL.1 and BKL.2 were calculated based on the standard error of the breaking point multiplied by 1.96, then added or subtracted from the breaking point to get the CI ranges.

2.5 Statistical Analysis

All mean growth parameters, survival and whole fish proximate and amino acid analyses were analyzed using a one-way analysis of variance (ANOVA) to determine the significant difference ($P \leq 0.05$) among the treatment means followed by the Student-Newman-Keuls multiple range test to determine differences among treatment means using SAS (V9.3 SAS Institute, Cary, NC). The dependent variables used to obtain dietary lysine requirements from BQM, 4-SKM, 5-SKM, BKL.1 and BKL.2 were statistically analyzed using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) with the additional packages "minpack.lm", "MuMIn", "boot", "easyreg", "qpcR", "nlme" and "segmented." These packages were used to evaluate model fit and confidence intervals around the estimated requirement.

3. Results

Water quality results for the experimental trial are presented in Table 3. For the growth trial, mean final fish weight for treatments ranged from 33.43 to 48.78 g, PWG was between 156.53 and 269.76%, TGC varied from 0.0553 to 0.0826 FCR ranged from 1.74 to 2.84, feed intake was between 56.35 and 64.03, and survival ranged from 75 to 95% (Table 4). Results indicated significant differences in mean final weight, PWG, TGC, FCR and survival (Table 4). Fish fed the 1.42% lysine (by dry weight) diet performed poorly and was significantly different in the growth

parameters from those of the rest of the fish reared on the other experimental diets; whereas, mean survival was significantly higher in the fish fed the 1.57% lysine dry weight diet compared to fish reared on the 2.12% lysine dry weight diet. A dose response and plateau in growth parameters existed in fish reared on diets other than the basal diet.

Proximate composition analyses of whole body fish are presented in Table 5. No significant differences were observed in percentage of crude protein (P = 0.1531), crude fat (P= 0.6357), crude fiber (P= 0.2704), moisture (P= 0.5835) and ash (P= 0.4067) across all dietary treatments. Amino acid composition analysis of whole body fish are also presented in Table 5. Significant differences were observed in aspartic acid, glutamic acid, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Aspartic acid, isoleucine, leucine, phenylalanine, threonine, tyrosine and valine values were significantly higher in the fish fed the 2.43% lysine dry weight diet compared to those of the fish reared on the first 2 diets containing 1.43-1.57% lysine by dry weight. Fish fed the 2.26-2.43% lysine by dry weight diets had significantly higher amounts of lysine compared to that of the fish fed the 1.42% lysine by dry weight diet. Methionine was significantly different in fish reared on the 2.43% dry weight diet in comparison to that of fish fed the 1.42% lysine by dry weight diet. Pompano fed the 2.43% lysine by dry weight diet contained a significantly higher level of glutamic acid compared to that of the fish fed the 1.57% lysine by dry weight diet.

Results of the dietary lysine requirement R model fittings are presented in Table 6. In all cases, the R^2 for all growth parameters and models conducted were low, with the exception being 100% R^2 for PWG when measured and fitted by a 4-SKM model. Thermal growth coefficient and PWG were best fitted by a 4-SKM model. The BKL.1 model was the most parsimonious for FW and PRE. Lastly, the BQM model was the most parsimonious for LRE. Figure 1 displays the

relationship between the LRE of fish and the various diets fed to them. Thermal growth coefficient was the response variable used to estimate the lysine requirement using a 4-SKM model. The model estimated that optimal growth is obtained with a dietary lysine requirement of 1.67%, based on dry weight. (Figure 2).

4. Discussion

Lysine is an essential amino acid (EAA) that must be provided in sufficient quantity in the feed for optimal growth and survival (Li et al. 2009). Lysine is often used as a reference amino acid, not only due to its critical role in protein deposition, interaction with other amino acids and critical physiological roles (Ball *et al.* 2007, Robinson and Li 2007), but also due to its utilization to estimate requirements of the rest of the amino acids based on the ideal protein concept, where both indispensable and dispensable amino acids are limiting (van Milgen and Dourmad 2015). Peanut meal was the primary protein source utilized in the experimental diets. As a protein source, peanut meal contains low amounts of lysine. Peanut meal was selected as our primary protein source so that we could start off with lower levels of lysine in the experimental diets in hopes of receiving a dose response in our dietary treatments. In a study conducted by Batal et al. (2005), they concluded that peanut meal contains 1.54% lysine based on a 90% dry weight basis from seven different peanut meal samples. In the present study, all growth parameters, including FW, PWG, TGC and FCR, significantly improved when lysine increased from 1.42 to 1.57% dry weight before plateauing (Table 4). Lysine deficiency, as shown in the basal diet, causes loss of appetite, resulting in low diet intake and depressed growth. Similarly, juvenile totoaba, *Totoaba macdonaldi*, final weight and percent weight gain significantly increased with increasing dietary lysine levels from 1.60 to 1.77% of the dry weight (Madrid *et al.* 2019). Moreover, Craig and Gatlin (1992) observed that red drum, *Sciaenops ocellatus*, percent weight gain was dramatically

enhanced from 254 to 336% with increasing lysine levels from 1.25 up to 1.50% of the dry diet. Survival was variable among all treatments in the present study. Florida pompano fed the 2.12% dry weight lysine diet experienced the most mortality with a survival of 75%. This treatment was significantly worse compared to the fish fed 1.57% dry weight lysine. The discrepancies in survival may be because of the high variability in Florida pompano individual weights encountered during and at the conclusion of the trial (Gilbert 1986, Weirich et al. 2009).

Dietary lysine levels did not have a significant effect on proximate composition of fish whole body in the present study (Table 5). Generally, the highest percentage of crude fat and ash reported in the fish were fed the first two (lowest) dietary lysine diets, which also performed the worst in growth parameters. The inverse of this is that fish fed these two dietary levels of lysine contained the lowest amount of crude protein. Totoaba did not contain any significant differences in their whole body proximate compositions when fed various levels of lysine (Madrid et al. 2019). Additionally, dietary lysine did not significantly affect whole body composition in turbot, *Scophthalmus maximus*, (Peres and Oliva-Teles 2008) and milkfish, *Chanos chanos*, (Borlongan and Benitez 1990). However, lysine levels in marine species such as Japanese seabass, *Lateolabrax japonicas*, (Mai et al. 2006) and cobia, *Rachycentron canadum*, *(*Zhou *et al. 2007)* displayed significant differences in crude protein in their whole body proximate compositions. Amino acid analyses of fish whole body displayed significant differences in lysine and methionine content (Table 5). In the fish, lysine slowly increased across all treatments; whereas, methionine kept increasing then slowly decreased at 2.12% dry weight before slowly increasing back up through the rest of the diets. Lysine increases muscle growth in fish by rapidly increasing size and length of muscle fibers (Valente et al. 2013, Michelato *et al.* 2016), which may explain the worst growth and whole body lysine content in the most deficient lysine diet. Lysine retention efficiency

depicted in Figure 1 displays that it was steadily increasing until around 1.61% dietary lysine, and then it began to decrease sharply over the rest of the experimental diets. This suggests that lysine could be utilized efficiently when dietary lysine was deficient and poorly utilized when it was in excess; thus, noting that an excess of lysine in feed formulations is detrimental to costs. Finke *et al.* (1987) and Gahl *et al.* (1991) reported that decreasing lysine retention at an excess level of dietary lysine might develop due to diminishing returns in a dose-response approach in which efficiency decreases as the growth response approached the maximum.

