2015-04-30

Maximizing Fishmeal Replacement in Commercial Diets for Florida Pompano, Trachinotus Carolinus: Digestibility, Growth and Performance of Soy Protein Products

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MAXIMIZING FISHMEAL REPLACEMENT IN COMMERCIAL DIETS FOR FLORIDA POMPANO, *TRACHINOTUS CAROLINUS*: DIGESTIBILITY, GROWTH AND PERFORMANCE OF SOY PROTEIN PRODUCTS

By

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A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

Coral Gables, Florida

May 2015

UNIVERSITY OF MIAMI

A thesis submitted in partial fulfillment of
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Maximizing Fishmeal Replacement in Commercial Diets for Florida Pompano, *Trachinotus carolinus,* Digestibility, Growth and Performance of Soy Protein Products (May 2015)

Abstract of a thesis at the University of Miami.

Thesis supervised by Professor Daniel D. Benetti.
No. of pages in text. (38)

Efforts to reduce the use of fishmeal in aquafeeds for carnivorous marine fish have been a focal point of aquaculture nutrition research in recent years. Building upon such research, this study was designed to compare the digestibility, growth and performance of three soy protein products as potential replacements for fishmeal in feeds for Florida pompano, *Trachinotus carolinus*. Two soy protein concentrates (Solae SPC™ Profine® and Selecta SPC™) and a fermented soy protein product (Hamlet protein™) were initially tested for digestibility of crude protein, crude energy and 17 amino acids in adult Florida pompano (average weight 577g) using the indirect method of fecal collection. In the second experiment, growth and performance of diets formulated with these ingredients were evaluated in juveniles (average initial weight 27.8g) and sub-adults (average initial weight of 127.7g). The two soy protein concentrates demonstrated a significantly higher apparent digestibility of energy than the fermented soy protein ($p=0.00$). Solae SPC yielded the highest apparent digestibility of crude protein ($p= 0.001$). No significant differences were found in the digestibility of important indispensable amino acids arginine and methionine. However, Selecta SPC and Solae SPC yielded the highest digestibility of the remaining essential amino acids. In the juvenile growth experiment, performance metrics of average daily growth and gross

protein intake were significantly greater in the fish fed the Solae SPC diet (*p= 0.02*). The feed conversion ratio (FCR) and fish in fish out (FIFO) ratio, commonly used in evaluating economic and environmental feed efficiency, were significantly lowest for the Solae SPC diet (*p=0.049*). In a third experiment on sub-adult pompano (127.7g average weight at stocking), a repeat of the growth experiment was conducted. Undetermined factor(s) in the manufacturing of three of the four diets led to severely reduced palatability, yielding poor performance and unreliable data through all metrics. While a comparison of the soy protein products was undermined, a commercial diet and a control diet had similar and healthy consumption rates and could be compared. While these diets were neither iso-proteic nor iso-lipidic, a comparison of their performance metrics yielded interesting results. Across almost all growth and performance metrics in sub-adult Florida pompano, the control diet (with 13.1% soybean meal), performed equally or better than the more expensive commercial diet, supporting the increasingly common notion that the diets should be both species and size-specific, as nutritional requirements appear to change throughout life. These preliminary results also corroborated the main hypothesis of this study, that a Florida pompano diet whose protein is supplemented with soybean meal is not only viable but economically and environmentally preferable to a fishmeal rich, high-protein diet.

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CHAPTER 1: INTRODUCTION

Responsible aquaculture is essential to the future of seafood production. Given the current trends in world population growth, wild fisheries and global seafood consumption, ecologically and economically efficient aquaculture seems to be the only way to meet a growing demand for fish consumption. Consider that of the 158 million tons of seafood produced worldwide in 2012, 66.6 million tons were produced by aquaculture, while 91.3 million tons were reported from wild fisheries. To illustrate the recent trends, those numbers can be compared with 49.9 million tons produced by aquaculture and 90.8 million tons by wild capture fisheries only a few years earlier in 2007, (FAO 2014). These straightforward trends have been documented and monitored by FAO and other organizations for decades. Projections into the future vary widely, but all suggest increases in aquaculture production and little to no increase in wild fish landings. Regardless of how liberal or conservative the projection, one thing is certain: more aquaculture feeds will need to be produced in the coming years than ever before. Poorly understood issues surround aquaculture feeds and beg the question of exactly how aquaculture's growth will transpire.

The first and foremost issue called to light by this looming deficiency is the need for an adequate replacement for fishmeal. Fishmeal (FM) and/or fish oil (FO) are key ingredients in aquaculture feeds for carnivorous species. A major contentious issue in aquaculture involves the capture of pelagic fishes for reduction into fishmeal and fish oil. This process transforms small, low market value fish into high value products with agricultural and industrial applications (Naylor et al. 2000). Across the globe, wild stocks of small pelagics are straining under the pressure of widespread commercial fishing activities (Hamre 2004; Boyer et al., 2001; MSG 2009; Zeller et al. 2011). This pressure is exacerbated by demand for FM and FO for inclusion into aquaculture feeds. FM is currently accepted as the best dietary protein source for aquaculture production of carnivorous fish because it is known for having high essential amino acid and fatty acid content, and low levels of carbohydrates and anti-nutritional factors (Zhou et al. 2004), as well as highly digestible protein, energy, dry matter and high availability of essential amino acids (Rawles et al. 2010). As a result, during 2006, 68.2 percent of the FM and 88.5 percent of FO produced globally was used by the aquaculture sector (Tacon and Metian 2008).

A significant obstacle presents itself if we consider the anticipated growth of aquaculture alongside the ten-year FM/FO projections made by the Food and Agriculture Organization. They project the price of FM and FO to increase by 25 to 50% (FAO 2014) by 2022, while the price of aquaculture products are expected to see a much smaller increase. Unless the inclusion rates of these ingredients in feeds decrease via protein supplementation or replacement, there is simply no way for high performance species to be raised cost effectively now or in the future.

The highest cost in any aquaculture operation generally comes from the feed, (usually 50-70%), with protein being its most expensive component (Bassompierre et al., 1997). Moreover, quality of feed is the most important factor in growth performance. With the recent interest in high market-value carnivorous species, cost concerns are increasingly relevant since carnivorous fish require greater amounts of protein and fishmeal represents the primary protein source in feeds formulations. Fortunately, recent developments in ingredient processing have resulted in an increased number of novel protein products from plants that may become price-competitive replacements for fishmeal in carnivorous fish feeds (Gaylord et al., 2010).

One such alternative to FM and FO that has gained popularity for use in aquaculture feeds is the soybean, in the form of soybean meal (SBM) and soybean oil (SBO). SBM has been deemed to be among the highest quality plant-based ingredients because it is protein-rich and contains an amino acid profile that largely meets the essential amino acid requirement of many fishes, although is it known to be deficient in methionine and histidine when compared to FM (NRC, 2011). Soybean products for inclusion in aquaculture feeds are also highly available, with large production tonnages worldwide (U.S. Department for Agriculture 2007). It has become the goal of many soybean-producing organizations to determine which characteristics of soybeans are most valuable when choosing a SBM for inclusion into aquaculture feeds and to develop soybean strains that exhibit these qualities. Favorable attributes include low anti-nutritional factors and high levels of necessary fatty and amino acids, thereby replicating fishmeal as closely as possible.

