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Evaluation of selected plant products as dietary protein sources for Florida pompano (Trachinotus carolinus)

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EVALUATION OF SELECTED PLANT PRODUCTS AS DIETARY PROTEIN SOURCES FOR FLORIDA POMPANO (*TRACHINOTUS CAROLINUS*)

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ABSTRACT

Profitability of aquaculture enterprises worldwide has been affected by rising fish meal prices. Plant proteins are promising replacements for fish meal because of their lower cost and worldwide availability. The goal of this research was to provide nutritional information for the development of all-plant diets for Florida pompano by evaluating growth of fish fed soy-based, fish-meal-free diets and assessing the nutritional value of several plant protein sources.

In the first experiment, juvenile Florida pompano were fed five, soy-based, fish-meal-free diets containing graded levels of soybean meal (SBM, or M) at 0, 20, 25, 30, or 35%, with the remaining protein provided by soy protein concentrate (SPC, or C) at 59, 46, 43, 39, or 36%, respectively. These diets were compared to a control diet that contained equal levels (30%) of SBM and SPC in combination with 10% menhaden fish meal. Weight gain of fish fed the control diet (ten times initial weight) was significantly higher than gain of fish fed M0/C59, M20/C46, or M35/C36 diets, but was not different ($P > 0.05$) from fish fed the M25/C43 or M30/C39 diets. No apparent trends in whole-body composition of pompano were found. Differences in growth appeared to be due to decreased feed intake related to palatability of some diets. Results indicated that fish-meal-free diets that contained 25-30% SBM and 39-43% SPC were as effective as a diet of similar composition containing 10% menhaden fish meal.

The second experiment determined apparent digestibility coefficients (ADCs) for canola meal (CM), corn gluten meal (CGM), and distiller's dried grains with solubles (DDGS). A reference diet and test diets containing 70% reference diet mixture and 30% test ingredient (CM, CGM, or DDGS) were used following typical methods for digestibility trials. Apparent crude protein digestibility of CGM (57.2%) was significantly higher than DDGS (20.6%) but similar to CM (38.6%). Apparent energy digestibility of DDGS (30.7%) was significantly lower than

CGM (57.1%) but significantly higher than CM (21.3%). The reference diet formulation was hypothesized to be an additional factor that can influence the calculation of ADCs, in addition to the chemical and physical attributes of the test ingredient.

CHAPTER 1. INTRODUCTION

Aquaculture production has steadily increased over the last few decades to meet the growing demand for seafood. During this time period, wild-harvest fisheries production has remained stagnant, providing new opportunities for aquaculture, especially for the production of marine species. Many types of marine finfish aquaculture require the use of compounded diets that contain high protein levels (> 40 percent). Protein in fish diets is commonly provided from animal by-products, in particular fish meal. Fish meal is one of the best protein sources for fish feeds because of its nutritional value and high palatability to fish. Fish meal contains high levels of dietary essential amino acids and essential fatty acids (omega-6 and omega-3 highly unsaturated fatty acids). However, fish meal is a finite resource and is expensive ($1,400 per ton; Muirhead, 2011) relative to other protein supplements in the ingredient market. Rising fish meal prices worldwide will continue to increase feed prices unless more economical alternatives are developed and utilized. Several ingredients have the potential to replace fish meal in marine-fish diets. Plant ingredients are attractive replacements for fish meal because of their lower cost and widespread availability. The reduction or elimination of fish meal use in commercial diets can greatly reduce feed costs for aquaculturists.

Soybeans are one of the most promising plant-based substitutes for fish meal. Soybean products commonly available for use in commercial diets include full-fat soybeans; dehulled, solvent-extracted soybean meal (SBM); soy protein concentrate (SPC); and soy protein isolate. SBM is obtained after heat treatment and extraction of oil from full-fat soybeans, resulting in a mash (i.e., meal composed of different particle sizes) with a protein level around 49 percent (as fed). SBM prices currently range from $319 to $402 per ton, about one-third the price of fish meal (Muirhead, 2011), making it an affordable protein source. Soluble-carbohydrate extraction of SBM produces SPC, a product with protein levels at or above 65 percent (as fed). Processing SBM into SPC increases amino acid levels and eliminates or deactivates most of the compounds found in soybean that negatively affect digestion in fishes. Because of this, SPC has shown greater potential than SBM as a fish meal replacement.

SBM and SPC have been used to reduce fish meal levels in the diets of various fishes (Peres and Lim, 2008). However, wider use of soybean-based diets is hindered by negative effects associated with high soybean inclusion levels. High inclusion levels of soybean products in fish diets has caused reduced diet intake, decreased growth, poor nutrient utilization and digestibility, negative physiological effects, and histological abnormalities (Francis et al., 2001; Krogdahl et al., 2010). SBM, and to a lesser extent SPC, are deficient in the dietary essential amino acids, lysine and methionine, and contain various compounds that affect digestion. Compounds present in SBM and SPC are proteinase inhibitors, lectins, and saponins. Proteinase inhibitors decrease protein digestibility by binding with trypsin and/or chymotrypsin, enzymes which hydrolyze protein in the gastrointestinal tract (Krogdahl et al., 2010). Lectins cause histological abnormalities in gastrointestinal tract cells, reduce nutrient uptake, and affect enzyme activity (Francis et al., 2001). Saponins increase mucosal cell membrane permeability, decrease active uptake of nutrients, and lead to enteritis in salmonids fed SBM-based diets (Krogdahl et al., 2010).

Plant ingredients often contain high levels of carbohydrates, which can provide pellet stability and an inexpensive source of energy in diets. However, many fishes have limited ability to digest carbohydrates, which can have negative effects when carbohydrate is present in large quantity in the diet. Carbohydrates commonly found in soybeans include sucrose, raffinose, and stachyose. Raffinose and stachyose are oligosaccharide (i.e., three and four saccharide units, respectively) carbohydrates that are not digested well by fish or other mono-gastric animals. High levels of these carbohydrates in the diet can cause diarrhea and reduced nutrient absorption (Krogdahl et al., 2010). Carbohydrates in soybeans also can change micro-flora populations in the gastrointestinal tract of animals; such effects on fish are under investigation (Krogdahl et al., 2010).