Growth and/or net nutrient deposition are the most accurate and important tools in studying fish feed efficiency and nutrient requirements. Appropriate mathematical models have to be used in order to predict the quantitative relationship between nutrient intake and growth (Belal 2005). In the present study, the R mathematical models used to make informed decisions on the dietary lysine requirements for each parameter were chosen based on AICc, R^2 and akaike weights. The AICc and akaike weights used in selection for the appropriate model were based on the lowest and highest values, respectively (Table 6). However, that is not always the case due to disadvantages or limitations in model selection. In order to overcome these limitations, a saturation kinetic model was developed. The saturation kinetic model fulfills the requirement of covering the full range of growth and has a biological basis (based on the law of mass action and the enzyme kinetics), in addition to having four or five parameters that have biological meaning (Morgan *et al.* 1975). Mercer (1982) further explained this type of model theoretically by stating that the effect of a nutrient in a physiological system may be regarded as the result of physio-chemical interaction between the nutrient and various micro-molecular components of the organism. As a result, the 4-SKM was the model used to determine the dietary lysine requirement. Thermal growth coefficient was the parameter utilized to fit a 4-SKM model and determine the quantitative lysine requirement.

Thermal growth coefficient accounts for growth disparities due to temperature variations (unless extreme) and body size differences (Iwama and Tautz 1981). Although this growth model is without limitations (Jobling 2003), it is evidently better suited for cross study comparisons than other models, such as specific growth rate or percent weight gain. Thus, when TGC is fitted to dietary lysine, a clear dose response emerges (Figure 2) and allows for the fitting of a non-linear model like the parameter saturation kinetic model.

The estimated lysine requirements from this study closely resemble the lysine requirement of red drum. In that study, Craig and Gatlin (1992) concluded that the estimated lysine requirement for red drum was 1.55% of the dry weight based on growth data and feed efficiency. Madrid et al. (2019) estimated that juvenile totoaba require 1.93% lysine, based on dry weight, using TGC as their growth model from their experimental diets. Japanese yellowtail, *Seriola quinqueradiata*, lysine requirement was determined to be 1.78% of the dry diet from weight gain and feed efficiency (Ruchimat *et al.* 1997). On the contrary, other species of fish have been reported to require a greater or lower amount of dietary lysine in their diets to support optimal growth. Adesola *et al.* (2017) found that dietary lysine levels of 3.17% of the dry weight resulted in maximum growth for dusky kob, *Argyrosomus japonicus*. A previous study reported a dietary lysine requirement of 2.33% of the dry diet for cobia, on the basis of specific growth rate (Zhou et al. 2007). Juvenile hybrid striped bass, *Morone chrysops x Morone saxatilis*, requires a lysine content of 1.40% of the dry diet based on a broken-line analysis of weight gain and feed efficiency (Griffin *et al.* 1992). Espe *et al.* (2007) reported optimal growth was achieved with a dietary lysine level of 1.26% of the dry weight in diets fed to Atlantic salmon, *Salmo salar*. In a previous research study conducted with Florida pompano determining the dietary lysine requirement, Riche (2011) reported that 2.25% lysine, on the basis of dry weight, is required. Silver pompano, *T. blochii*,

lysine requirement was identified at 2.40-2.45% of the dry diet (Ebeneezar et al. 2019). In both of these studies, it is obvious that they reported a higher dietary lysine requirement in comparison to this study. The reason for this may be due to the fact that in both studies, taurine was not included in experimental diets. Taurine has been shown to be an essential nutrient for numerous fish species in larval and juvenile stages and is involved in growth, osmoregulation, bile salt conjugation, immunomodulation and anti-oxidation processes (Salze and Davis 2015).

The lower dietary lysine requirement for Florida pompano compared to other species may be due to the fact that it has a maximum weight gain of 2.8-3.4 g/day during its production cycle depending on stocking density (Weirich *et al.* 2008). The variation of the estimated values of dietary lysine requirement among the various finfish compared with our study may be due to different protein sources, culture conditions, and the methods used (Salze *et al.* 2017). Other factors reported to influence lysine requirement are fish life-stage, amount of food consumed, culture conditions, and protein sources in addition with their digestibility (Hauler and Carter 2001, De Silva *et al.* 2000). Furthermore, feeding practices, proximate composition of diets, and fish strains can have effects on the dietary lysine requirement (Kim *et al.* 1992). Based on selection of TGC as our parameter fitted against dietary lysine levels, a lysine requirement of 1.67% of the dry weight is needed to fully enhance Florida pompano growth and feed utilization.

5. Conclusion

There was clear evidence that Florida pompano fed a diet containing 1.57% lysine or higher had positive growth trends and were significantly different than the basal diet. Based on the R modeling, a 4-SKM model and a one-slope broken line model were most selected to quantify various lysine requirements (Table 6). Among all of the different models utilized, a minimum dietary lysine requirement of 1.67% dry weight is needed to optimize Florida pompano growth.

Table 1. Composition of diets containing graded levels of lysine added into the basal diet and fed to juvenile Florida pompano for 8 weeks.

Ingredient (g/100g, as-is)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Poultry by-product meal[1]	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Soybean meal[2]	13.40	13.40	13.40	13.40	13.40	13.40	13.40	13.40
Peanut meal[3]	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Corn protein concentrate[4]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Menhaden fish oil[5]	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Soy Lecithin[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Corn Starch[7]	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Whole wheat[7]	29.55	29.55	29.55	29.55	29.55	29.55	29.55	29.55
Mineral premix[8]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride[7]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin C[10]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[7]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Lysine	0.00	0.15	0.30	0.45	0.65	0.85	1.05	1.25
Methionine	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Phenylalanine	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Glutamic Acid[11]	0.625	0.55	0.475	0.40	0.30	0.20	0.10	0.00
Glycine[12]	0.625	0.55	0.475	0.40	0.30	0.20	0.10	0.00
Taurine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

[1]Darling Ingredients Inc., Dallas, Texas, USA.
[2]Solvent Extracted Soybean Meal, Auburn University, Auburn, Alabama, USA.
[3]Golden Peanut and Tree Nuts, Alpharetta, Georgia, USA
[4]Empyreal 75 TM Cargill Corn Milling, Cargill Inc., Blair, Nebraska, USA.
[5]Omega Protein Inc., Reedville, Virginia, USA.
[6]The Solae Company, St. Louis, Missouri, USA.
[7]MP Biochemicals Inc., Solon, Ohio, USA.
[8]ASA Premix (g/100 g premix): cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250, ferrous sulphate heptahydrate, 4.0, manganous sulphate anhydrous, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193, and α cellulose 81.826.
[9]ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate, 20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU/g), 2.40; vitamin D3 (400,000 IU/g), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.
[10]Stay C, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
[11]VWR Chemicals LLC, Solon, Ohio, USA
[12]Alfa Aesar, Ward Hill, Massachusetts, USA

Table 2. Proximate and amino acid (AA) analysis of the experimental diets (g/100g, dry weight) fed to juvenile Florida pompano for 8 weeks.