Soybeans have been introduced experimentally and commercially in diets for many aquacultured species, such as trout (i.e. Glencross et al. 2005, Satoh et al. 2002), salmon (i.e. Knudson et al. 2006), catfish, shrimp, tilapia, sea bream, cobia (i.e. Zhou et al. 2004, Suarez et al. 2013) and several species of pompano (i.e. Lech and Reigh 2012, Riche and Williams 2009, Quintero et al. 2012). Florida Pompano (*Trachinotus carolinus*) has demonstrated promise as an aquaculture species for some time and recently caught the eye of the industry, (Jory et. al 1985; Cuevas 1978; Riche 2009; Riche and Williams 2010). It can be raised in marine or low-salinity environments, tolerates low dissolved oxygen and handling stress, accepts balanced feeds and can be spawned in captivity (Riche 2009; Riche and Williams 2010). Pompano is a member of the Carangidae family, which includes many important aquacultured fishes; namely jacks and other species of pompano around the world. Commercial carangid production, like most marine finfish aquaculture, is still limited by the availability of affordable and high-quality feeds that meet the nutritional demands of carnivores. What's more, high quality feeds for high performance fish historically include high levels of fishmeal and fish oil. This tendency results in high Fish-In Fish-Out (FIFO) ratios and Feed Fish Dependency Ratios (FFDR), which limit the efficiency and capacity of marine fish aquaculture and negatively impact its public perception. The impetus for this study was to improve economic and ecological efficiency of feeds, without compromising the health, growth and quality of the animals produced. Simultaneously, such a study would undoubtedly provide more insight into the nutritional needs of Florida Pompano and assess the viability of a range of soy protein products as potential fishmeal replacements or supplements.

The evaluation of feed ingredients is crucial to nutrition research and feed development for any aquacultured species. In evaluating ingredients for use in aquaculture feeds, there are several important aspects that must be understood to enable the judicious use of a particular ingredient in feed formulation (Glencross et al. 2007). The determination of nutrient digestibility is the first step in evaluating the potential of an ingredient for use in the diet of an aquaculture species (Allan et al. 2000). The terms "digestibility" and "availability" refer to the amount or proportion of nutrients or categories of nutrients, such as crude protein or amino acids that disappear from a feed item as they pass through the digestive system and are excreted in feces (NRC 2011). Measuring the apparent digestive coefficient (ADC) of nutrients in an ingredient or feed provides a very practical estimate of nutrient availability (Smith & Talbrett 2004). An advantage to this method over testing single ingredients is that test ingredients might be more palatable to animals when supplied in combination with other ingredients and may lead to more normal levels of feed intake (Sales and Britz 2002). After experimentation, differences between aquafeed ingredients and diets may be explained by what is learned from digestibility studies regarding the amount of indigestible energy, poorly digestible ingredients in the formulation, and/or leaching.

As stated, soy products have been considered suitable and stable ingredients with adequate nutritional value for replacing fishmeal (FM). However, SBM inclusion levels are limited by species-specific digestive physiology and by the presence of both heat-resistant and thermo-labile anti-nutritional factors (ANFs). While the process of solvent-extracting and cooking may significantly reduce the biological activity of temperature-sensitive protease inhibitors, this processing also renders protein less available for absorption at the gastrointestinal level (NRC 2011, Suarez et al. 2013).

The present study was conducted to evaluate the apparent digestibility coefficients (ADCs) of protein, energy and amino acids in Brazilian soybean protein concentrate (Selecta SPC), Hamlet protein and a U.S. produced soybean protein concentrate (Solae SPC). In addition, two studies comparing FM replacement in Florida pompano diets, using juveniles (25-50g) and sub-adults (127-225g) were performed in order to optimize soy-based aquafeeds. The overarching objectives were to improve both the ecological and economic efficiencies of commercially formulated feeds for Florida pompano, *Trachinotus carolinus*.

CHAPTER 2: DIGESTIBILITY

2.1 Materials and Methods

2.1.1 Materials

Care and handling of the fish as well as procedures used in this study were reviewed and approved by the University of Miami Animal Care and Use Committee. Selecta SPC™, Hamlet protein™ and Solae SPC™ were purchased from regional distributors.

Selecta SPC™ is a soy protein concentrate produced by a Brazilian company (Selecta). It is intended for incorporation in various animal feeds.

Hamlet protein™ is produced by a Danish company (Hamlet Protein), and goes through a 'bioconversion' or fermentation process that reduces the number of anti-nutritional factors to safe levels for various farmed animals.

Solae SPC™ (Profine®) is a soy protein concentrate made by The Solae Company (of Dupont/Danisco) for inclusion in animal feeds. The company makes soy-based products for human consumption, but also has two products processed for use in animal feeds.

The nutrient compositions of the test ingredients are shown in Table 1.

Table 1. Nutrient composition (%) of test ingredients (as feed)

Proximate components	[1]Hamlet protein	[2]SPC Selecta	[3]SPC Solae
Dry Matter	92.6	95.1	90.7
Crude Protein	53.8	63.6	61.9
Crude Lipid	3.4	2.6	1.5
Ash	5.6	4.9	5.1
Amino acid			
Alanine	2.4	3.2	2.9
Arginine	3.7	5.2	5.0
Aspartate + Asparagine	3.9	5.3	4.8
Cystine	0.2	0.3	0.3
Glutamate + Glutamine	6.0	8.2	7.6
Glycine	2.5	3.4	3.2
Histidine	1.4	1.8	1.8
Isoleucine	2.5	3.4	3.1
Leucine	4.2	5.9	5.4
Lysine	3.1	4.1	3.7
Methionine	0.6	0.7	0.7
Phenylalanine	2.9	4.1	4.0
Proline	2.9	3.9	3.8
Serine	2.5	3.4	3.4
Taurine	0.0	0.0	0.0
Threonine	2.2	2.9	2.7
Tryptophan	n.d.	n.d.	n.d.
Tyrosine	1.8	2.5	2.5
Valine	2.7	3.6	3.3

[1] Hamlet Protein Inc. OH-USA
[2] Soybean Protein Concentrate (Selecta, Brazil)
[3] Soybean Protein Concentrate (The Solae Company, St. Louis, MO)

2.1.2 Experimental Digestibility Diets

Diets were made at the UMEH Nutrition Laboratory. All dry ingredients were milled to one micron and mixed for ten minutes in a bowl mixer, (Hobart, Troy, OH. USA). Menhaden oil was mixed in for an additional ten minutes before a mixture of carboxylmethyl cellulose sodium and hot water was added to create an appropriate consistency for pelletizing. Then the mix was pushed through a 6mm stainless steel plate and dried in a convection oven under low heat (60°C) for approximately 24 hours. Each batch was tested for humidity to ensure moisture levels around 10% (± 2%), vacuum-sealed in plastic bags, and stored in an air-conditioned room until it was fed.

Digestibility diets (Table 2) were comprised of a 70:30 mixture of the reference diet and the test ingredients. Yttrium oxide (Y_2O_3) was included at 0.5% of the reference diet as an inert, indigestible marker.

Table 2. Reference and test diets for determination of apparent digestibility coefficients in Florida pompano.

Ingredient (g/kg of diet)	Reference diet (g/kg of diet)		Test diet (g/kg of diet)
Fishmeal	620.0		700.0
Dextrin	120.0		
Menhaden Oil[a]	120.0		
Mineral & Vitamin Premix[b]	60.0		
Carboxylmethyl cellulose[c]	15.0		
Cellufil[d]	65.0		
Yttrium oxide[h]		5.0	
Test Ingredient	---		300.0
Total	1,000		1,000

[a] Menhaden Fish Oil Omega Protein, (g/kg) 617 crude protein, 900.7 dry matter, 120.6 crude lipid.

[b] Composition (g/kg): $Ca(H_2PO_4)_2 \cdot H_2O$, 136.00; $Ca(C_6H_{10}O_6) \cdot 5H_2O$, 348.553; $FeSO_4 \cdot 7H_2O$, 5.00; $MgSO_4 \cdot 7H_2O$, 132.00; K_2HPO_4, 240.00; $NaH_2PO_4 \cdot H_2O$, 88.00; NaCl, 45.00; $AlCl_3 \cdot 6H_2O$, 0.084; KI, 0.15; $CuSO_4 \cdot 5H_2O$, 0.50; $MnSO_4 \cdot H_2O$, 0.70; $CoCl_2 \cdot 6H_2O$, 1.00; $ZnSO_4 \cdot 7H_2O$, 3.00; $NaSeO_3$, 0.0127 Ascorbic acid, 50; DL-calcium pantothenate, 5.0; choline chloride, 36.2; inositol, 5.0; menadione sodium bisulfite, 2.0; niacin, 5.0; pyridoxine HCl, 1.0; riboflavin, 3.0; thiamine mononitrate, 0.5; DL-α-tocopherol acetate (250 IU/g), 8.0; vitamin A palmitate (500,000 IU/g), 0.2; micro-mix, 10.0; cellulose, 874.1. Micro-mix composition (g/100g): Biotin, 0.50; folic acid, 1.8; vitamin B12, 0.02; cholecalciferol (40 IU/µg), 0.02; cellulose, 97.66.