One of the attractive qualities of soybean as an alternative to fish meal is soybean's much lower price. However, soybean prices can be expected to rise as worldwide demand for soybean continues to increase. Other reasonably priced plant-based ingredients which have shown potential for use in aquafeeds include canola meal (CM), corn gluten meal (CGM), and distiller's dried grains with solubles (DDGS). These ingredients may be useful as partial replacements for soybean products when market price warrants reduced soybean inclusion as a feed-cost control strategy.

Canola is a variety of rapeseed genetically selected for low levels of erucic acid, a fatty acid and known carcinogen. Canola oil is used in human foods and as a replacement for fish oil because of an excellent fatty acid profile. Due to high demand for canola oil, CM, a by-product of canola oil production, is an affordable and commercially available commodity for aquafeeds. CM is the material remaining after the oil is extracted from rapeseed. It contains 34-48 percent crude protein (dry matter basis) and varying lipid levels depending on the oil-extraction method and the source country. CM is currently priced $244-295 per ton (Muirhead, 2011), making it cheaper than SBM. CM has an excellent dietary essential amino acid profile, which is comparable to fish meal, and has been researched as a potential protein replacement for fish meal with varying results (Glencross et al., 2004; Erdogan and Olmez, 2010). Inclusion levels of CM in aquafeeds are limited by the presence of carbohydrates; tannins; the alkaloid, sinapine; and glucosinolates, which can cause depressed feed intake, decreased nutrient availability, and disrupted digestion (Burel and Kaushik, 2008).

Corn is one of the most cultivated plants in the United States and has many uses and by-products. CGM is obtained when starch is extracted from corn and the protein portion (gluten) is separated from the remaining mash. CGM contains approximately 60 percent crude protein (as fed) and is currently priced $575-630 per ton (Muirhead, 2011), making CGM more expensive than SBM and CM. CGM has a good dietary-essential amino acid profile, except for lysine and arginine, and it does not contain any compounds that negatively affect digestion. CGM has potential as a fish meal replacement and is commonly incorporated into commercial diets (Pereira and Oliva-Teles, 2003).

DDGS is a by-product of ethanol production. It can be produced from a variety of grains, but most DDGS produced in the USA is a by-product of the yeast fermentation of corn grain. To be classified as DDGS, at least 75 percent of the solids present in the stillage (i.e., mash remaining after the removal of alcohol by distillation) must be retained (Webster et al., 2008). DDGS is affordable ($200-257 per ton; Muirhead, 2011) and commercially available due to increased ethanol production for motor vehicle fuel. Crude protein content of DDGS is approximately 27 percent (as fed), with low levels of the dietary-essential amino acids lysine and methionine. Although DDGS contains 35-40 percent carbohydrate, it has shown promise as a protein and energy source for some fishes (Zhou et al., 2010).

The goal of the research reported here was to provide nutritional information to assist the development of all-plant diets for Florida pompano (*Trachinotus carolinus*), a promising aquaculture candidate in the United States. Florida pompano tolerate a wide range of culture conditions and historically have attracted high market prices. High feed costs can greatly affect the profitability of commercial pompano production. Development of effective all-plant diets for Florida pompano could increase the profitability of pompano aquaculture, facilitate increased commercial pompano production, and assist the creation of sustainable aquaculture methods for related marine fish species.

CHAPTER 2. UTILIZATION OF ALL-PLANT PROTEIN, SOY-BASED DIETS BY FLORIDA POMPANO (*TRACHINOTUS CAROLINUS*)

2.1 Introduction

Rising prices and growing demand for fish meal are expected to continue to increase aquaculture feed prices worldwide. Soybean protein offers one of the best alternatives to fish meal and soybean products are currently incorporated into most aquafeeds (Gatlin et al., 2007). Soybeans are suitable alternatives to fish meal because of a balanced amino acid profile, relatively low price, and consistent supply (Bonaldo et al., 2006). While the use of soybeans in aquafeeds is increasing, there are negative effects associated with soybean use in diets for some species. High inclusion levels of soybeans can cause poor diet palatability, enteritis, reduced growth, decreased digestibility of dietary protein and lipid, and negative physiological conditions in some fishes (Francis et al., 2001; Krogdahl et al., 2010). These effects are attributed to anti-nutritional factors (compounds that inhibit growth) in soybeans, such as proteinase inhibitors, saponins, lectins, and oligosaccharides (Krogdahl et al., 2010), and nutrient deficiencies that can be created when soybean is used to replace some animal protein products (Zhao et al., 2009; Salze et al. 2010).

Soybean meal (SBM) and soy protein concentrate (SPC) have shown differing effects among species at different inclusion levels. Partial fish-meal replacement with SBM and SPC has been reported in cobia, *Rachycentron canadum* (Salze et al., 2010), red sea bream, *Pagrus major* (Biswas et al., 2007; Matsunari et al., 2008; Takagi et al., 2010), Japanese flounder, *Paralichthys olivaceus* (Park et al., 2002; Deng et al., 2006), Atlantic cod, *Gadus morhua* (Hansen et al., 2007), and yellowtail, *Seriola quinqueradiata* (Takagi et al., 2008) among others. Complete replacement of fish meal with soybean products has been reported in Nile tilapia, *Oreochromis niloticus* (Zhao et al., 2009) and Atlantic cod, *Gadus morhua* (Walker et al., 2010).

Factors that may prohibit high inclusion of soybean products are inadequate levels of some dietary essential (limiting) amino acids, presence of phytic acid, reduced diet palatability and digestibility, and low levels of taurine (Kissil et al., 2000; Sajjadi and Carter, 2004; Kader et al., 2010; Takagi et al., 2010). Crystalline amino acids can be included in diets to eliminate amino acid deficiencies or to serve as attractants (Khan et al., 2003; Deng et al., 2006). When phytase, an enzyme which breaks down phytic acid, is added to diets, bound minerals, proteins, and other compounds are released, thereby increasing dietary nutrient availability (Denstadli et al., 2006; Biswas et al., 2007). Taurine is reported as a dietary essential nutrient for yellowtail and improves growth and physiological condition in other species fed plant-protein based diets (Takagi et al., 2010). Taurine is suggested to increase growth by aiding lipid digestion and/or by acting as a feeding stimulant (Chatzifotis et al., 2008). Optimum dietary inclusion levels of soybean products must be determined individually for each cultured species due to variability in soybean tolerance among species.