AA (g/100g, dry weight)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Alanine	2.19	2.17	2.20	2.16	2.10	2.12	2.12	2.11
Arginine	2.99	3.00	3.02	2.91	2.90	2.96	2.95	2.95
Aspartic Acid	3.78	3.77	3.82	3.72	3.66	3.76	3.69	3.72
Cysteine	0.61	0.60	0.61	0.61	0.58	0.59	0.59	0.61
Glutamic Acid	8.64	8.81	8.62	8.25	7.97	7.73	8.02	7.97
Glycine	2.74	2.66	2.56	2.42	2.31	2.28	1.98	1.86
Histidine	0.94	0.93	0.94	0.93	0.91	0.91	0.92	0.91
Hydroxyproline	0.29	0.31	0.29	0.29	0.31	0.41	0.26	0.27
Isoleucine	1.64	1.62	1.65	1.65	1.61	1.63	1.63	1.62
Leucine	3.68	3.64	3.74	3.66	3.52	3.55	3.58	3.55
Lysine	**1.42**	**1.57**	**1.69**	**1.78**	**1.93**	**2.12**	**2.26**	**2.43**
Methionine	0.94	0.92	0.91	0.88	0.88	0.90	0.90	0.87
Phenylalanine	2.20	2.18	2.24	2.17	2.13	2.14	2.14	2.14
Proline	2.46	2.41	2.41	2.33	2.43	2.31	2.41	2.42
Serine	1.65	1.89	1.72	1.56	1.54	1.58	1.57	1.61
Taurine	0.72	0.72	0.72	0.70	0.70	0.72	0.72	0.72
Threonine	1.30	1.31	1.34	1.26	1.24	1.26	1.26	1.26
Tryptophan	0.39	0.39	0.39	0.38	0.39	0.38	0.38	0.39
Tyrosine	1.61	1.62	1.64	1.45	1.46	1.54	1.51	1.54
Valine	1.94	1.88	1.92	1.94	1.90	1.91	1.91	1.89
Proximate analysis (g/100g, dry weight)								
Crude Protein (%)	42.93	42.75	43.20	42.55	42.36	42.48	42.54	42.80
Moisture (%)	5.35	6.03	11.07	9.75	5.66	7.34	6.80	7.98
Crude Fat (%)	8.49	8.31	8.59	8.34	8.81	8.52	8.67	8.75
Crude Fiber (%)	3.06	2.89	3.13	2.66	2.94	2.64	2.62	2.73
Ash (%)	6.90	6.99	6.86	6.89	6.96	6.87	6.94	6.86

[1]Analysis by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Table 3. Mean water quality parameters for Florida pompano reared in a growth trial in a recirculating system for 8 weeks.

Parameters	Mean	± std. dev.
Temperature (°C)	27.97	± 1.59
Salinity (g/L)	35.48	± 3.43
pH	7.79	± 0.33
Dissolved oxygen (mg/L)	5.49	± 0.51
Total ammonia nitrogen (mg/L)	0.27	± 0.35
Nitrite (mg/L)	0.84	± 0.82
Nitrate (mg/L)	38.55	± 35.98

Table 4. Mean growth performance of juvenile Florida pompano (mean initial weight 13.07 ± 0.46) fed experimental diets containing different levels of lysine (1.42-2.43%, dry weight) for 8 weeks. Values represent the mean of four replicates. Results in the same columns with different superscript letters are significantly different ($P \leq 0.05$) based on analysis of variance followed by Student-Newman-Keuls multiple range tests.

Analyzed lysine (dry weight)	Final mean weight (g)	PWG[1]	TGC[2]	FCR[3]	Feed intake	Survival (%)
1.42%	33.43[b]	156.53[b]	0.0553[b]	2.84[b]	56.35	82.50[ab]
1.57%	41.66[a]	219.77[a]	0.0713[a]	2.24[a]	64.03	95.00[a]
1.69%	41.91[a]	221.21[a]	0.0717[a]	2.22[a]	63.94	91.25[ab]
1.78%	43.76[a]	236.38[a]	0.0751[a]	2.04[a]	62.75	82.50[ab]
1.93%	43.55[a]	234.68[a]	0.0745[a]	2.09[a]	62.92	86.25[ab]
2.12%	48.78[a]	269.76[a]	0.0826[a]	1.74[a]	61.54	75.00[b]
2.26%	44.61[a]	241.57[a]	0.0763[a]	1.89[a]	59.28	78.75[ab]
2.43%	44.30[a]	234.00[a]	0.0749[a]	2.04[a]	62.86	86.25[ab]
P-value	0.0045	0.0009	0.0007	<0.0001	0.3305	0.0360
PSE[4]	2.1588	14.0061	0.0033	0.1154	2.3923	3.9857

[1]PWG: Percent weight gain.
[2]TGC: Thermal growth coefficient.
[3]FCR: Feed conversion ratio.
[4]PSE: Pooled standard error.

Table 5. Proximate and amino acid analysis of whole fish from the growth trial fed graded levels of lysine over 8 weeks. Values represent the mean of four replicates. Results in the same columns with different superscript letters are significantly different ($P \leq 0.05$) based on analysis of variance followed by Student-Newman-Keuls multiple range tests.

Composition (g/100g), dry weight	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	P-value	PSE[2]
Alanine	3.66	3.61	3.75	3.83	3.71	3.84	3.86	3.93	0.1008	0.0765
Arginine	3.51	3.53	3.66	3.73	3.67	3.78	3.80	4.03	0.0585	0.1097
Aspartic Acid	4.89^{b}	4.90^{b}	5.25^{ab}	5.32^{ab}	5.23^{ab}	5.38^{ab}	5.44^{b}	5.68^{a}	0.0060	0.1360
Cysteine	0.46	0.45	0.48	0.48	0.48	0.49	0.49	0.51	0.5926	0.0217
Glutamic Acid	7.09^{bc}	6.92^{c}	7.40^{abc}	7.72^{abc}	7.43^{abc}	7.71^{abc}	7.78^{ab}	8.10^{a}	0.0037	0.1884
Glycine	4.43	4.36	4.25	4.41	4.16	4.40	4.32	4.37	0.8905	0.1451
Histidine	1.19	1.23	1.31	1.31	1.32	1.36	1.36	1.05	0.6255	0.1230
Hydroxyproline	1.11	1.08	0.91	1.02	0.88	0.99	0.89	0.82	0.0488	0.0664
Isoleucine	2.37^{b}	2.39^{b}	2.60^{ab}	2.65^{ab}	2.62^{ab}	2.68^{ab}	2.69^{ab}	2.86^{a}	0.0036	0.0775
Leucine	3.81^{b}	3.83^{b}	4.14^{ab}	4.16^{ab}	4.16^{ab}	4.26^{ab}	4.31^{ab}	4.48^{a}	0.0062	0.1165
Lysine	3.92^{c}	4.03^{bc}	4.36^{abc}	4.44^{ab}	4.46^{ab}	4.54^{ab}	4.62^{a}	4.83^{a}	0.0008	0.1287
Methionine	1.45^{c}	1.49^{bc}	1.57^{abc}	1.63^{ab}	1.60^{abc}	1.65^{ab}	1.66^{ab}	1.75^{a}	0.0012	0.0430
Phenylalanine	2.16^{b}	2.17^{b}	2.34^{ab}	2.33^{ab}	2.32^{ab}	2.36^{ab}	2.40^{ab}	2.54^{a}	0.0224	0.0708
Proline	2.75	2.71	2.61	2.69	2.57	2.65	2.68	2.66	0.8757	0.0887
Serine	1.93	1.88	1.98	2.05	1.98	2.02	2.06	2.11	0.2510	0.0635
Taurine	1.01	1.03	1.12	1.05	1.13	1.03	1.18	1.15	0.5101	0.0669
Threonine	2.28^{b}	2.30^{b}	2.46^{ab}	2.46^{ab}	2.45^{ab}	2.51^{ab}	2.55^{ab}	2.60^{a}	0.0114	0.0602
Tryptophan	0.56	0.56	0.59	0.60	0.63	0.61	0.57	0.64	0.5302	0.0315

Tyrosine	1.61[b]	1.67[b]	1.81[ab]	1.73[ab]	1.82[ab]	1.84[ab]	1.82[ab]	2.05[a]	0.0367	0.0804
Valine	2.74[b]	2.76[b]	2.98[ab]	2.97[ab]	2.98[ab]	3.04[ab]	3.05[ab]	3.23	0.0090	0.0837
Proximate analysis (g/100g, dry weight)										
Crude Protein (%)	57.85	57.38	61.13	59.83	60.70	60.75	62.28	61.78	0.1531	1.3497
Moisture (%)	3.34	3.10	3.40	3.41	3.04	3.18	3.40	3.01	0.5835	0.1903
Crude Fat (%)	29.27	30.19	27.05	29.18	28.08	28.48	26.76	26.25	0.6357	1.5975
Crude Fiber (%)	0.09	0.08	0.10	0.05	0.14	0.12	0.04	0.13	0.2704	0.0314
Ash (%)	12.69	12.48	11.70	11.05	11.62	11.38	11.92	11.44	0.4067	0.5350