[c and d] USB-Affymetrix, Cleveland, OH.

[e] Sigma-Aldrich Company, St. Louis, MO.

2.1.3 Experimental Design

The Florida pompano used in this experiment were spawned and reared at the University of Miami Experimental Hatchery (UMEH). Ten fish (average weight of 577g) were randomly distributed into a flow-through system consisting of twelve 1,000-liter cylindrical polyethylene tanks, each tank containing one oxygen stone, at a stocking rate of 6.6 kg/m^3. Each treatment had three replicates, and three treatments are reported for comparisons of digestibility. Tanks were fed once per day to apparent satiation, which equaled about 2-3% of the total biomass of the pompano. Prior to the onset of the fecal collection period, the fish were fed their respective diets for a week as an acclimatization period (Glencross 2007). Feces were collected by manual stripping, which has been deemed the most practical collection method for digestibility studies (Rawles et al. 2010; Glencross et al. 2005; Vandenberg and de la Noue 2001). The fish were fed four hours prior to fecal collection (the optimal amount of time as determined by a preliminary fecal collection trial at UMEH). Collection was performed on all ten fish from each tank. Fish were dip-netted from the tank and a solution of MS-222 (500 mg/L; Finiquel, Argent Chemical, Redmond WA) was sprayed onto the gill cavity and the opercula held closed until complete relaxation of abdominal muscles was attained. Immediately afterward, the gills were rinsed with a gentle stream of seawater for 30 seconds, flushing residual anesthetic. To extract feces, gentle pressure was applied down the lower abdomen of the fish with the thumb, pushing feces from its distal intestinal tract. Care was taken to exclude urine, mucus, and other contaminants from fecal samples. After fecal collection, fish were introduced into a large polyethylene tank with oxygenated seawater until complete recovery. Fecal samples were lyophilized, or freeze-dried, for 24 hours and sent to the Fish

Nutrition Laboratory of Texas A&M University for analyses. Yttrium oxide analyses were conducted in duplicate by inductivity coupled plasma mass spectrometry as performed in Suarez et al. 2013. Yttrium oxide was analyzed by inductivity coupled plasma mass spectrometry (ICP- AES analysis, Perkin Elmer Optia 3000DV; Perkin Elmer, Wellesley, MA, USA) at 371 nm. All yttrium analyses were conducted in duplicate. Proximate composition of diets and fecal samples were analyzed using established methodologies for dry matter, crude protein (AOAC, 2000), lipids (Folch et al. 1957) and ash (AOAC 2000). Crude protein was estimated by measuring total nitrogen via the Dumas method (Ebling, 1968) and multiplied by 6.25. Dry matter was determined by heating the samples at 125 °C for 3 h, and ash was quantified after heating at 650 °C for 3 h (AOAC, 1990). Crude lipid was determined by chloroform and methanol extraction (Folch et al. 1957). Gross energy content was measured by combustion via bomb calorimeter (Parr Instrument Company, Moline, IL, USA) using benzoic acid as a standard. Amino acid content was quantified after acid hydrolysis with 6N HCl according to procedures described by Pohlenz et al. 2012. Calcium and phosphorus were quantified by Inductively Coupled Plasma Spectroscopy. Plasma amino acids were assayed via HPLC following a fluorometric technique (Buentello and Gatlin 2000) using pre-column derivatization with o-phthaldialdehyde (Sigma, St. Louis, MO).

2.1.4 Statistical Methods

All statistical analyses were performed using Statgraphics Centurion version 16.1.11. Data from each treatment were tested for homogeneity of variance using Levene’s test and then subjected to a one-way analysis of variance (ANOVA) if the variances were indeed homogenous. Duncan's multiple range tests were applied, first to determine whether significant differences existed among the dietary treatments and second, to identify where they occurred. Results were considered statistically significant at $p < 0.05$.

Apparent digestibility coefficients (ADCs) for energy, protein and amino acids were calculated using three fecal samples from each treatment according to the formulas set forth by NRC (2011). The formulas for calculating ADC of test ingredients are below.

$$ADC_{Nutrient} = 1 - \frac{Y_2O_3\ in\ feed}{Y_2O_3\ in\ feces}\ x\ \frac{Nutrient\ content\ of\ feces}{Nutrient\ content\ of\ feed}$$

$$ADC_{test\ ingredient} = ADC_{test\ diet} + \left(ADC_{test\ diet} - ADC_{ref\ diet}\right)\ x\ \left(\frac{0.7\ D_{ref}}{0.3\ D_{ingredient}}\right)$$

where ‘ref’ is reference, ‘D_{ref}’ is the percentage of nutrient or kcal/g of gross energy of the reference diet and ‘$D_{ingredient}$’ is the percentage of nutrient or kcal/g of gross energy of the ingredient.

2.2 Digestibility Results

Both sets of diets formulated and processed at UMEH were readily consumed and apparently digested by adult pompano, suggesting that palatability and texture were consistent and acceptable.

Throughout the week-long experimental period, average water temperature values were 25.1 ± .1°C, salinity was 33.1 ± .05 parts per thousand (p.p.t.), and dissolved oxygen (D.O.) was maintained above saturation at an average of 7.24 mg/L. The ADC values for energy, protein and amino acids in adult pompano were determined for the experimental ingredients as described in Methods section 2.1.3 and are presented in Table 3. Amino acids are separated into indispensable and dispensable amino acids.

Table 3. Apparent digestibility coefficient (ADC) of dietary protein, energy and amino acids of the evaluated vegetable feed ingredients for adult Florida pompano.

Ingredient	Selecta	Hamlet	Solae	*p-value*	*P.S.E.*
Crude protein ADC	74.0^{c}	76.0^{b}	77.6^{a}	0.0014	1.63
Energy ADC	68.2^{a}	58.5^{b}	68.6^{a}	0.0000	5.00
Indispensable AA					
Arginine	85.9	85.6	86.2	0.757	0.84
Histidine	84.2^{a}	81.1^{b}	80.7^{b}	0.0080	1.91
Isoleucine	82.3^{a}	77.9^{c}	80.1^{b}	0.0036	2.11
Leucine	87.2^{a}	83.9^{b}	86.9^{a}	0.0050	1.71
Lysine	84.2^{a}	75.6^{b}	83.0^{a}	0.0053	2.27
Methionine	87.6	89.6	87.2	0.0959	1.50
Phenylalanine	85.0^{a}	82.2^{b}	85.7^{a}	0.0044	1.75
Threonine	78.0^{a}	73.8^{b}	72.2^{b}	0.0178	3.02
Valine	81.5^{a}	76.9^{b}	79.1^{ab}	0.0124	2.27
Dispensable AA					
Alanine	85.6^{a}	82.3^{b}	83.1^{b}	0.0121	1.68
Aspertate[1]	71.1^{a}	66.3^{ab}	61.9^{b}	0.0101	4.55
Cysteine	76.0	68.5	70.2	0.0705	4.43
Glutamate[2]	83.6	82.5	80.8	0.0901	1.70
Glycine	71.9^{a}	66.0^{b}	60.0^{c}	0.0022	5.56
Proline	82.9^{a}	79.8^{b}	76.5^{b}	0.0154	2.27
Serine	80.1	77.1	76.8	0.0633	2.00
Tyrosine	86.5	84.1	85.5	0.0527	1.31

[*]Values in a column that do not have the same superscript letters are significantly different according to Duncan's multiple range test ($P < 0.05$).
[1] Aspartic acid and asparagine
[2] Glutamic acid and glutamine

Solae SPC™ showed the highest apparent digestibility of crude protein at 77.6 and Selecta SPC™ yielded the lowest at 74.0. Interestingly, both SPCs showed the highest digestibility of energy, both above 68, as Hamlet Protein™ yielded an ADC significantly lower at 58.5.