Florida pompano (*Trachinotus carolinus*) is a promising aquaculture species native to the Gulf of Mexico and the southeastern Atlantic coast of the United States. Pompano exhibit favorable qualities for aquaculture, including ease of reproduction in captivity, high market price, and a wide tolerance of salinities (Weirich et al., 2006). Interest in pompano aquaculture has persisted for over 50 years, but only recently has knowledge of the nutritional needs of pompano begun to increase (Lazo et al., 1998; Riche, 2009; Gonzalez-Felix et al., 2010; Gothreaux et al., 2010; Riche and Williams, 2010). Studies have determined that Florida pompano utilize the energy and amino acids in soybean products effectively, which suggests that soybean products can be suitable replacements for fish meal in compounded diets (Gothreaux et al., 2010; Riche and Williams, 2010). The objective of this study was to determine the effects of fish-meal-free (FMF) diets containing graded levels of SBM and SPC on weight gain and body composition of Florida pompano.

2.2 Materials and Methods

Diet Preparation

Six isonitrogenous and isocaloric diets were formulated to match or exceed the dietary essential amino acid levels in whole-body pompano (Table 1 & 2). Five experimental diets were formulated to contain 0, 20, 25, 30, and 35 percent SBM, with the remaining protein provided by SPC at levels of 59, 46, 43, 39 and 36 percent, respectively. These diets were tested with a control diet that contained 30 percent SBM and 30 percent SPC in combination with 10 percent menhaden fish meal. All diets were supplemented with 0.5 percent taurine and 1,000 FTU of fungal phytase (Natuphos™, BASF Corp., Geismar, Louisiana) per kilogram diet. Diets were prepared by grinding ingredients to < 0.6-mm particle size with a Wiley mill (Thomas Scientific, Swedesboro, New Jersey). Dry ingredients were mixed for 60 minutes in a twin-shell laboratory mixer (Patterson-Kelley Co., East Stroudsburg, Pennsylvania), then moved to a Hobart food mixer (Rapids Machinery, Troy, Ohio) where oil and water were added and the wet mash was mixed to a dough-like consistency. Diets were pelleted through a 3-mm die using a pasta maker (Ronco Inventions, Chatsworth, California). Pellets were air-dried overnight, stored in sealed bags and refrigerated (4 ^{o}C) until used.

Table 1. Formulation and chemical composition of the experimental diets (g/kg, as fed)[1]

Ingredient	Control	M0/C59	M20/C46	M25/C43	M30/C39	M35/C36
Soybean meal	300		200	250	300	350
Soy protein concentrate	300	594.9	460.8	427.2	393.7	360.1
Fish meal	100					
Wheat flour	128.3	227.3	159.8	143	126.1	109.2
Fish oil	94.1	99.8	100.7	100.9	101.2	101.4
Corn gluten meal	20	20	20	20	20	20
$CaH_4(PO_4)_2 \cdot H_2O$	16	16	16	16	16	16
CMC[2]	10	10	10	10	10	10
Methionine	7.3	7.6	8.3	8.5	8.7	8.9
Taurine	5	5	5	5	5	5
Vitamin mix[3]	5	5	5	5	5	5
Mineral mix[4]	2.5	2.5	2.5	2.5	2.5	2.5
Soy lecithin	16	16	16	16	16	16
Choline chloride	0.9	0.9	0.9	0.9	0.9	0.9
Stay-C[5]	0.6	0.6	0.6	0.6	0.6	0.6
Phytase	0.2	0.2	0.2	0.2	0.2	0.2
Ethoxyquin	0.4	0.4	0.4	0.4	0.4	0.4
Chemical composition (g/kg, as fed)						
Dry matter	917	915	920	912	926	932
Crude protein	399	388	400	395	415	423
Lipid	112	98	94	89	87	103
Ash	76	56	60	59	65	70
Fiber	39	31	37	46	37	37
NFE	290	342	330	323	323	299
Gross energy (kcal/g)	4.5	4.4	4.6	4.6	4.7	4.7

[1]M = soybean meal; C = soy protein concentrate.
[2]Carboxymethylcellulose sodium salt.
[3]Per kg diet: vitamin A, 6000 IU; vitamin D, 1000 IU; vitamin E, 0.1 g; biotin, 0.2 g; folic acid, 9 mg; niacin, 0.2 g; pantothenic acid, 0.1 g; vitamin B-6, 25 mg; riboflavin, 40 mg; thiamin, 40 mg; vitamin B-12, 20 mg.
[4]Per kg diet: iron, 0.1 g; manganese, 25 mg; copper, 10 mg; zinc, 0.1 g; iodine, 4.5 mg; cobalt, 50 µg; selenium, 0.5 mg.
[5]Stay-C stabilized vitamin C (L-ascorbyl-2-polyphosphate), 35% ascorbic acid activity.

Table 2. Amino acid composition of whole-body pompano and experimental diets[1]

Amino acid	WB[2]	Control	M0/C59	M20/C46	M25/C43	M30/C39	M35/C36
Arginine	0.60	0.69	0.76	0.83	0.76	0.71	0.72
Histidine	0.22	0.30	0.30	0.36	0.31	0.29	0.29
Isoleucine	0.56	0.72	0.79	0.87	0.79	0.74	0.75
Leucine	0.92	1.12	1.23	1.35	1.21	1.15	1.17
Lysine	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methionine	0.30	0.27	0.25	0.29	0.26	0.23	0.24
Phenyalanine	0.39	0.57	0.63	0.69	0.63	0.59	0.60
Threonine	0.52	0.52	0.54	0.61	0.55	0.51	0.52
Valine	0.74	0.85	0.93	1.02	0.92	0.86	0.88

[1]Values expressed as amino acid/lysine, %, dry matter basis.
[2]WB (whole-body pompano).