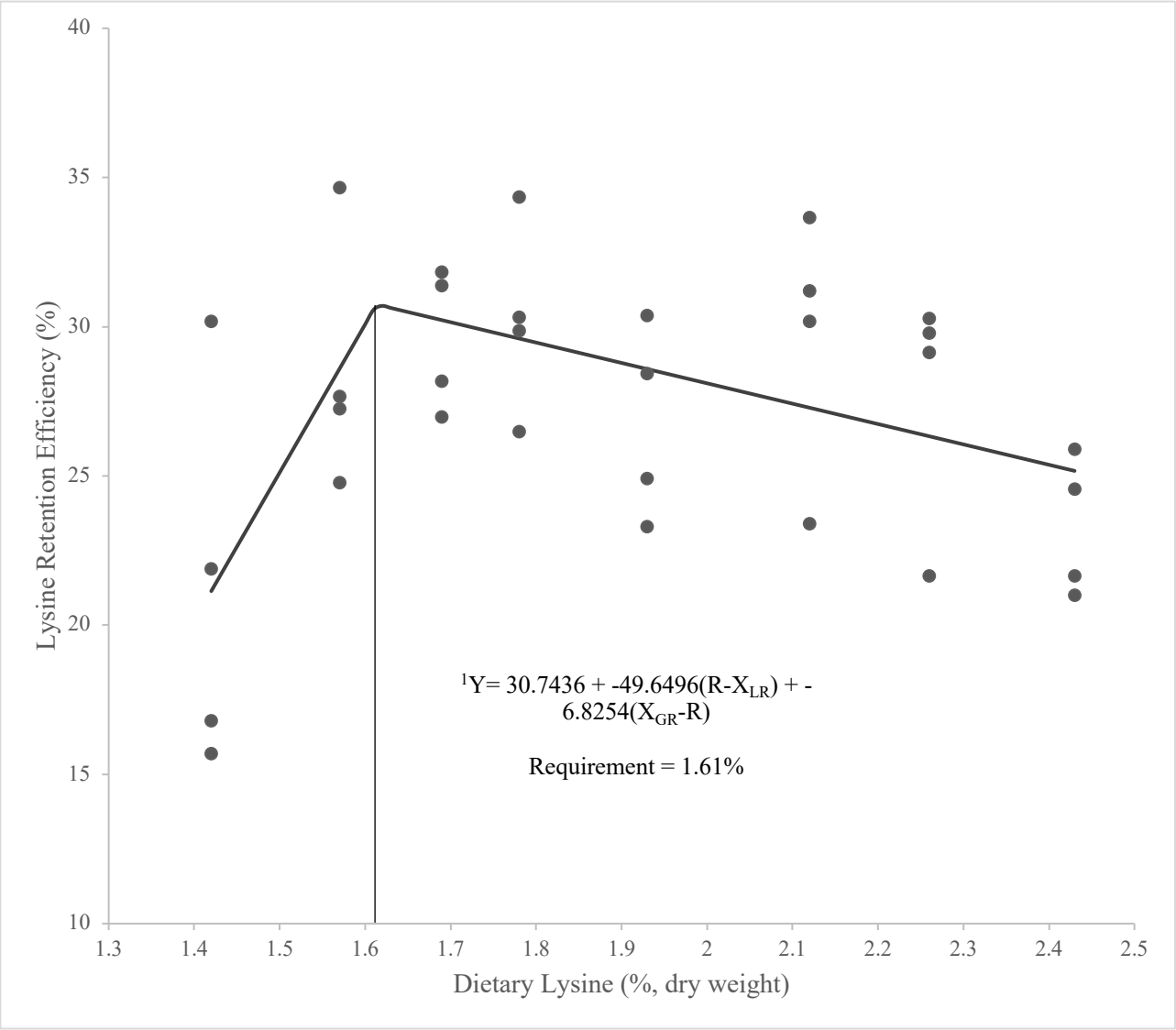
[1]Analysis by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).
[2]PSE = Pooled Standard Error

Table 6. Regression models calculating lysine estimate requirements for juvenile Florida pompano on the basis of mean final weight (FW), thermal growth coefficient (TGC), percent weight gain (PWG), protein retention efficiency (PRE) and lysine retention efficiency (LRE) reared over an 8-week period.

Parameter	Model	R^2	AICc	Akaike Weight	Requirement (%, dry weight) [95% CI]
FW	bqm	0.4872	191.3662	0.2678	2.12 (1.75-2.49)
	4-Skm	0.4840	191.5639	0.2426	1.71 (1.51-2.17)
	5-Skm	0.4902	194.0070	0.0715	1.74-2.55
	bkl.1	**0.4506**	**190.9462**	**0.3304**	**1.75 (1.58-1.91)**
	bkl.2	0.4501	193.6028	0.0875	1.81 (1.59-2.04)
TGC	bqm	0.5505	-217.4724	0.2347	1.66 (1.41-1.91)
	4-Skm	**0.5608**	**-218.2168**	**0.3406**	**1.67 (1.50-1.93)**
	5-Skm	0.5679	-215.9148	0.1077	1.64-2.62
	bkl.1	0.5137	-217.5839	0.2482	1.78 (1.66-1.91)
	bkl.2	0.5146	-215.0186	0.0688	1.80 (1.62-1.99)
PWG	bqm	0.5490	311.8392	<0.0001	2.12 (1.81-2.43)
	4-Skm	**1.0000**	**-218.2148**	**1.0000**	**1.73 (1.51-2.02)**
	5-Skm	0.5537	314.3349	<0.0001	1.85-2.30
	bkl.1	0.5021	312.3847	<0.0001	1.79 (1.66-1.93)
	bkl.2	0.5043	314.8651	<0.0001	1.82 (1.63-2.00)
PRE	bqm	0.5331	153.2392	0.1723	1.88 (1.49-2.26)
	4-Skm	0.5463	152.3197	0.2729	1.84 (1.60-2.12)
	5-Skm	0.5659	153.7318	0.1347	1.91-2.29
	bkl.1	**0.5134**	**151.9340**	**0.3309**	**1.80 (1.67-1.94)**
	bkl.2	0.5134	154.5579	0.0891	1.80 (1.60-2.00)
LRE	bqm	0.3069	189.8076	0.0914	2.04 (1.80-2.28)
	4-Skm	0.2605	191.8808	0.0324	1.46[1]
	5-Skm	0.4111	187.4173	0.3020	1.49-2.35
	bkl.1	0.2608	188.8094	0.1506	1.57 (1.14-2.00)
	bkl.2	**0.3702**	**186.7402**	**0.4236**	**1.61 (1.51-1.72)**