Among Selecta SPC™, Hamlet Protein™ and Solae SPC™ Profine®**,** there were statistically significant differences for several indispensable and dispensable amino acids. Two very important indispensable amino acids for marine fish: arginine and methionine, showed no significant differences in digestibility among the three ingredients. For histidine and threonine, Selecta SPC™ was the most digestible ingredient. Both SPCs were significantly higher than Hamlet Protein™ for the remaining indispensable amino acid ADCs, which include isoleucine, leucine, lysine, phenylalanine and valine.

In comparative analysis of the eight dispensable amino acids measured, exactly half were found to have ADCs that were statistically significant. Selecta SPC™ was the most digestible of the three soy protein ingredients in instances wherever statistically significant differences were found.

2.3 Digestibility Discussion

The experiment demonstrated several significant differences in the apparent ability of adult pompano to digest soy proteins. It is unsurprising that Solae SPC was the most digestible of the three soy ingredients tested, as this company also produces a handful of human food-grade products. Solae SPC is made with less processed, high-protein soy before being cooked and ultimately processed, while Selecta SPC is made via cooking a pre-toasted soy that is higher in plant carbohydrates. These are known to be more difficult for fish to digest and ultimately utilize. Hamlet Protein appeared to be fairly digestible in this study, but needs to be evaluated further for growth performance, as it contains less crude protein (53.8g/100g) than its counterparts Selecta (63.6g/100g) and Solae (61.9g/100g)(Figure 1). Its manufacturing process is less well known, so an explanation of its performance is more speculative.

Overall, the indirect method of nutrient collection used in this study yielded ADC's that are slightly lower than studies of other marine species (Riche and Williams 2010; Santinha et al. 1999; Zhou et al 2004). In addition to the sources of error regularly presented by the indirect method of manual stripping (contamination of fecal samples by impurities such as urine, mucus and undigested food), Florida pompano present another challenge with their compact body shape, short digestive tract and short transit time (Williams et al., 1985). The smaller, rounder body cavity makes extracting fecal matter from only the end of the digestive tract more difficult and less precise. One has less ability to exert pressure exclusively on the intestine if it is coiled among all of the other internal organs. Elongated fishes provide a simpler subject for fecal extraction via manual stripping. Additionally, a short time of digestion combined with the voracious eating habits of pompano to eat until they appear bloated may lead to a decrease in digestion as this may limit absorption of nutrients (Williams et al. 1985; Lazo et al. 1998; Weirich et al. 2006; Riche 2009, Taynor 2012). Despite its challenges, this study provides a valuable relative comparison of some readily available and novel fishmeal replacement candidates, and demonstrated ADC values in the range of what has been published for this species in full salinity studies in the past, (Riche and Williams 2010).

CHAPTER 3: GROWTH AND PERFORMANCE

3.1 Materials and Methods

3.1.1 Materials

As in the digestibility study, care and handling of the fish as well as procedures used in this study were reviewed and approved by the University of Miami Animal Care and Use Committee. Selecta SPC™, Hamlet protein™ and Solae SPC™ were purchased from various regional distributors in the Americas and sent to their respective manufacture sites as explained below. Otohime was purchased through a domestic distributor, Reed Mariculture.

3.1.2 Making- Juvenile Growout Diets

A balanced FM-based control diet (diet 1) – similar to commercial Florida pompano diets was formulated to contain 41% crude protein from menhaden FM (Omega Protein, Houston, TX) and commodity-grade soybean meal (Archer Daniels Midland, Decatur, IL) (Table 3). Menhaden oil (Omega Protein) was included as a lipid source and provided in combination with FM for a total of 13% lipid and an estimated gross energy level of 20 kJ/g. Three experimental diets were derived from the control diet by replacing a portion of the FM protein with an increased level of Selecta SPC™ (diet 2), Hamlet protein™ (diet 3) and Solae SPC™ (diet 4) while maintaining the iso-nitrogenous, iso-lipidic and iso-energetic integrity of the diets. As plant protein sources increased in the diet, an increased supplementation of dietary taurine was needed. When necessary, crystalline L-methionine was used to balance dietary levels of sulfur-containing amino acids. Diets met the currently established nutritional requirements for juvenile Florida pompano, as suggested by several other studies (Lazo et al. 1998, Richie 2009, Rossi and Davis 2012).

Table 4. Formulation of the experimental diets (g/100 g as feed) fed to juvenile Florida pompano, Trachinotus carolinus.

Ingredient	Experimental diets			
	D1 Control diet	D2 Selecta SPC	D3 Hamlet protein	D4 Solae SPC
Menhaden meal [a]	30.0	15.0	15.0	15.0
Soybean meal[b]	35.0	35.0	35.0	35.0
SPC (Brazilian Selecta)[c]		24.0		
Hamlet protein[d]			24.0	
SPC (Solae)[e]				24.0
Corn gluten	10.0	1.0	4.5	1.5
Menhaden oil	6.6	8.0	8.0	8.0
Lecithin (Soy refined)	1.0	1.0	1.0	1.0
Mineral-vitamin premix[f]	6.0	6.0	6.0	6.0
Choline chloride	0.5	0.5	0.5	0.5
Taurine[g]	0.47	0.58	0.58	0.58
DL-Methionine[g]		0.34	0.29	0.33
Ca P-dibasic	0.5	1.0	1.0	1.0
Dextrin	9.0	6.50	4.13	6.91
Cellufil	0.93	1.08		0.18
Theoretical analysis g/100g (as feed)				
Crude protein (g/100g)	41	41	41	41
Crude lipid (g/100g)	13	13	13	13
Energy (kJ/g)	20	20	20	20

[a] Omega Protein Corp. (Hammond, LA, USA), food-grade. 617.0 crude protein, 907.0 dry matter, 126.0 crude lipid, 187.0 ash

[b] Archer Daniels Midland, Decatur, IL, 480.0 crude protein, 933.8 dry matter, 18.4 crude lipid, 38.0 crude fiber, 61.9 ash, commodity roasted/cooked and hexane extracted, genetically modified soy.

[c] Brazilian Selecta, 636.0 crude protein, 951.0 dry matter, 20.6 crude lipid, 49.0 ash.

[d] Hamlet Protein, 538.0 crude protein, 926.0 dry matter, 34.0 crude lipid, 56.0 ash.

[e] Solae Profine VP, 619.0 crude protein, 907.0 dry matter, 15.0 crude lipid, 51.0 ash.

[f] Mineral Premix composition (g/kg): Ca(H2PO4)2 ·H2O, 136.00; Ca(C6H10O6) ·5H2O, 348.553; FeSO4 ·7H2O, 5.00; MgSO4 ·7H2O, 132.00; K2HPO4, 240.00; NaH2PO4 ·H2O, 88.00; NaCl, 45.00; AlCl3 ·6H2O, 0.084; KI, 0.15; CuSO4 ·5H2O, 0.50; MnSO4 ·H2O, 0.70; CoCl2 ·6H2O, 1.00; ZnSO4 ·7H2O, 3.00; NaSeO3, 0.0127. Vitamin Premix composition (g/kg): Ascorbic acid, 50; dl-calcium pantothenate, 5.0; Choline chloride, 36.2; Inositol, 5.0; Menadione sodium bisulfite, 2.0; Niacin, 5.0; Pyridoxine HCl, 1.0; Riboflavin, 3.0; Thiamine mononitrate, 0.5; dl-alpha-tocopherol acetate (250 IU/g), 8.0; Vitamin A palmitate (500,000 IU/g), 0.2; Vitamin micro-mixc, 10.0; Cellulose, 874.1 Vitamin Micro-mix composition (g/100g): Biotin, 0.50; Folic acid, 1.8; Vitamin B12, 0.02; Cholecalciferol (40 IU/ug), 0.02; Cellulose, 97.66

[g] USB-Affymetrix, Cleveland, OH.