Experimental Fish and Culture System

Wild pompano were obtained with a beach seine from the surf zone of Grand Isle and Holly Beach, Louisiana. Fish were transported to the Louisiana State University Agricultural Center Aquaculture Research Station, Baton Rouge, Louisiana and subjected to a 35-day quarantine period during which they were treated to eliminate external and internal parasites (Appendix A). The fish were then stocked into a recirculation culture system and given a one-week acclimation period prior to the start of the experiment. The recirculation system consisted of 18, circular, 227-L fiberglass tanks; a 0.3-mm mechanical particle filter (CeTus, Evolution Aqua Ltd, United Kingdom); a biological bead filter (Sweetwater, Aquatic Ecosystems Inc., Apopka, Florida); two, 120-watt, ultraviolet light sterilizers (Emperor Aquatics, Pottstown, Pennsylvania); and a corona-discharge ozone generator (CD-7, Del Ozone, San Luis Obispo, California). Salinity was maintained with synthetic sea salt (Crystal Sea, Marine Enterprises International, Baltimore, Maryland). Aeration was supplied to each tank with an airstone attached to a regenerative blower. Weekly water exchanges of approximately five percent of system volume were performed with freshly made sea water. Mean water quality parameters ± SD were: temperature, 30.1 ± 0.9 ^{o}C; salinity, 10.2 ± 0.8 g/L; alkalinity, 97 ± 40 mg/L; total ammonia-nitrogen, 0.3 ± 0.4 mg/L; and nitrite-nitrogen, 0.52 ± 0.55 mg/L. Twenty pompano (initial weight of 7.0 g per fish) were stocked in each tank, with each diet assigned to three tanks. Fish were fed to apparent satiation twice daily for ten weeks. Apparent satiation was achieved by allowing each tank to consume as much feed as possible in two, two-minute periods. Fish in each tank were batch weighed bi-weekly. Before weighing, fish were anesthetized with MS-222 (150 mg/L) and allowed to recover before being returned to the tank. Prior to the experiment, 15 fish were euthanized and stored frozen (-20 ^{o}C) for proximate analysis. This was repeated at the conclusion of the 10-week feeding trial with three fish collected from each tank.

Chemical and Statistical Analysis

Proximate analysis of pompano tissue (whole-body) and the experimental diets was conducted by standard methods (AOAC, 2002). Dry matter content of diets was determined by 24-hr oven drying at 100 ^{o}C; dry matter content of pompano whole-body was determined by 48-hr freeze drying. Crude protein, lipid, and fiber contents of samples were determined by the Louisiana State University Agricultural Center Department of Agricultural Chemistry. Ash content was measured by incineration at 600 ^{o}C in a muffle furnace. Gross energy was determined by bomb calorimetry (Model C5000, IKA Works Inc., Wilmington, North Carolina). Nitrogen free extract (NFE) was calculated as: NFE (%) = dry matter – (crude protein + lipid + ash + fiber). Amino acid concentrations were determined with high performance liquid chromatography after hydrolysis (1100 Series, Agilent Technologies, Wilmington, Delaware).

Data were subjected to one-way analysis of variance (ANOVA) using the mixed procedure of SAS version 9.1 (SAS, Cary, North Carolina). Prior to ANOVA, percentage data were arcsine-transformed to correct for normality. All hypotheses were tested at a significance level of $\alpha = 0.05$. If a significant difference ($P < 0.05$) was found, differences among means were identified with the Tukey-Kramer multiple range test.

2.3 Results

After two weeks, consumption of the M0/C59 diet was noticeably decreased. Pompano fed the M0/C59 diet had significantly lower weight gain, specific growth rate (SGR) and feed intake (FI), and significantly higher (poorer) feed conversion ratio (FCR), than fish fed the other diets (Table 3). Protein efficiency ratio (PER) of M0/C59-fed fish did not differ ($P > 0.05$) from PER of fish fed M30/C39, but was lower than PER of fish fed the other diets (Table 3). Significant differences in survival were identified, but there was no apparent relationship related to dietary treatment (Table 3). Fish fed the control diet had significantly higher weight gain than fish fed M0/C59, M20/C46, or M35/C36, but did not differ ($P > 0.05$) in weight gain from fish fed M25/C43 or M30/C39 (Table 3). With the exception of the control diet which contained fish meal, no significant differences in weight gain, FCR, SGR, PER, FI, or survival were observed among pompano in the treatment groups that received diets containing both soybean meal (20-35 percent) and soy protein concentrate (36-46 percent) as protein sources (Table 3).

Table 3. Growth parameters of Florida pompano fed the experimental diets for 10 weeks[1]

	Weight gain[2]	FCR[3]	SGR[4]	PER[5]	FI[6]	Survival (%)
Control	1075 ± 71[a]	1.56 ± 0.04[a]	3.52 ± 0.09[a]	1.60 ± 0.04[a]	1.60 ± 0.12[a]	98 ± 2[ab]
M0/C59	410 ± 42[c]	2.18 ± 0.15[b]	2.32 ± 0.11[c]	1.20 ± 0.08[b]	0.80 ± 0.07[c]	82 ± 6[b]
M20/C46	758 ± 50[b]	1.76 ± 0.06[a]	3.06 ± 0.08[b]	1.43 ± 0.05[a]	1.33 ± 0.03[ab]	95 ± 5[ab]
M25/C43	875 ± 55[ab]	1.61 ± 0.01[a]	3.25 ± 0.08[ab]	1.58 ± 0.01[a]	1.42 ± 0.10[ab]	100[a]
M30/C39	878 ± 29[ab]	1.69 ± 0.01[a]	3.26 ± 0.04[ab]	1.42 ± 0.01[ab]	1.50 ± 0.03[ab]	100[a]
M35/C36	793 ± 73[b]	1.64 ± 0.05[a]	3.12 ± 0.12[ab]	1.44 ± 0.05[a]	1.28 ± 0.08[b]	93 ± 4[ab]

[1]Mean values ± SE (n=3), values in each column with different letters are significantly different ($P < 0.05$).
[2]Weight gain (%) = 100 x (final weight – initial weight)/initial weight.
[3]FCR (feed conversion ratio) = total feed consumed/wet weight gain.
[4]SGR (specific growth rate) = 100% x (ln(final weight) – ln(initial weight))/days.
[5]PER (protein efficiency ratio) = 100% x wet weight gain/protein intake.
[6]FI (feed intake), g/fish/day.