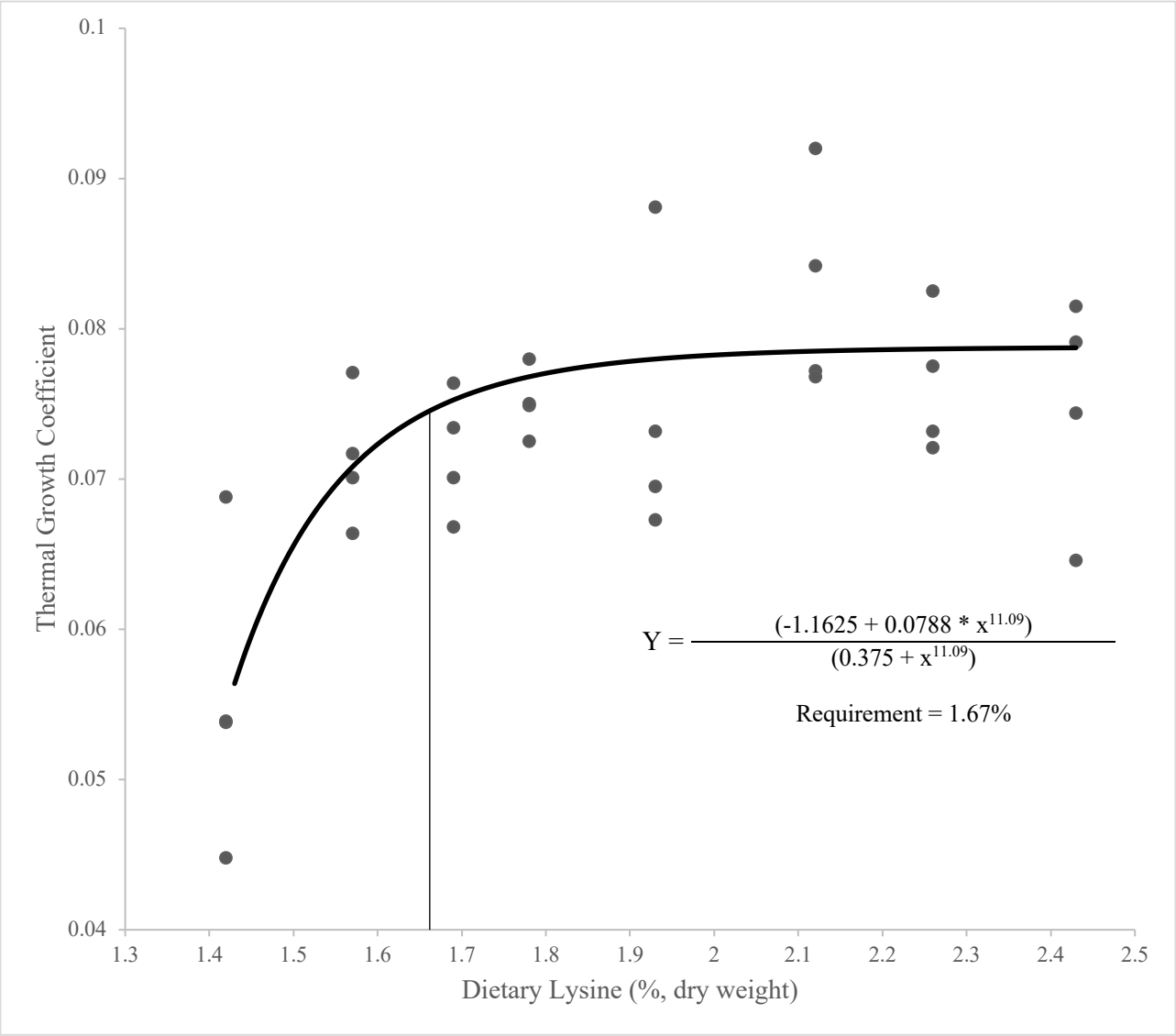
Note: Bold font indicates the model selected for calculation of dietary lysine requirement.
[1]Could not calculate confidence intervals.

Figure 1. Lysine retention efficiency of juvenile Florida pompano to graded levels of lysine as dry weight of diet. The dietary lysine requirement was fitted by a two-slope broken line model.



[1]See materials and methods for full parameterization of the model.

Figure 2. Thermal growth coefficient of juvenile Florida pompano to graded levels of lysine. The dietary lysine requirement was fitted by the 4-parameter saturation kinetic model.



[1]See materials and methods for full parameterization of the model.

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CHAPTER III

EFFECTS OF CARBOHYDRASE SUPPLEMENTATION ON THE GROWTH AND APPARENT DIGESTIBILITY COEFFICIENTS IN FLORIDA POMPANO, Trachinotus carolinus

Abstract

Florida pompano growth and feed utilization can be optimized even when fed soy-based, low animal protein diets. Plant-based diets contain anti-nutritional factors and carbohydrates that limit digestibility in Florida pompano. Dietary carbohydrase enzymes have been used in feeds to improve carbohydrate digestibility in a number of species and may be beneficial for low animal meal diets for the Florida pompano. Thus, the present study was designed to evaluate the effects of using a commercial carbohydrase enzyme complex composed of endo-1,4-ß-xylanase and endo-1,4-ß-glucanase on growth performance, feed utilization, and digestibility in diets fed to juvenile Florida pompano. A growth trial was conducted with iso-nitrogenous (40%) and iso-lipidic (8%) test diets containing 0, 0.015, 0.030, and 0.045% of commercial carbohydrase enzymes endo-1,4-ß-xylanase and endo-1,4-ß-glucanase. The trial was conducted in a semi-closed recirculating system with 12 1000-L fiberglass tanks. Twenty fish (mean weight: 23.34 ± 1.49 g) were stocked into each tank and assigned to triplicate tanks in a randomized design. Growth results indicated that there were no significant differences, however, TGC displayed significant differences. All enzymatic treatments displayed significant differences in apparent protein digestibility (ADP) compared to the basal diet. Results indicate that endo-1,4-ß-xylanase and endo-1,4-ß-glucanase enzyme complex can impact ADP but did not have greatly affect growth performance under the reported conditions.

KEYWORDS: enzyme, digestibility, growth

1. Introduction

Marine aquaculture feed production has been largely dependent on animal proteins such as fishmeal (FM) to supply the essential nutrients needed by marine species. Due to the increasing demand for FM, marine aquaculture must shift feed formulations toward increased use of plant-based feedstuffs if they are going to meet the global aquaculture demand (Gatlin III et al. 2007, National Research Council 2011). Plant-based proteins are a sustainable and economical protein source (Castillo and Gatlin III 2015). In addition, plant-based proteins are the primary source for numerous species in aquaculture (National Research Council 2011). Plant-based protein sources, such as soybean meal, canola meal and corn gluten meal, are cost-effective compared to animal proteins but must be used within nutritional limits of any given species. Plant-based proteins compared to FM have lower levels of protein and are quite often low in one or more essential amino acids (EAA). Depending on the ingredient and fish species, it may also have lower digestibility and palatability issues (Gatlin III et al. 2007, Glencross *et al.* 2007b).

Anti-nutritional factors such as non-starch polysaccharides (NSP) are present in plant-based protein sources, but they are also found in the cereal grains and other carbohydrate sources that are used in fish feeds. When anti-nutritional factors are present, they may also impair fish health and growth performance (Francis et al. 2001, National Research Council 2011). Most fish and other monogastric animals poorly utilize NSPs because they lack the necessary endogenous enzymes to hydrolyze the β-glycosidic bonds of NSPs (Krogdahl et al. 2005, Yigit and Olmez 2011). Furthermore, NSP can impair fish performance by interfering with absorption and digestion of nutrients, reducing digestibility and feed efficiency of the diet (Castillo and Gatlin III 2015, National Research Council 2011). Higher inclusion of plant proteins and the lower inclusion of animal proteins increase the carbohydrate content in feeds. Freshwater species such as common

carp have the ability to digest 89-99% of the carbohydrate content of the diet (Medale *et al.* 1999, Yamamoto *et al.* 2001). On the flipside, Florida pompano have a reported 50% carbohydrate digestibility (Williams *et al.* 1985). Thus, undigested carbohydrates can reduce digestion of other nutrients such as protein.

Dietary supplementation with exogenous β-glycosidases improve NSP utilization in animals since they lack the proper enzymes that hydrolyze the β-glycosidic bonds (Adeola and Cowieson 2011, Castillo and Gatlin III 2015, Sinha et al. 2011). Exogenous non-starch polysaccharidases (NSPase) hydrolyze NSPs, which results in improved carbohydrate digestibility (Adeola and Bedford 2004). Enzyme supplementation is extensively used in non-ruminant farm animal feeds (Adeola and Cowieson 2011, Bedford 2000). Commercial feeds in aquaculture may use phytase as an exogenous enzyme supplementation to improve phosphorus availability (Kumar *et al.* 2012). However, exogenous NSPase supplementation in commercial diets is very limited in aquaculture (Castillo and Gatlin III 2015). Therefore, further investigation in the use of enzymes, such as carbohydrases, in aquaculture diets is needed.