3.1.3 Making Sub Adult Growout Diets

Four diets were formulated using the optimal protein (48%) and lipid (17%) levels suggested from a previous study performed at the University of Miami Experimental Fish

Hatchery, (Taynor 2013). It has been suggested that digestible protein/digestible energy ratios decrease as fish grow larger (Einen and Roem 1997, Lupatsch et al. 2001b, NRC 2011), so a diet formulated for the next size class might include a lower percentage of crude protein. Formulations were sent to a commercial aquafeeds manufacturer in Peru named Nicovita. Ingredients were shipped to the manufacturer over the course of a few months, gathered and then manufactured according to the proprietary methods used at Nicovita. Table 5 represents Nicovita's final formulations based on the guidelines we set forth and the manner in which that their nutritionists create their commercial diets. In addition to the control diet and three test diets, we also purchased and fed Otohime, a very high quality commercial growout feed of the same size (6mm). Samples of all diets were sent to Eurofins Nutrition Analysis Center in DeMoines, Iowa for proximate analysis.

Table 5. Formulation and subsequent analysis of the experimental diets (g/100 g as feed) fed to sub-adult Florida pompano. Sources of all ingredients not made available by manufacturer.

Ingredient	Experimental diets				
	D1 Control diet	D2 Selecta SPC	D3 Hamlet protein	D4 Solae SPC	D5 Commercial Otohime*
Fish meal	40.0	11.9	12	12	> 45
Squid/Krill meal					> 40
Soybean meal	13.1	15.0	9.2	14.8	
SPC (Brazilian Selecta)		28.0			
Hamlet protein			28.0		
SPC (Solae)				28.0	
Corn gluten	13.4	5.1	15	13.6	
Wheat gluten	4.1				< 5
Starch	7.8	2.5	2.5	2.5	< 5
Poultry Meal	5	4.9	7	7	
Menhaden oil	10.7	13.2	12.6	13.2	
Lecithin (Soy refined)	2.0	0.8	0	0	< 5
Vitamin premix	0.5	0.5	0.5	0.5	< 2.5
Mineral premix	0.44	1.38	1.18	1.18	< 2.5
Choline (60%)	0.23	0.1	0.1	0.1	
Oenophosphate	1.25	4.4	4.5	4.3	< 5

Antimicotic&antioxidant	0.2	0.2	0.2	0.2	
Taurine	1.66	1.92	1.91	1.9	
Vitamin C	0.15	0.15	0.15	0.15	
DL-Methionine		0.03	0.04	0.06	
Proximate analysis g/100g (as feed)					
Crude protein (g/100g)	48.8	48.5	48.9	48.8	57.7
Crude lipid (g/100g)	17.5	16.7	17.6	17.3	13.8
Energy (kJ/g)	20.7	20.3	20.5	20.5	20.3

* Otohime formulation is very approximate based on proximate analysis of their S2 diet, proximate analysis of all diets is exact.

3.1.4 Experimental Setup

The juvenile portion of this study was performed in the same system and with only a few differences from the digestibility study. 35 juvenile pompano with an average weight of 27.8 ± 8g were stocked at the equivalent of just over 1 kg/m^3. The justification for this number was that they would grow into a 5 kg/m^3 stocking density by the end of the study period. Fish were fed twice per day for ten minutes or until apparent satiation, unless water quality appeared compromised. The experiment lasted 34 days total. Each treatment employed three replicates.

The sub-adult study was performed one year later with similarly raised fish. Each tank was stocked with a dozen fish with an average weight of 127.8 ± 15g, yielding an average initial stocking density of 1.7 kg/m^3. As in the juvenile study, fish were fed twice per day unless water quality appeared to be visually compromised. This experiment also lasted 34 days.

For both studies, the following variables were determined for fish growth and diet performance:

ADG = average daily gain; SRG = specific growth rate; MDI = mean daily intake; GEI = gross energy intake; GPI = gross protein intake; FE = feed efficiency; PER = protein efficiency ratio; FCR = feed conversion ratio; FIFO = fish in: fish out; VSI= visceral somatic index; HSI= hepato-somatic index. Calculations are found below.

a. ADG (g/d)

b. SGR = 100 (*ln* average final weight- *ln* average initial weight)/numbers of days

c. MDI = (g/fish/day)

d. GEI = (kcal/fish/day)

e. GPI = (g/fish/day)

f. FE = (weigh final-weight initial)/total feed intake

g. PER = weight gain in g/ protein intake

h. FCR = feed intake/ wet weight gain

i. FIFO ratio = (level of fishmeal in the diet + level of fish oil in the diet) / (yield of fishmeal from wild fish + yield of fish oil from wild fish) * FCR

Yield of fishmeal from wild fish = 22.5 (Jackson, 2010).

Yield of fish oil from wild fish = 5.0 (Jackson, 2010).

j. VSI= visceral weight * 100/body weight (AOAC, 1994)

k. HSI= liver weight * 100/body weight (AOAC, 1994)

3.2 Results

3.2.1 Juvenile Growout Results

Water quality parameters were sufficiently suitable for juvenile Florida pompano survival and growth (Weirich and Richie 2006). The experiment spanned August and September 2013 when water temperature (25.2 ± .2°C) and salinity (35.8 ± .1 p.p.t.), were slightly higher than the digestibility experiment. Well water pH remained the same at 7.25 ± .03. During the 34-day trial, no mortality occurred. No significant differences were found in mean daily intake (MDI), suggesting that there were no major issues with feed palatability. Eleven performance metrics are presented for each diet in Table 7. For each analysis, a p-value <0.05 indicates statistical significance.

The control diet and Solae SPC diets performed statistically higher than Hamlet and Selecta in final weight and average daily growth (ADG). Fish consumption resulted in a significantly lower gross protein intake for Hamlet protein than the three other diets, at 0.48 grams/day. The effectiveness of the control diet is not surprising given that it contained double the amount of fishmeal as the test diets.

Feed conversion metrics FCR (Feed Conversion Ratio) and FIFO (Fish in- Fish out) showed significant differences in this study. Selecta SPC had the highest, or worst FCR at 2.69 while Solae SPC was significantly lower at 2.16. The control diet was also significantly lower than Selecta SPC with a 2.18 FCR. Hamlet soy fell in between but was not significant different from any other diets. Finally, Solae SPC stood alone when viewed in terms of its FIFO, with a score of 1.80.

The liver and viscera of three randomly selected fish from each replicate were removed and weighed in relation to the whole fish weight in order to calculate standard post-mortem biological indices hepato-somatic index (HSI) and visceralsomatic index (VSI). In this case, both indices indicated no significant differences among the four treatments (Table 6). This analysis suggests that the diets did not physiologically alter the distribution of mass within the viscera of the fish and that growth was normal and balanced. It should be noted that the experiment was ended after 34 days when a bacterial infection occurred in several tanks.