Whole-body composition of pompano fed the M0/C59 diet contained significantly less crude protein per gram (dry weight) than whole-body of fish fed the control diet but not different ($P > 0.05$) than any of the other diets (Table 4). Moisture content of fish fed M0/C59 was higher ($P < 0.05$) than that of fish fed the control diet or the M30/C39 diet, but not different ($P > 0.05$) from the moisture content of fish fed M20/C46, M25/C43, or M35/C36. No trend in lipid, ash, or energy content of fish whole-body was apparent among treatment groups, indicating no observed effect of soy-product inclusion levels on these components of body composition.

Table 4. Whole body composition of Florida pompano fed the experimental diets (g/100g)[1]

Diet	Moisture	Crude protein[2]	Lipid	Ash	GE[3]
Initial	68.9	14.9	10.5	3.0	6.1
Control	69.7 ± 0.4^{b}	17.3 ± 0.0^{a}	9.0 ± 0.4^{ab}	2.9 ± 0.0^{b}	5.8 ± 0.8^{ab}
M0/C59	73.5 ± 0.9^{a}	16.3 ± 0.3^{b}	5.2 ± 1.2^{b}	3.3 ± 0.0^{a}	5.3 ± 1.8^{b}
M20/C46	69.9 ± 0.6^{ab}	17.0 ± 0.2^{ab}	8.9 ± 0.4^{ab}	2.9 ± 0.1^{b}	5.8 ± 0.3^{ab}
M25/C43	71.5 ± 1.1^{ab}	16.5 ± 0.2^{ab}	7.5 ± 1.3^{ab}	3.0 ± 0.1^{ab}	5.6 ± 1.6^{ab}
M30/C39	68.7 ± 1.1^{b}	16.8 ± 0.1^{ab}	9.8 ± 0.9^{a}	2.8 ± 0.1^{b}	5.9 ± 1.2^{a}
M35/C36	70.9 ± 0.3^{ab}	17.0 ± 0.3^{ab}	7.8 ± 0.1^{ab}	3.0 ± 0.1^{ab}	5.7 ± 0.5^{ab}

[1]Mean values ± SE (n=3), values in each column with different letters are significantly different ($P < 0.05$).
[2]Calculated as nitrogen (%) x 6.25.
[3]GE (gross energy), kcal/g.

2.4 Discussion

Results of the current study suggest that replacement of fish meal with mixtures of SBM and SPC is feasible for Florida pompano, but it appears that factors other than the amino acid profile of these ingredients affect fish performance at different levels of soybean-product inclusion. Certainly, the significantly lower feed intake of fish fed M0/C59 was a primary cause of the poor weight gain of fish in this treatment group due to reduced nutrient intake relative to fish fed the other diets (Table 3). It is possible that one of the factors affecting feed intake is the attractiveness or palatability of soybean products. Feed intake data suggest that a diet (M0/C59) composed primarily of SPC (59 percent), wheat flour, fish oil, and corn gluten meal was not as attractive and/or palatable as diets of similar composition and nutritional value that contained 20-35 percent SBM and reduced quantities of SPC (36-46 percent of diet). Why this would be the case is not readily apparent. However, the nutritional equivalence — i.e., amino acid (Table 2) and energy content (Table 1) — among diets suggests that nutrient deficiencies were unlikely to be the cause. There is no evidence from previous research conducted in this laboratory, or from the literature, to suggest that SBM in prepared diets is attractive to pompano, but it is possible that SPC could be more unattractive than SBM, such that replacement of 13 percent SPC in the M0/C59 diet with SBM in the M20/C46 diet significantly increased feed intake to a level not different ($P > 0.05$) from that of fish fed the control diet (Table 3). This effect appears to be limited, however, since feed intake and weight gain of pompano fed M35/C36, which contained the largest amount of soy products (710 g/kg diet), also was significantly below that of fish fed the control diet.

Weight gain, FCR, SGR, and PER did not differ significantly among Florida pompano fed the control diet, M25/C43 or M30/C39, indicating that a 40 percent protein, FMF diet containing 680-690 g/kg (68-69 percent) of SPC and SBM produced growth performance of pompano equivalent to a similar diet (600 g/kg SPC and SBM) containing 10 percent (100 g/kg) menhaden fish meal.

Studies with other species have shown reduced growth of fish fed diets that contained SPC as a major protein source. Kissil et al. (2000) reported that increased levels of SPC and phytic acid in diets for gilthead seabream caused reduced feed intake and weight gain due to low diet palatability. Deng et al. (2006) improved palatability of soy-based diets for Japanese flounder by incorporating 0.5 percent taurine as a feeding stimulant, and reduced phytic acid content by addition of phytase at a concentration of 750 FTU/kg diet. Nonetheless, replacement of 87.5 and 100 percent of fish meal with SPC resulted in decreased growth due to reduced diet acceptability.

Zhao et al. (2009) completely replaced fish meal with SPC in Nile tilapia diets by increasing feeding frequency. Nile tilapia fed a FMF soybean-based diet six times per day exhibited feed intake and weight gain not different from that of fish fed a fish-meal based diet twice per day. Walker et al. (2010) reported no negative effects of SPC inclusion level on growth or feed intake of Atlantic cod fed FMF diets. However, hydrolyzed fish protein concentrate and blood meal, which are likely feeding stimulants, also were included in all diets.

Soybean products are among the most promising replacements for fish meal in aquafeeds. However, to be effective, FMF diets must be consumed in quantities sufficient to support rapid fish growth and cost-efficient production. Development of nutritious, palatable, FMF diets for Florida pompano, and other fishes, will require the continued identification and testing of new alternatives to fish meal. Identification of suitable ingredients and effective combinations of feedstuffs that optimize fish growth and feed cost, while minimizing fish meal use, will improve the profitability and sustainability of pompano aquaculture, and contribute to the long-term growth of pompano farming in the United States.