Florida pompano, *Trachinotus carolinus*, is an important species for aquaculture and fisheries (Hoese and Moore 1992) with a high market value (Weirich 2011). Closely related species such as the golden pompano, *T. ovatus*, is of economic importance in Southeast Asia (Lin et al. 2012). Carbohydrase enzymes have been shown to improve apparent digestibility coefficients (ADCs) in diets for rainbow trout (Dalsgaard et al. 2016). Enhancing carbohydrate digestibility in plant-based diets can restore nutrient utilization efficiency as observed in low carbohydrate dietary formulations and/or those with lower levels of nondigestible carbohydrates, with benefits on growth, nutrient digestibility, and economic performance of the feed (Roe *et al.* 2018). Carbohydrase enzymes, such as xylanase and glucanase, dominate the global carbohydrase market

by more than 80% (Castillo and Gatlin III 2015). Presently, limited information exists on dietary supplementation of NSPase enzymes in plant-based diets fed to Florida pompano and a clear need to increase the level of plant-based ingredients. Therefore, the purpose of this study was to evaluate the efficacy of using a commercial carbohydrase enzyme complex composed of *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase on growth performance, feed utilization, and digestibility in diets fed to juvenile *T. carolinus*.

2. Materials and Methods

2.1 Experimental diets

Four experimental diets were formulated to be iso-nitrogenous and iso-lipidic (40% protein and 8% lipid). The four diets were designed to contain soybean meal, poultry meal and corn protein concentrate as the dietary protein sources. The basal diet was modified to contain graded levels (0, 0.015, 0.030 and 0.045%) of highly purified NSP-splitting enzymes *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase (Natugrain TS L, BASF Corporation, Ludwigshafen, Germany) (Table 1). Digestibility diets were created using the same ingredients as mentioned above with all treatments having 1% chromic oxide inert marker added at the expense of corn starch. Test diets were prepared at the Aquatic Animal Nutrition Laboratory, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, AL., USA. Pre-ground dry ingredients and fish oil were first weighed before being simultaneously mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Boiling water was then blended into the mixture to attain a proper consistency for pelleting. The moist mash from each diet was then passed through a 3-mm diameter die in a meat grinder. Then, the diets were air dried at 35°C to attain a moisture content of <10% and stored at room temperature. After cooling, the enzyme was diluted with deionized water and then was top coated to the diets to produce four levels of enzyme, respectively.

2.2 Culture Systems

The feeding trial was conducted at the Claude Peteet Mariculture Center (CPMC), Gulf Shores, AL, USA. Florida pompano fingerlings were spawned at the Alabama Marine Resources Division at CPMC. They were then nursed in an indoor recirculating system of CPMC and fed with a commercial diet (FF Starter, Zeigler Bros., Inc. Gardners, PA, USA) until reaching a suitable size. The trial was conducted in a recirculating system with 12 1000-L fiberglass tanks that were equipped with a reservoir tank, biological filter, supplemental aeration and circulation pump. The tanks were located under a greenhouse. At the beginning of the trial, twenty fish (mean weight = 23.34 ± 1.49 g) were stocked into each tank and assigned to triplicate tanks in a randomized design. The fish were subjected to natural lighting of approximately 14-hour daylight/ 10-hour darkness. Temperature, dissolved oxygen (DO), salinity, and pH were monitored and recorded twice daily using a YSI Proplus multiprobe meter (Yellow Spring Instruments Co., Yellow Springs, OH, USA). Total ammonia nitrogen (TAN) was recorded twice a week using an ion selective electrode (Orion, EA 940, Thermo Electron Corporation, Beverly, MA, USA). Nitrite and nitrate were tested weekly with a LaMotte test kit (3354-01, 3352-01, LaMotte Company, Chestertown, MD, USA).

2.3 Feed Management and Sample Collection

Fish were fed four times per day and the daily ration was adjusted to apparent satiation. In addition, feed inputs were calculated every two weeks after each sampling to adjust for growth and mortalities. The growth trial lasted for 56 days. At the conclusion of the trial, four fish from each tank were euthanized and frozen for later analysis. These whole-body fish samples were then homogenized in a food processor and analyzed for dry matter, protein and energy content. Growth performance indices, including percentage weight gain (100 x (final mean weight-initial mean

weight)/initial mean weight), thermal growth coefficient 100 x [final mean weight$^{1/3}$ – initial mean weight$^{1/3}$ / ∑DxT], where D is number of days and T is the overall average water temperature degrees Celsius, feed intake (average total feed per fish), feed conversion ratio (feed intake/ weight gain) and percent survival (Initial fish number – Final fish number)/Initial fish number × 100), were calculated at the conclusion of the trial. Apparent net protein retention (ANPR, %) was calculated as: (final weight x final protein content) – (initial weight x initial protein content) x 100 / protein intake. Apparent net energy retention (ANER, %) was calculated as (final weight x final energy content) – (initial weight x initial energy content) x 100 / energy intake.

2.4 Digestibility Trial and Biochemical Analysis

The apparent digestibility coefficients of dry matter (ADDM), protein (ADP), and energy (ADE) were investigated. Pompano were fed the same diets from the growth trial with an inert marker added (chromic oxide at 1% of the diet). Fish were acclimated to the diet three days prior to manual stripping and were fed on the same schedule as the growth trials at 0700, 1100, 1500, and 1900 h based on a fixed percentage of body weight. Fish were randomly assigned to a treatment diet (n = 3 tanks per treatment). On the day of stripping, fish were fed on a staggered feeding schedule. Four tanks were fed per feeding block and stripping occurred 3 hours after feeding to allow appropriate time for digestion. Each feeding block contained one tank from each treatment to minimize any influence of the time of day on sampling. Feeding occurred at 0600, 0800, and 1000 h, with stripping at 0900, 1100, and 1300 h. Fish were anesthetized using MS-222 and then manually stripped by applying pressure to the gut to defecate and collect feces. Each fecal sample was dried in an oven at 105°C until a constant weight was obtained. Feces were then stored and analyzed by the individual tank and treatment. Protein, used to determine ADP, was determined

using the standard micro-Kjeldahl method (Ma and Zuazaga 1942). Gross energy content (ADE) was determined using a semi-micro bomb calorimeter (Model 6725, Parr Instrument Co. Moline, IL, USA), and chromic oxide content was measured using a protocol developed by McGinnis and Kasting (1964). Digestibility coefficients were calculated according to Cho *et al.* (1982) as depicted in the following formulas:

ADMD (%) = 100 - [100 x (%Cr2O3 in feed/%Cr2O3 in feces)]

ADP or ADE (%) = 100 - [100 x (%Cr2O3 in feed/%Cr2O3 in feces x %nutrient feces/%nutrient feed)]

2.5 Statistical Analyses

Statistical analyses were conducted using SAS (V9.3 SAS Institute, Cary, NC). Mean initial weight, final weight, percent weight gain (PWG), thermal growth coefficient (TGC), feed conversion rate (FCR), feed intake, survival, ANPR, ANER and digestibility coefficients were analyzed using a one-way analysis of variance (ANOVA). Significant differences ($p \leq 0.05$) were determined by Student-Newman-Keuls tests.

3. Results

3.1 Water Quality

During the experimental study, temperature (28.41 ± 1.38), DO (5.60 ± 0.61), salinity (24.83 ± 2.81), pH (7.45 ± 0.29), TAN (0.025 ± 0.056), nitrite (0.78 1 ± 0.45) and nitrate (50.91 ± 17.76) were determined (Table 2).