*Table 6. Post-mortem biological indices of juvenile pompano at the end of the growth trial**

Dietary treatment	VSI[1]	HSI[2]
Control	5.30	1.72
Selecta	5.65	1.79
Hamlet	5.51	1.75
Solae	5.03	1.62
p-value	0.33	0.76
P.S.E	0.71	0.31

[1]VSI: Visceral-somatic index = Viscera weight * 100/body weight
[2]HSI: Hepato-somatic index = Liver weight * 100/body weight

Table 7. Performance indicators for juvenile Florida Pompano fed experimental diets

Dietary treatment	Final weight	WG[2]	ADG[3]	SGR[4]	MDI[5]	GEI[6]	GPI[7]	FE[8]	PER[9]	FCR[10]	FIFO[11]
	g	*%*	*g/d*	*%/d*	*g/d*	*kcal/d*	*g/d*	*g gain/g fed*	*%*	*g fed/g gain*	
Sample period 1 – 34 d[1]											
D1. Control	46.4^{a}	64.6	0.54^{a}	1.47	1.17	23.2	0.53^{a}	0.46	1.02	2.18^{a}	2.90^{c}
D2. Selecta	42.1^{b}	53.6	0.43^{b}	1.26	1.15	23.0	0.52^{a}	0.38	0.83	2.69^{b}	2.24^{b}
D3. Hamlet	41.6^{b}	57.6	0.45^{b}	1.34	1.09	21.8	0.48^{b}	0.41	0.93	2.45^{ab}	2.05^{ab}
D4. Solae	46.0^{a}	65.3	0.53^{a}	1.48	1.15	23.0	0.52^{a}	0.47	1.04	2.16^{a}	1.80^{a}
p-value	0.015	0.065	0.018	0.063	0.053	0.051	0.020	0.061	0.056	0.049	0.001
P.S.E.	2.72	6.75	0.06	0.124	0.039	0.80	0.021	0.051	0.113	0.29	0.456

[*]Values in a column that do not have the same superscript letters are significantly different according to Duncan's multiple range test ($P < 0.05$).
[1]Initial weight: 27.8 ± 8.8 g.
[2]Weight gain = ((final weight – initial weight) x 100)/ initial weight
[3]ADG = Average daily gain
[4]SGR = 100 x (ln average final weight - ln average initial weight)/numbers of days
[5]MDI = Mean daily intake
[6]GEI = Gross energy intake
[7]GPI = Gross protein intake
[8]FE = Feed efficiency
[9]PER = Protein efficiency ratio
[10]FCR = Feed conversion ratio
[11]FIFO = Fish in: fish out ratio = (g fishmeal in the diet + g fish oil in the diet) / (g fishmeal from wild fish + g fish oil from wild fish) x FCR

3.2.2 Sub Adult Growout Results

This trial was performed in the same system in August and September of 2014 and also spanned 34 days. Water temperature (25.5± .3°C) and salinity (34.9± .1 p.p.t.) were fairly consistent with previous trials, and well within the range of conditions that Florida pompano experience in the wild. Low pH (7.32 ± .1) persisted throughout this trial, as it did in the previous. Some unidentified manufacturing problems, presumably affecting palatability, undermined several of the test diets and made analysis difficult to reliably trust. Throughout the trial, fish did not appear to eat the Hamlet, Solae and Selecta diets as voraciously as the others. This is reflected in lower MDI's of Hamlet and Solae in the following table. (Selecta was withheld from the table due to its extreme outlying nature. Fish in this treatment did not eat enough to be included in a fair analysis of the ingredient, as subsequent growth was negligible). The table is being presented in this thesis to demonstrate due diligence, but unfortunately cannot be responsibly evaluated for statistical significance.

Notably, between the two diets that had statistically similar MDI's, the control diet containing 13.1% regular SBM performed similarly to the commercial diet (Otohime) across most metrics, which contains no soy. As a result of its highly animal based makeup, the estimated FIFO ratio for the commercial diet was over 5, while the control diet was calculated to be 2.77.

The visceralsomatic index (VSI) indicated no significant differences among the five treatments for this study (Table 8). Interestingly, the hepato-somatic index (HSI) or ratio of liver weight to body weight was significantly elevated in the control treatment, at 1.58.

Table 8. Post-mortem biological indices of sub-adult pompano at the end of the growth trial

Dietary treatment	VSI[1]	HSI[2]
Control	5.11	1.58*
Commercial	4.40	1.07
Selecta	5.45	1.21
Hamlet	5.55	0.97
Solae	5.02	0.86
p-value	0.06	0.00
F-statistic	8.12	3.18

[1]VSI: Visceral-somatic index = Viscera weight * 100/body weight
[2]HSI: Hepato-somatic index = Liver weight * 100/body weight
* Indicates significant difference within column

Table 9. Performance indicators for sub-adult Florida pompano fed experimental diets

Dietary treatment	Final weight	WG[2]	ADG[3]	SGR[4]	MDI[5]	GEI[6]	GPI[7]	FE[8]	PER[9]	FCR[10]	FIFO[11]
	g	*%*	*g/d*	*%/d*	*g/d*	*kcal/ d*	*g/d*	*g gain/g fed*	*%*	*g fed/g gain*	
Sample period 1 – 34 d[1]											
D1. Control	97.7	78.5	2.87^{a}	1.70^{a}	4.66^{a}	96.7	2.27^{ab}	0.62^{a}	1.27	1.64	2.77^{b}
D2. Commercial	94.1	74.2	2.77^{a}	1.63^{a}	4.63^{a}	96.5	2.67^{a}	0.60^{a}	1.03	1.68	5.36^{a}
D3. Hamlet	50.0	39.1	1.47^{b}	0.97^{b}	2.62^{b}	56.7	1.35^{c}	0.53^{b}	1.12	1.83	1.33^{c}
D4. Solae	65.7	50.3	1.93^{b}	1.19^{b}	3.53^{b}	76.3	1.81^{bc}	0.52^{b}	1.12	1.87	1.36^{c}
p-value	0.006	0.004	0.006	0.003	0.003	0.004	0.002	0.218	0.256	0.50	0.000
F-statistic	8.99	10.7	8.95	11.14	11.8	10.11	13.53	1.84	1.64	0.86	0.76

[*]Values in a column that do not have the same superscript letters are significantly different according to Duncan's multiple range test ($P < 0.05$).
[1]Initial weight: 127.8 ± 15g.
[2]Weight gain = ((final weight – initial weight) x 100)/ initial weight
[3]ADG = Average daily gain
[4]SGR = 100 x (ln average final weight - ln average initial weight)/numbers of days
[5]MDI = Mean daily intake
[6]GEI = Gross energy intake
[7]GPI = Gross protein intake
[8]FE = Feed efficiency
[9]PER = Protein efficiency ratio
[10]FCR = Feed conversion ratio
[11]FIFO = Fish in: fish out ratio = (g fishmeal in the diet + g fish oil in the diet) / (g fishmeal from wild fish + g fish oil from wild fish) x FCR

3.3 Discussion

3.3.1 Juvenile Growout Discussion

Examinations of food conversion ratios (FCR) and fish-in, fish-out (FIFO) ratios for these experimental diets highlight the most intriguing results in this study, both because significant differences were found and because these parameters will most directly affect the bottom line of a production-minded business. The control diet was designed to represent an average commercial diet using a conservative amount of commodity soy, and other test diets were intended to compare other processed soy products in addition to 35% regular soybean meal. Despite its adequate and expected performance in other parameters, the control diet was significantly worst in terms of its use of wild fish to grow fish, as indicated by a 2.90 FIFO ratio. Hamlet Protein yielded a 2.05 while Selecta SPC produced a FIFO of 2.24. Solae SPC, at 1.80 was superior in this category. In an ideal commercial setting, a balance between a low FCR and low FIFO ratio must be struck. This is because FCR speaks directly to economic viability; while a low FIFO generally only means that the environmental impact on forage fish has been minimized.

3.3.2 Sub-Adult Growout Discussion

After execution of both the digestibility and juvenile growout portions of the study, we hired an industry professional to commercially produce test diets within basic parameters that we set forth. While it is difficult to know exactly what happened in the process, several diets exhibited poor palatability upon feeding. Future research should exhibit more control over the acquisition and storage of ingredients, as well as a more transparent understanding of the exact manufacturing process. It is known that heat extrusion can alter the digestibility and performance of soy ingredients (Knudson et al. 2006, Gaylord et al. 2010), but the exact temperature of extrusion was not known for those conducting the study. For experimental purposes, we need to exercise more control and correct the factor(s) that cause these diets to be so unattractive to the fish. The diets with significantly low mean daily intakes (MDIs) included Hamlet, Selecta SPC and to a lesser degree, Solae SPC.