CHAPTER 3. DETERMINING DIGESTIBILITY COEFFICIENTS OF CANOLA MEAL, CORN GLUTEN MEAL, AND DISTILLER'S DRIED GRAINS WITH SOLUBLES FOR FLORIDA POMPANO

3.1 Introduction

Rising feed prices have prompted investigation of alternative, less expensive combinations of ingredients for aquafeeds. However, alternative ingredients have wide-ranging nutrient compositions that can vary in digestibility (nutrient availability) among fishes. Digestibility is quantified by calculating apparent digestibility coefficients (ADCs) of individual nutrients in ingredients. ADCs determine the availability of nutrients in an ingredient by measuring differences in nutrient levels between diet and feces. The most commonly used method of calculating ADCs (i.e., indirect method) involves the use of a test diet and reference diet, both of which contain an inert, indigestible compound, such as chromic oxide, as a dietary marker (Cho et al., 1982). A series of equations are used to calculate apparent digestibility coefficients of individual nutrients based on differences in the amount of marker and the amount of each nutrient of interest in the test diet, reference diet, and feces (Cho et al., 1982; Forster, 1999). When digestibility coefficients are not corrected for non-dietary sources of fecal nutrients, such as nutrients from cells that are continuously sloughed from the intestinal lining, they are termed "apparent". Most digestibility values reported in the literature are apparent digestibility coefficients because the effort required to measure very small differences between apparent and "true" digestibility coefficients is of little practical value (Glencross et al., 2007).

ADCs can range from zero to 100 percent, although values outside this range can occur due to inherent measurement errors, especially when working with very small quantities of nutrients. ADCs can be used in diet formulation to produce species-specific diets at least-cost. Least-cost feed formulation requires knowledge of the ADCs of multiple ingredients and the nutritional requirements (e.g., amino acid requirements) of the target species (Allan et al., 2000b). However, ingredient ADCs have not been determined for most cultured fishes, resulting in continued reliance on fish meal — a high-quality, high-cost protein source — as a primary ingredient in some manufactured diets. Development of less expensive diets that provide acceptable fish growth would provide greater value for aquaculturists.

Measurement of ingredient digestibility requires collection of feces by one of three methods: settling, stripping, or dissection (Sullivan and Reigh, 1995). Each method has limitations and benefits. The settling method, which uses gravity to passively collect feces on a screen or at the bottom of a sedimentation column, is least stressful to fish. However, the settling method typically overestimates digestibility due to leaching losses of soluble nutrients exposed to water (Smith et al., 1980). Stripping, forces feces from the posterior portion of the digestive tract by application of gentle pressure on the abdomen (Austreng, 1978). The dissection method involves surgical removal of feces from the distal intestine. Dissection can result in contamination of the sample with mucus, blood and bits of tissue, and can cause death of the fish if the wound does not heal properly. Stripping and dissection methods can underestimate digestibility if undigested material is collected (Glencross et al., 2007). Factors other than fecal collection method that can affect the accuracy of ADCs include environmental conditions in which the fish were fed, ingredient composition of the diet, the feed manufacturing process used, and fish feeding practices (McGoogan and Reigh, 1996; Cheng and Hardy, 2003).

Florida pompano (*Trachinotus carolinus*) is a promising aquaculture species for which digestibility coefficients of a limited number of feedstuffs have recently been determined (Gonzalez-Felix et al., 2010; Gothreaux et al., 2010; Riche and Williams, 2010). The objective of this study was to expand the ingredient digestibility database for Florida pompano by determining digestibility of crude protein and energy, and amino acid availability, of canola meal (CM), corn gluten meal (CGM), and distiller's dried grains with solubles (DDGS), three practical ingredients available for use in formulated diets.

3.2 Materials and Methods

Wild Florida pompano were obtained with a beach seine from the surf zone of Grand Isle and Holly Beach, Louisiana. Fish were transported to the Louisiana State University Agricultural Center Aquaculture Research Station, Baton Rouge, Louisiana and subjected to a 35-day quarantine period during which they were treated to eliminate external and internal parasites (Appendix A). The fish were then stocked into a recirculation culture system and grown to a mean individual weight of at least 80 g before digestibility trials were initiated. During the grow-out period fish were fed a commercial marine starter diet (Aquaxcel, Cargill, Franklinton, Louisiana. The recirculation system consisted of 12, circular, 227-L fiberglass tanks; a 0.3-mm mechanical particle filter (CeTus, Evolution Aqua Ltd, United Kingdom); a biological bead filter (Sweetwater, Aquatic Ecosystems, Apopka, Florida); two, 120-watt, ultraviolet light sterilizers (Emperor Aquatics Inc., Pottstown, Pennsylvania); and a corona-discharge ozone generator (CD-7, Del Ozone, San Luis Obispo, California). Salinity was maintained with synthetic sea salt (Crystal Sea, Marine Enterprises International, Baltimore, Maryland). Aeration was supplied to each tank with an airstone on a regenerative blower. Weekly water exchanges of approximately five percent of system volume were performed with freshly made sea water. Mean water quality parameters ± SD were: temperature, 26.0 ± 0.7 ^{o}C; salinity, 9.9 ± 0.6 g/L; alkalinity, 91 ± 15 mg/L; total ammonia-nitrogen, 0.3 ± 0.2 mg/L; and nitrite-nitrogen, 0.4 ± 0.2 mg/L. Twenty five pompano (mean weight of 83 g per fish) were stocked into each tank, with each diet assigned to three tanks.

A reference diet and three test diets containing 30 percent test ingredient (CM, CGM, or DDGS) and 70 percent reference diet were formulated (Table 5). Chromic oxide was used as an inert marker (10 g/kg diet). Diets were prepared as described in Chapter 2, after which the chemical compositions of the finished diets and the test ingredients were determined by analysis (Table 6).