3.2 Growth Performance and nutrient retention

For the growth trial, initial mean weight (23.34 ± 1.49) was not significantly different (P=0.99) among fish assigned to various treatments. Mean final weights (85.1 to 99.3 g) were not significantly different (P=0.13). Average PWG (263.0 to 326.3%) of fish were not significantly

influenced (P=0.082) by the different inclusion levels of the enzyme in diets. Mean TGC were significantly different (P=0.0499) among fish fed diets with enzyme levels (0.0982 to 0.1135). FCR (1.72 to 2.01) were not affected (P=0.071) by the level of enzyme in the diet. Mean feed intakes (123.33 to 130.71) were not significantly different (P=0.62) amongst the different treatments. Mean survival (92.0 to 100%) were not affected (P=0.11) by the level of enzyme in the diet. Mean ANPR (P=0.19; 17.16 to 22.02%) and ANER (P=0.65; 21.13 to 25.63%) did not indicate any significant differences amongst fish fed the different inclusion levels of the enzyme (Table 3).

3.3 Digestibility

Statistically significant differences (P=0.029) were found in ADP ranging from 72.93 to 82.56% amongst the various enzyme treatments (Table 4). Apparent digestibility of dry matter and ADE were not significantly different; ADDM ranged from 48.95 to 52.12% and ADE from 65.24 to 70.26% (Table 4)

4. Discussion

Plant-based diets are being investigated among different species of fish with the goal of completely replacing fish meal (FM) in production diets (Tacon et al. 2011). The ability of some species to utilize low-FM or all-plant diets varies by species presumably due to natural food habits as well as the level of nutritional information available (National Research Council 2011). As the inclusion of plant protein sources continue to increase and levels of animal meals decrease, the wider inclusion causes limitations in feeds such as imbalanced AA profiles (Aragao et al. 2003, Fagbenro and Davies 2001) and the presence of anti-nutritional factors (ANFs) like lectins, phytic acid, saponins, phytosterols, allergens and NSPs (National Research Council 2011). In previous studies with Florida pompano, complete removal or lowering the reference level of animal meal

in the diets resulted with significant reduction in growth performance (Rhodes et al. 2017, Rossi Jr and Davis 2012). In the present study, poultry by-product meal was maintained at 10% of the diets with soybean meal and corn protein concentrate providing the bulk of protein. To offset the increased presence of anti-nutritional factors present in plant-based diets, a commercial carbohydrase enzyme complex consisting of endo-1,4-ß-xylanase and endo-1,4-ß-glucanase was implemented in these diets to see if the fish can utilize the plant-based proteins more efficiently without negative growth trends.

Carbohydrases have been shown to significantly improve growth performance and feed conversion in some fish. In research, the usage of *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase in Florida pompano diets mainly composed of plant-based materials has the potential to enhance growth performance. During the present study, fish reared on the 0.015% carbohydrase enzyme complex diet significantly outperformed the fish fed the 0.030% carbohydrase enzyme complex diet in TGC. Japanese seabass, *Lateolabrax japonicus*, fed with non-starch polysaccharide enzyme diets significantly enhanced their specific growth rate and feed efficiency ratio (Ai *et al.* 2007). There were significant differences in growth rates and feed conversion ratios in Caspian salmon, *Salmo trutta*, when fed 0.05% Natuzyme (Bioprotin Pty Ltd., Sunnybank, Australia) and 0.05% Hemicell (ChemGen Corp, Gaithersburg, MD, USA) in combination compared to the other diets (Zamini *et al.* 2014). Specific growth rates in tilapia, *Oreochromis niloticus* showed significant improvements when fed diets containing 1 and 2% exogenous enzymes (Goda *et al.* 2012). However, some species did not yield any significant improvements in growth when supplemented with an exogenous enzyme. In a research study with Pacific white shrimp, *Litopenaeus vannamei,* results indicated no significant differences in final biomass, final weight, weight gain percentage and FCR with any addition of the supplemental carbohydrase (Rovabio Excel LC Alpharetta, GA,

USA) (Qiu and Davis 2017). White seabream, *Diplodus sargus*, fed with supplemental 0.04% *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase enzyme diets also displayed no significant differences in final body weight, weight gain and daily growth index compared to enzyme-free diets (Magalhães *et al.* 2016). Thus, the effects of carbohydrases on fish and shrimp are inconsistent.

Supplementation of carbohydrases in plant-based feeds increase energy digestibility by improving energy-yielding nutrient digestibility in starches and fats (Adeola and Bedford 2004). However, the AED coefficients and ANER showed no significant differences in energy digestibility and retention amongst the various treatments. In another study conducted by Stone *et al.* (2003), they reported that supplementing commercial α-amylase, *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase in wheat-based and lupin-based diets did not significantly improve energy digestibility in silver perch, *Bidyanus bidyanus*. Similarly, Pacific white shrimp fed carbohydrase diets with an inclusion level of 0.02% did not increase energy digestibility (Qiu and Davis 2017). On the contrary, rainbow trout, *Onchorynchus mykiss*, apparent gross energy digestibility improved when fed lupin-based diets containing hemicellulases (Farhangi and Carter 2007). Research data in different aquaculture species regarding results of the effects of energy are inconsistent. Carbohydrase supplementation did not produce any significant differences in ADDM, but the 0.045% enzyme diet had the highest dry matter out of all the diets. This diet may have had greater dry matter digestibility because there was more carbohydrase available to break down the NSPs and carbohydrates. Pacific white shrimp exhibited no significant differences in ADDM when fed supplemental enzyme diets (Qiu and Davis 2017). However, Synergen (Alltech Inc., Nicholasville, KY, USA) and Natugrain enzymes supplemented in European seabass,

Dicentrarchus labrax, diets showed that ADDM was significantly different than the basal diet (Magalhães *et al.* 2018).

Exogenous enzymes improve protein digestibility by increasing contact between the digesta and proteases, thus increasing the access to protein for digestive protease (Tahir et al. 2008). Apparent digestibility of protein significantly improved in fish fed any amount of enzyme in the experimental diets. However, there were no significant differences observed in ANPR. The lack of significance in ANPR may be due to the high variability in Florida pompano individual weights usually encountered at the conclusion of growth trials (Gilbert 1986, Weirich et al. 2009). Growth performance and ANPR of Florida pompano did not reflect the ADP results in this study. Digestibility and growth are not always in direct correlation for a variety of reasons (Zhou *et al.* 2015). On average, protein digestibility in all the enzyme treated diets was 8.42% higher compared to the basal diet, which in effect increases around 3.37% available protein to the fish. This would not be detectable in a growth trial but is a major calculable saving in feed formulations when formulated on a digestible protein basis. Additional studies have indicated significant improvements in protein digestibility with supplementation of carbohydrases in plant-based diets fed to Pacific white shrimp and rainbow trout (Dalsgaard et al. 2016, Qiu and Davis 2017). However, Magalhães et al. (2016) indicated that rainbow trout fed any combination of fishmeal and carbohydrase enzyme inclusion levels did not significantly affect their ADP. Dalsgaard *et al.* (2012) indicated significant differences were observed in rainbow trout fed ß-xylanase and ß-glucanase in soybean diets, but no differences were observed when the rainbow trout were fed the supplemental enzymes in sunflower and rapeseed meal. The variations in the outcomes of the different studies may be due to different carbohydrase enzymes, physiological differences in digestive systems (primarily pH), and the plant-based protein source.

5. Conclusion

Results indicate that the inclusion of *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase significantly improved thermal growth coefficient of Florida pompano. Apparent digestibility of protein (ADP) increased significantly once the enzyme was included in the diets compared to the basal diet. Based on the results of the study, a calculated improvement of 3.37% available protein based on digestible protein was found in Florida pompano fed any amount of the enzyme. Further research is needed to test the efficacy of different types of exogenous enzymes in plant-based diets fed to Florida pompano.