While the poor performance of three diets precluded further analysis, an interesting takeaway remains from this portion of the study. When comparing the control diet and the commercial diet (Otohime), some interesting points arise. Metrics regarding growth and intake (FW, WG, ADG, SGR, MDI, GEI, GPI) showed little to no difference between the two diets when a simple one-way ANOVA was run between only those two treatments. In metrics that measure efficiency (such as FE, PER, FCR, FIFO), the control diet actually outperformed the well-respected commercial diet, namely in protein efficiency ratio (PER) and Fish-In Fish-Out ratio (FIFO). Because it uses high levels of animal protein and has a higher crude protein inclusion (57% to 48%), Otohime's PER was lower than the control diet which contained 13.1% SBM. Due to the amount of fish, squid and krill meal used, Otohime is clearly not a viable growout option in terms of environmental impact for the future of feeds. Otohime is formulated to be one of the most effective larval weaning and early juvenile feeds on the market. To attain and keep such a reputation, its smaller diameter feeds are not formulated so much for economic efficiency but primarily for biological performance. When feeding small quantities to small fish, performance is often favored over cost effectiveness in the industry and especially the research setting. Experiments such as this would help to strike a balance between the two measures, not only to help reduce the impact on the environment, but also to produce more cost effective diets without sacrificing performance. According to this data and for both of these reasons, soybean meal (SBM) could, and should be included at some level to supplement traditional animal meals at least in sub-adult Florida pompano (~125-225g).

The significantly higher VSI of the control diet indicates a larger liver. This could indicate an excess of energy in the control diet, but it is more likely that the other treatments yielded fish with smaller than average livers because of reduced energy intake. Further histological analysis is needed to know for certain the exact condition of the cells in the liver, but is likely that a large liver in this case actually speaks positively toward the performance of the control diet versus the others.

Throughout the digestibility and growout portions of this research, we observed slightly reduced performances when compared with similar research for this and other species (Riche and Williams 2010; Quintero et al. 2012, Santinha et al. 1999; Zhou et al. 2004). While it is desirable to control as many variables as possible in any diligent scientific experiment, commercial aquaculture nutrition research is often conducted in a practical and limited setting. Even at UMEH, the number of ongoing projects and the quantity of clean seawater limits our ability to control variables as much as we would like to as scientists. This experiment was conducted in a flow-through system using makeup water from an existing and aging saltwater well. Of legitimate concern is the recent pH of this water. Through all experiments, the pH ranged from 7.20-7.40, well below the average 8.0- 8.1 that our oceans currently exhibit. Detrimental effects on marine finfish metabolism have been observed in low pH (and consequently hypercapnic) situations, mostly as a result of fish having to do additional work to maintain acid-base balance (Heuer and Grosell 2014). Water quality is essential for achieving optimal results in any aquaculture research, and will need to be improved before further work is performed in this system. Fortunately, all replicates experienced the same conditions and so it is reasonable to compare results between treatments.

CHAPTER 4: COMPREHENSIVE DISCUSSION AND CONCLUSION

We initially hypothesized that higher apparent digestibility values across all nutrients for Florida pompano diets would correspond with higher growth rates. We saw that this was not necessarily true in the juvenile growout portion of this study, but the results may have been more affected by the batch quality of the soy products than the digestibility of the diet ingredients. Future research should perform similar studies with various larger size classes of pompano and with novel fishmeal replacement products, since digestibility and (healthy) growth in this trial were observed for two different size classes, ~575 gram adults and ~30 gram juveniles, respectively. Most animals are known to have different nutritional requirements at different points in their lives, and pinpointing nutritional requirements for each size class is vital for maximizing the efficiency of marine fish feeds. Several of the publications cited in this thesis have attempted to address this issue for specific species, but such a complex issue will only be resolved after years of focused research.

Our data reinforce the notion that digestibility of amino acids is not be the only factor to consider when attempting to maximize growth in a juvenile fish diet. Consider Selecta SPC, which appeared to be highly digestible by adult pompano across virtually all ADCs of amino acids, yet performed less well when incorporated into a balanced juvenile pompano growout diet. There are a handful of potential explanations for this. A likely explanation might lie in the different nutritional requirements of juvenile fish and adults for growth. It has been suggested in research that juvenile fish fed soy products early on in life perform better on soy-based growout diets, (NRC 2011).

Another explanation could lie in the relative importance of the digestibility of energy. It is possible that high digestibility of energy aided growth performance more than the digestibility of certain amino acids. After all, Florida pompano juveniles and adults have to be active swimmers to be found in high-energy coastal environments and surf zones, (Bellinger and Avault Jr. 1970; Ross and Lancaster 2002). In this experiment, the apparent digestibility of energy was significantly highest in both of the SPC diets, of which Solae experienced the best growth. It is however confounding that Selecta SPC did not perform as well in growth parameters. Further analysis of anti-nutritionals in these diets is needed to pinpoint exactly what made the American (Solae) product perform much better than the Brazilian (Selecta) in most growth performance metrics. While no rancidity was detected, manufacture or storage defects are always a remote but possible source of error. Given that our diets were homemade and not extruded, it is likely that some enzyme inhibitors, lectins, saponins and other anti-nutritionals were not denatured, thereby affecting growth performance and digestibility, (Francis et al. 2001; Knudson et al. 2006; NRC 2011; Satoh et al. 2003).

While it is encouraging that a domestic soy product performed well, a concern in moving forward commercially with Solae SPC is its cost. According to the World Bank, soybean meal as a commodity was recently trading around $420 USD per metric ton in November 2014, while fishmeal was trading at $2,200 USD at the same time. The price of most soy protein concentrate is about two to two and a half times the price of soybean meal while Solae products sell for close to four times that ($1600 USD/metric ton). While still cheaper than fishmeal by a fair margin, we need to continue to explore other soy protein options and figure out how to better supplement those that are most promising, like Solae SPC. Fermented products like Hamlet protein (972$/ ton) are digestible and encouraging, but remain much more expensive than regular de-hulled solvent-extracted soybean meal, without proving drastically more effective, probably due to a reduced percentage of crude protein (~55% compared to ~65% in Solae).

The second growout study exemplified one important concept, despite its inability to evaluate all of the soy protein ingredients being tested. It highlighted another industry-wide issue, which is how and when to strike a balance between cost-effectiveness and performance in juvenile feeds. There is no doubt that Otohime is a leading manufacturer in the industry, and for good reason, but this study demonstrates that we can do better, at least for growout of young pompano. Not only did our control diet keep pace with an expensive commercial diet; it did so while containing commodity-grade soy. The data show that a simple diet supplemented with 13% soy can actually outcompete Otohime, at a fraction of the cost and footprint on wild and reduction fisheries. After all, Otohime is priced at around $6.50/kg when sold in 20 kg bags. That means that even with an FCR of 1:1, (which has proven nearly impossible for adult carnivorous marine finfish), a farmer must sell a kg of fish for significantly more than the $6.50/kg that he or she put into it in order to make a profit. One can quickly see how the use of such a feed becomes cost-prohibitive when the biomass increases beyond larval stages. Environmentally, the choice becomes simple when similar growth and performance are derived from diets whose FIFOs are 2.77 (Control w/ 13% soy) and 5.36 (Otohime).

As aquaculture continues to grow and wild catch remains stable at best, aquafeeds need to increase efficiency with respect to alternatives for fishmeal and fish oil. Over the past two decades, some plant-based protein sources have been demonstrated to be more appropriate for commercial use than others. Despite some concerns over digestibility and anti-nutritional factors, soymeal and soymeal products remain a legitimate and promising substitute for fishmeal in carnivorous fish feeds, so long as we are aware of their limitations. By subjecting ingredients to digestibility and growth studies like this one, we are assessing the potential of each novel product. When implemented in a proven and repeatable experimental design, continuing research about soy and species-specific nutritional requirements should lead to an economically and environmentally viable solution to this increasingly poignant global problem.