Table 5. Ingredient composition of the diets (g/kg, as fed)

Ingredient	Reference diet	Test diet
Fish meal, menhaden	379.4	265.6
Soybean meal (49% CP)	250.0	175.0
Soy protein concentrate	100.0	70.0
Wheat middlings	153.3	107.3
Oil, menhaden	65.0	45.5
Soy lecithin	10.0	7.0
CMC[1]	20.0	14.0
Vitamin mix[2]	5.0	3.5
Mineral mix[3]	2.5	1.8
Stay-C[4]	0.6	0.4
Chromic oxide	10.0	7.0
Ethoxyquin	0.2	0.1
Choline chloride	4.0	2.8
Test ingredient[5]		300.0

[1]Carboxymethylcellulose sodium salt.
[2]Per kg diet: vitamin A, 6000 IU; vitamin D, 1000 IU; vitamin E, 0.1 g; biotin, 0.2 g; folic acid, 9 mg; niacin, 0.2 g; pantothenic acid, 0.1 g; vitamin B-6, 25 mg; riboflavin, 40 mg; thiamin, 40 mg; vitamin B-12, 20 mg.
[3]Per kg diet: iron, 0.1 g; manganese, 25 mg; copper, 10 mg; zinc, 0.1 g; iodine, 4.5 mg; cobalt, 50 μg; selenium, 0.5 mg.
[4]Stay-C stabilized vitamin C (L-ascorbyl-2-polyphosphate), 35% ascorbic acid activity.
[5]Canola meal, corn gluten meal, or distiller's dried grains with solubles.

Table 6. Chemical composition of the diets and test ingredients (%, as fed)[1]

	Diet				Ingredient		
	Reference	CM[2]	CGM[3]	DDGS[4]	CM	CGM	DDGS
Dry matter	92.70	91.98	91.39	91.94	89.2	89.4	87.6
Crude protein	46.91	43.95	51.61	39.78	36.3	61.9	25.8
Lipid	11.02	8.48	8.54	11.50	4.9	4.2	13.9
Ash	11.80	10.61	8.63	9.64	7.3	1.1	3.7
Chromic oxide	1.08	0.73	0.82	0.85			
GE (kcal/g)[5]	4.65	4.48	4.75	4.72	4.2	5.0	4.5
Fiber					9.7	0.3	5.8
NFE[6]					31.0	21.9	38.4

[1]As determined by analysis.
[2]Canola meal.
[3]Corn gluten meal.
[4]Distiller's dried grains with solubles.
[5]GE (gross energy).
[6]NFE (nitrogen free extract) = dry matter – (crude protein + lipid + ash + fiber).

Fish were fed once daily and were acclimated to diets for six days prior to fecal collection. Fecal material was collected weekly for five weeks. On collection days, fish were fed to apparent satiation and fecal material was manually stripped 3.5 to 4 hr after feeding by applying pressure to the abdomen. Prior to stripping, fish were anesthetized with MS-222 (150 mg/L) and allowed to recover before being returned to the tank. Fecal samples from individual fish were pooled, by tank, and stored frozen at -20 ^oC. Samples were freeze-dried for a minimum of 48-hr prior to chemical analysis. Diets and fecal material were analyzed with standard methods (AOAC, 2002). Chromium content was determined with inductively coupled plasma spectrometry by the Louisiana State University Agricultural Center Department of Agricultural Chemistry. Nitrogen was measured spectrophotometrically (Model FP-528, LECO Corp., St. Joseph, Michigan) and crude protein content was calculated as nitrogen x 6.25. Gross energy was determined with bomb calorimetry (Model C5000, IKA Works Inc., Wilmington, North Carolina). Amino acid concentrations were determined with high performance liquid chromatography after hydrolysis (1100 Series, Agilent Technologies, Wilmington, Delaware).

ADCs of the reference diet and test diets were calculated with Equation 1 (Appendix B). ADCs for crude protein and energy in each of the test ingredients were calculated with Equation 2 (Cho et al., 1982) and Equation 3 (Forster, 1999; Appendix B).

ADCs were subjected to one-way analysis of variance (ANOVA) and regression analysis with SAS version 9.1 (SAS, Cary, North Carolina). Percentage data were arcsine-transformed prior to ANOVA to correct for normality. Hypotheses were evaluated at a significance level of $\alpha = 0.05$. If a significant difference ($P < 0.05$) was found, differences among means were identified with the Tukey-Kramer multiple range test.

3.3 Results

The two equations used to calculate ADCs of protein and energy in the three test ingredients produced different (numerical) estimates of mean digestibility, although the range of individual ADCs calculated with each of the equations overlapped. The magnitude of the difference varied among ingredients. Discrepancies between mean ADCs in Table 7 result from a correction factor applied in Equation 3 that accounts for: (i) the concentration of the nutrient of interest in the 300 g (30 percent) of reference diet replaced by test ingredient in 1 kg of test diet, and (ii) the concentration of the nutrient of interest in the 300 g of test ingredient that replaced the reference diet mixture in 1 kg of test diet. Equation 2 does not make this adjustment. Thus, ADCs calculated with Equation 2 (Cho et al., 1982) will diverge from those calculated with Equation 3 (Forster, 1999) as the concentration of a given nutrient in 300 g of the test ingredient diverges from the concentration of that nutrient in 300 g of the reference diet. As an example, in this study DDGS contained 26 percent protein (as fed) and the reference diet contained 47 percent protein (as fed) resulting in a difference in calculated ACPD, between the two equations, of 17.6 percent (Table 7). Conversely, energy content of the three test ingredients was relatively similar to the energy content of an equal amount of the reference diet, so there was little difference among AED values calculated with the two equations (Table 7). Because the Forster (1999) modification has been gaining support in recent years as the more accurate of the two calculations for digestibility trials, ADCs calculated with Equation 3 will be used for reporting the results of the current study.

Table 7. Apparent digestibility coefficients for Florida pompano of crude protein (ACPD) and energy (AED) in the test ingredients[1,2]

	ACPD		AED	
Ingredient	Equation 2	Equation 3	Equation 2	Equation 3
Canola meal	41.8 ± 3.8	38.6 ± 4.3[ab]	22.7 ± 2.0[c]	21.3 ± 2.1[c]
Corn gluten meal	54.6 ± 3.1	57.2 ± 2.4[a]	57.3 ± 2.6[a]	57.1 ± 2.5[a]
Distiller's dried grains with solubles	38.2 ± 4.3	20.6 ± 7.0[b]	30.1 ± 1.4[b]	30.7 ± 1.4[b]

[1]Mean values ± SE (n=3), values in each column with different letters are significantly different ($P < 0.05$).
[2]Equation 2 (Cho et al., 1982); Equation 3 (Forster, 1999).