Table 1. Formulation (g/100g as is) of diets containing various levels of enzyme added to the basal diet and fed to juvenile Florida pompano for 8 weeks.

Ingredient (g/100g as is)	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Poultry by-product meal[1]	10.00	10.00	10.00	10.00
Soybean meal[2]	46.00	46.00	46.00	46.00
Corn protein concentrate[3]	12.00	12.00	12.00	12.00
Whole wheat[4]	21.22	21.22	21.22	21.22
Corn starch[4]	0.40	0.40	0.40	0.40
Menhaden fish oil[5]	5.33	5.33	5.33	5.33
Soy lecithin[6]	0.50	0.50	0.50	0.50
Mineral premix[7]	0.25	0.25	0.25	2.50
Vitamin premix[8]	0.50	0.50	0.50	0.50
Choline chloride[4]	0.20	0.20	0.20	0.20
Vitamin C[9]	0.10	0.10	0.10	0.10
Calcium monophosphate[6]	2.60	2.60	2.60	2.60
Lysine	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10
Taurine	0.50	0.50	0.50	0.50
Enzyme[10]	**0.00**	**0.015**	**0.030**	**0.045**

[1]Darling Ingredients Inc., Dallas, Texas, USA.
[2]Solvent Extracted Soybean Meal, Auburn University, Auburn, Alabama, USA.
[3]Empyreal 75 TM Cargill Corn Milling, Cargill, Inc., Blair, Nebraska, USA.
[4]MP Biochemicals Inc., Solon, Ohio, USA. For digestibility diets this was reduced by 1% and 1% Chromic Oxide was supplements (Alfa Aesar, Haverhill, Maryland, USA)
[5]Omega Protein Inc., Reedville, Virginia, USA.
[6]The Solae Company, St. Louis, Missouri, USA.
[7]ASA Premix (g/100 g premix): cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250, ferrous sulphate heptahydrate, 4.0, manganous sulphate anhydrous, 0.650; potassium iodide, 0.067;
sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193, and α cellulose 81.826.
[8]ASA Premix (g/kg Premix): thiamin HCL, 0.5; riboflavin, 8.0; pyridoxine HCl, 5.0; Ca-pantothenate,
20.0; niacin, 40.0; biotin, 0.040; folic acid, 1.80; cyanocobalamin, 0.002; vitamin A acetate (500,000 IU/g),
2.40; vitamin D3 (400,000 IU/g), 0.50; DL-α-tocopheryl acetate, 80.0; and α cellulose, 834.258.
[9]Stay C®, (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
[10]Natugrain TS-Feed Enzyme BASF Corporation, Ludwigshafen, Germany

Table 2. Mean water quality parameters for Florida pompano reared in a recirculating system over an 8-week period

Parameters	Mean ± std. dev.
Temperature (C)	28.41 ± 1.38
Dissolved oxygen (mg/L)	5.60 ± 0.61
Salinity (g/L)	24.83 ± 2.81
pH	7.45 ± 0.29
Total ammonia nitrogen (mg/L)	0.025 ± 0.056
Nitrite (mg/L)	6.83 1 ± 6.39
Nitrate (mg/L)	44.86 ± 25.87

Table 3. Growth performance of juvenile Florida pompano (mean initial weight 23.34 ± 1.49) offered practical diets with varying levels of enzyme over an 8-week growth trial. Values represent the mean of three replicates. Results in the same columns with different superscript letters are significantly different ($P \leq 0.05$) based on analysis of variance followed by the Student-Newman- Keuls multiple range test.

Treatment	Initial Mean Weight	Final Mean Weight	PWG[1]	TGC[2]	FCR[3]	Feed intake	Survival (%)	ANPR (%)	ANER (%)
0%	23.21	89.62	286.96	0.1038[ab]	1.92	127.48	100.00	21.51	25.63
0.015%	23.32	99.33	326.27	0.1135[a]	1.72	130.71	91.67	22.02	22.88
0.030%	23.47	85.12	262.98	0.0982[b]	2.01	123.33	100.00	17.16	21.13
0.045%	23.37	89.18	282.60	0.1029[ab]	1.90	124.56	96.67	20.85	24.16
P-value	0.998	0.132	0.0823	0.0499	0.0713	0.615	0.109	0.195	0.651
PSE[4]	1.052	3.805	14.762	0.0036	0.0649	4.144	2.357	1.564	2.534

[1]PWG: Percent weight gain.
[2]TGC: Thermal growth coefficient.
[3]FCR: Feed conversion ratio.
[4]PSE: Pooled standard error.

Table 4. Apparent digestibility coefficients for dry matter (ADDM), protein (ADP) and energy (ADE) of various levels of carbohydrase enzyme fed to juvenile Florida pompano for 8 weeks. Values represent the mean of three replicates. Results in the same columns with different superscript letters are significantly different ($P \leq 0.05$) based on analysis of variance followed by the Student-Newman- Keuls multiple range test.

Treatment	ADDM (%)	ADP (%)	ADE (%)
0%	50.81	72.93[b]	65.24
0.015%	49.11	80.95[a]	70.26
0.030%	48.95	82.56[a]	65.64
0.045%	52.12	80.53[a]	67.27
P-value	0.64	0.029	0.14
PSE[1]	1.97	1.89	0.90

[1]Pooled Standard Error

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CHAPTER IV

SUMMARY AND CONCLUSION

The use of plant proteins in Florida pompano, *Trachinotus carolinus* diets is becoming more common in research studies due to costs and decreasing availability of fishmeal. However, the replacement of fishmeal with plant proteins can impair amino acid profiles, productivity and digestion in diets and fish. Lysine, an essential amino acid, is found in low levels in plant-based ingredients. Plant-based ingredients contain antinutritional factors such as non-starch polysaccharides (NSPs) and carbohydrates, which impair nutrient utilization and digestibility because Florida pompano lack the enzymes to break down both. Determining the lysine requirement and supplementing exogenous enzymes in plant-based diets improves growth, feed utilization and digestibility of Florida pompano.

The first study was conducted to determine the dietary lysine requirement for Florida pompano. Practical diets were designed to contain 40% protein and 8% lipid using primarily peanut meal as its primary protein source. In the experimental growth trial, eight diets were formulated with graded levels of lysine (1.34-2.24%, dry weight). At the conclusion of the growth trial, the basal diet performed significantly worse compared to the rest of the diets in final weight, PWG, TGC and FCR. Survival was variable and diet 6 fish experienced significantly lower survival compared to diet 2 fish. Significant differences were observed in aspartic acid, glutamic acid, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine in whole body amino acid analysis of the fish, whereas there were no significant differences observed in whole body proximate composition of fish. A 4-parameter saturation kinetic regression model was fitted to model TGC to determine the dietary lysine requirement. The optimal dietary lysine requirement fed to Florida pompano was estimated to contain 1.67% of the dry diet.

The second study was carried out to evaluate growth performance, feed utilization and digestibility of Florida pompano fed plant-based diets containing carbohydrase enzymes comprised of *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase. A basal diet was designed to consist of 40% protein and 8% lipid utilizing soybean meal as its primary protein source. The basal diet was modified to produce four levels (0, 0.015, 0.030 and 0.045%) of enzymes *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase. Significant differences were observed in TGC of fish reared on the 0.015% carbohydrase diet compared to the fish reared on the 0.030% carbohydrase diet. Apparent digestibility of protein (ADP) was significantly different in all of the enzyme treated diets compared to the basal diet.

Based on the results of these growth trials, a dietary lysine requirement containing 1.67% of the dry diet is required to optimize Florida pompano growth. Supplementing *endo*-1,4-ß-xylanase and *endo*-1,4-ß-glucanase enzymes can significantly improve ADP in plant-based diets. More research is needed to test different types of exogenous enzymes supplemented in plant-based diets fed to Florida pompano.

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