REFERENCES

A.O.A.C., 1990. Official Methods of Analysis of AOAC. Association of Official Analytical Chemists, Washington, DC, USA.

A.O.A.C., 2000. Official Methods of Analysis of AOAC. Association of Official Analytical Chemist, Washington, DC, USA.

Bellinger, J. W. and Avault Jr., J. W. 1970. Seasonal Occurrence, Growth, and Length-Weight Relationship of Juvenile Pompano, *Trachinotus carolinus*, in Louisiana. Transactions of the American Fisheries Society, 99: 2.

Boyer, D.C., Boyer, H.J., Fossen, I., Kreiner, A., 2001. Changes in abundance of the northern Benguela sardine stock during the decade 1990–2000, with comments on the relative importance of fishing and the environment. South African Journal of Marine Science. 23(1): 67-84.

Buentello, J.A., and Gatlin III, D.M. 2000. The dietary arginine requirement of channel catfish (*Ictalurus punctatus*) is influenced by endogenous synthesis of arginine from glutamic acid. Aquaculture 188: 311-321.

Ebeling, M.E., 1968. The Dumas method for nitrogen in feed. Journal of the Association of Analytical Chemists 75, 401–413.

Einen, 0., and Roem, E.J. 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquaculture Nutrition 3:1, 15-126.

Folch, J., Lees, M., and Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 97, 383–394.

Francis, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture. 199, 197-227.

Gaylord, T.G., Barrows, F.T, Rawles, S.D., 2010. Apparent amino acid availability from feedstuffs in extruded diets for rainbow trout Oncorhynchus mykiss. Aquaculture Nutrition. 16, 400-406.

Glencross, B., Evans, D., Dods, K., McCafferty, P., Hawkins, W., Maas, R., Sipsas, S. 2005. Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement fecal collection methods. Aquaculture. 245, 211-220.

Glencross, B.D., Booth, M., Allan, G.L., 2007. A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquaculture Nutrition. 13, 17-34.

Hamre, J., 1994. Biodiversity and exploitation of the main fish stocks in the Norwegian-Barents Sea ecosystem. Biodiversity and Conservation. 3(6), 473-492.

Heuer, R and Grosell, M. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology. 307, R1061-R1084.

Jackson, A., 2010. Feeding Fish to Fish – is this a Responsible Practice? Humber Seafood Summit, Grimsby, UK.

Knudson, D., Ron, O., Baardsen, G., Smedsgaard, J., Koppe, W. and Froklaer, H.. 2006. Soyasaponins resist extrusion cooking and are not degraded during gut passage in Atlantic Salmon (*Salmo salar* L.). Journal of Agricultural and Food Chemistry 54, 6428-6435.

Lazo, J. P., D. A. Davis, and C. R. Arnold. 1998. The effects of dietary protein level on growth, feed efficiency and survival of juvenile Florida pompano (*Trachinotus carolinus*). Aquaculture. 169, 225–232.

Lech, G.P. and Reigh, R.C. 2012. Plant Products Affect Growth and Digestive Efficiency of Cultured Florida Pompano (*Trachinotus carolinus*) Fed Compounded Diets. PLos ONE. 7, 1-11.

Lupatsch, I., G. W. Kissil, D. Sklan, and E. Pfeffer. 2001b. Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (Sparus aurata L.). Aquaculture Nutrition. 7:71-80.

Maryland Sea Grant (MSG), 2009. Menhaden species team: background and issue briefs. Presented at the Ecosystem Based Fisheries Management for Chesapeake Bay, College Park, MD.

National Research Council (NRC), 2011. Nutrient Requirement of Fish and Shrimp. National Academy Press, Washington, DC. 253pp.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature. 405, 1017-1024.

Quintero, H., Davis, A.D., Rhodes, M., 2012. Soy protein concentrate as an alternative ingredient in Florida pompano (*Trachinotus carolinus*) diets. Journal of Applied Aquaculture. 24, 247-261.

Pohlenz, C., Buentello, A., Mwangi, W., and Gatlin III, D.M., 2012. Arginine and glutamine supplementation to culture media improves the performance of various channel catfish immune cells. Fish & Shellfish Immunology 32, 762-768.

Rawles, S.D., Thompson, K.R., Brady, Y.J., Metts, L.S., Gannam, A.L., Twibell, R.G., Webster, C.D., 2010. A comparison of two fecal collection methods for protein and amino acid digestibility coefficients of menhaden fish meal and two grades of poultry by-product meals for market-size sunshine bass (*Morone chrysops x M. saxatilis*). Aquaculture Nutrition. 16, 81-90.

Riche, M. 2009. Evaluation of digestible energy and protein for growth and nitrogen retention in juvenile Florida pompano, *Trachinotus carolinus.* Journal of the World Aquaculture Society. 40, 45–57.

Riche, M., and Williams, T.N. 2009. Apparent digestible protein, energy and amino acid availability of three plant proteins in Florida pompano, *Trachinotus carolinus* L. in seawater and low-salinity water. Aquaculture Nutrition. 16, 223–230.

Ross, S.W. and Lancaster, J.E. 2002. Movements and site fidelity of two juvenile fish species using surf zone nursery habitats along the southeastern North Carolina coast. Environmental Biology of Fishes, 63, 161-172.

Rossi Jr., W. and Davis, D.A. 2012. Replacement of fishmeal with poultry by-product meal in the diet of Florida pompano *Trachinotus carolinus L*. Aquaculture. 338-341, 160-166.

Sales, J. and Britz, P.J. 2002. Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae L.*). Aquaculture. 207, 113-123.

Santinha, P.J.M., Medale, F., Corraze G., and Gomes, E.F.S. 1999. Effects of the dietary protein: Lipid ratio on growth and nutrient utilization in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition. 5, 147-156.

Satoh, S., M. Takanezawa, A. Akimoto, V. Kiron, and Watanabe, T. 2002. Changes of phosphorus absorption from several feed ingredients in rainbow trout during growing stages and effect of extrusion of soybean meal. Fisheries Science. 68, 325–331.

Smith, D.M. and Tabrett, S.J., 2004. Accurate measurement of *in vivo* digestibility of shrimp feeds. Aquaculture. 232, 563-580.

Suarez J.A., Tudela, C., Davis, D., Daugherty, Z., Taynor, M., Glass, L., Hoenig, R., Buentello, A., Benetti, D. D. 2013. Replacement of fish meal by a novel non-GM variety of soybean meal in cobia, *Rachycentron canadum*: Ingredient nutrient digestibility and growth performance. Aquaculture. 416–417, 328-33

Tacon, A. G. and Metian, M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture. 285, 146-158.

Taynor, M. 2013. Evaluation of gross protein/energy ratios for adult Florida pompano, Trachinotus carolinus, fed practical diets, in relation to growth, feed utilization and nitrogen retention. M.S. Thesis. University of Miami. U.S.A.

Weirich, C. R., and Riche, M. 2006. Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L., to ammonia and nitrite at various salinities. Aquaculture Research 37, 855–861.

Williams, S., Lovell, R.T. and Hawke, J.P. (1985) Value of menhaden oil in diets of Florida pompano. Progressive Fish Culturist, 47, 159–165.

Vandenberg, G.W. and de la Noue, J., 2001. Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility marker. Aquaculture Nutrition. 7, 237-245.

Zeller, D., Rossing, P., Harper, S., Persson, L., Booth, S., Pauly, D., 2011. The Baltic Sea: Estimates of total fisheries removals 1950–2007. Fisheries Research. 108(2-3): 356-363.

Zhou, Q., Tan, B., Sen Mai, K., Liu, Y., 2004. Apparent digestibility of selected feed ingredients for juvenile cobia (*Rachycentron canadum*). Aquaculture. 241, 441-451.