Apparent crude protein digestibility (ACPD), as determined by Equation 3, ranged from 20.6 percent for DDGS to 57.2 percent for CGM (Table 7). Crude protein digestibility of CGM was significantly higher than that of DDGS, but neither CGM nor DDGS differed significantly from CM in protein digestibility. Apparent energy digestibility (AED) ranged from 21.3 percent to 57.1 percent (Table 7). AED of CGM was significantly higher than AED of CM or DDGS. DDGS also had higher ($P < 0.05$) energy digestibility than CM.

Apparent amino acid availability (AAAA) of leucine and tyrosine was significantly higher in CGM than in CM or DDGS (Table 8). AAAA of alanine and proline was significantly

Table 8. Apparent availability coefficients for Florida pompano of amino acids in the test ingredients[1]

Amino acid	CM	CGM	DDGS
Indispensable			
Arginine	53.8 ± 5.9	68.5 ± 10.8	35.0 ± 19.7
Histidine	46.9 ± 8.2	58.7 ± 6.1	30.0 ± 19.3
Isoleucine	50.4 ± 8.0	62.5 ± 9.8	40.9 ± 10.9
Leucine	46.8 ± 3.5[b]	70.8 ± 5.2[a]	55.6 ± 5.1[b]
Lysine	48.4 ± 3.1	47.9 ± 14.4	50.4 ± 41.2
Methionine	91.9 ± 0.4	84.9 ± 3.8	91.5 ± 4.6
Phenylalanine	54.2 ± 8.2	70.9 ± 6.6	55.5 ± 7.9
Threonine	44.6 ± 8.8	56.9 ± 10.5	37.6 ± 13.1
Valine	48.1 ± 6.1	64.7 ± 8.2	50.4 ± 7.9
Dispensable			
Alanine	32.7 ± 3.9[c]	68.3 ± 4.7[a]	44.9 ± 5.0[b]
Aspartic acid	17.5 ± 8.2[ab]	42.3 ± 10.4[a]	11.8 ± 12.5[b]
Cysteine	30.3 ± 15.6	42.5 ± 19.2	23.0 ± 20.8
Glutamic acid	49.8 ± 6.0	61.1 ± 5.3	42.2 ± 10.2
Glycine	17.7 ± 6.9[ab]	34.9 ± 13.4[a]	8.3 ± 8.5[b]
Proline	32.1 ± 4.0[c]	61.0 ± 5.1[a]	46.4 ± 5.3[b]
Serine	45.4 ± 20.9	81.1 ± 2.3	63.1 ± 20.5
Tyrosine	44.0 ± 0.6[b]	77.5 ± 2.1[a]	49.0 ± 2.8[b]
Mean	44.4 ± 16.7	62.0 ± 14.0	43.3 ± 19.5

[1]Mean values ± SD (n = 3), values in each row with different letters are significantly different ($P < 0.05$).

higher in CGM than in CM or DDGS, while availability of these amino acids in CM was significantly below their availability in DDGS. AAAA of aspartic acid and glycine in CGM was significantly higher than in DDGS but not different ($P > 0.05$) than availability of these amino acids in CM. No other significant differences were detected in AAAA among ingredients. Statistically significant differences in mean AAAA of amino acid groups also were not identified among ingredients (Table 9).

Table 9. Apparent availability coefficients for Florida pompano of amino acids in the test ingredients, by amino acid type (mean percentage availability ± SD)

Group[1]	CM	CGM	DDGS
Acidic	33.6 ± 22.9	51.7 ± 13.3	27.0 ± 21.5
Basic	49.7 ± 3.6	58.4 ± 10.3	38.5 ± 10.6
Non polar	50.9 ± 20.0	69.0 ± 8.0	55.0 ± 17.0
Uncharged polar	36.4 ± 12.2	58.6 ± 20.5	36.2 ± 21.5

[1]Acidic: aspartic acid, glutamic acid; Basic: arginine, histidine, lysine; Non polar: alanine, isoleucine, leucine, methionine, phenylalanine, proline, valine; Uncharged polar: cysteine, glycine, serine, threonine, tyrosine.

3.4 Discussion

Results indicated that protein digestibility of CGM was significantly higher than that of DDGS, but not significantly different from protein digestibility of CM, suggesting one or more negative attributes of DDGS that could reduce its value as a protein source for pompano diets. DDGS contains 9-11 percent fiber but no antinutritional factors other than phytic acid which, as the primary storage form of phosphorus in plants, is ubiquitous in feedstuffs of plant origin (National Research Council, 2011). Phytic acid can bind to protein, amino acids, and minerals in the diet causing decreased availability (Denstadli et al., 2006). CM contains 12-13 percent cellulose fiber, 1-2 percent indigestible oligosaccharides in dehulled meal (higher in common meal) and, in addition to phytic acid, a number of antinutritional factors (i.e., tannins, sinapine, glucosinolates) that can reduce the nutritional value of CM by negatively affecting digestion and nutrient absorption (Burel and Kaushik, 2008). However, many of these antinutritional compounds are located in the canola seed hull and concentrations are greatly reduced in dehulled CM of the type used in this study. In comparison, CGM contains less than three percent fiber and no antinutritional factors (Pereira and Olivia-Teles, 2003).

High quantities of indigestible starch, fiber, and antinutritional factors in a feedstuff can be expected to negatively affect protein and energy digestibility. Figure 1 confirms the inverse relationship between the starch (NFE) and fiber content of CM, CGM and DDGS, and the protein and energy digestibility of these ingredients. ACPD was negatively correlated (r = -0.91) with NFE content (i.e., CGM > CM > DDGS), while AED was negatively correlated (r = -0.97) with fiber content (i.e., CGM > DDGS > CM; Figure 1).

Plant products incorporated into feeds as protein sources also contain carbohydrates and fiber. Many fishes have limited ability to digest carbohydrates and no ability to digest fiber. In the current study, increased dietary NFE was associated with decreased ACPD, supporting the contention that dietary carbohydrate level affects protein digestion. The mechanism that produces this effect is thought to be primarily physical. Nutrient absorption is maximized when there is sufficient gut-retention time to allow full digestion of foods and absorption of the nutrients released. Nutrients can be absorbed only if they come in contact with appropriate cells lining the gastrointestinal tract. As in most carnivorous fishes, the gastrointestinal tract of

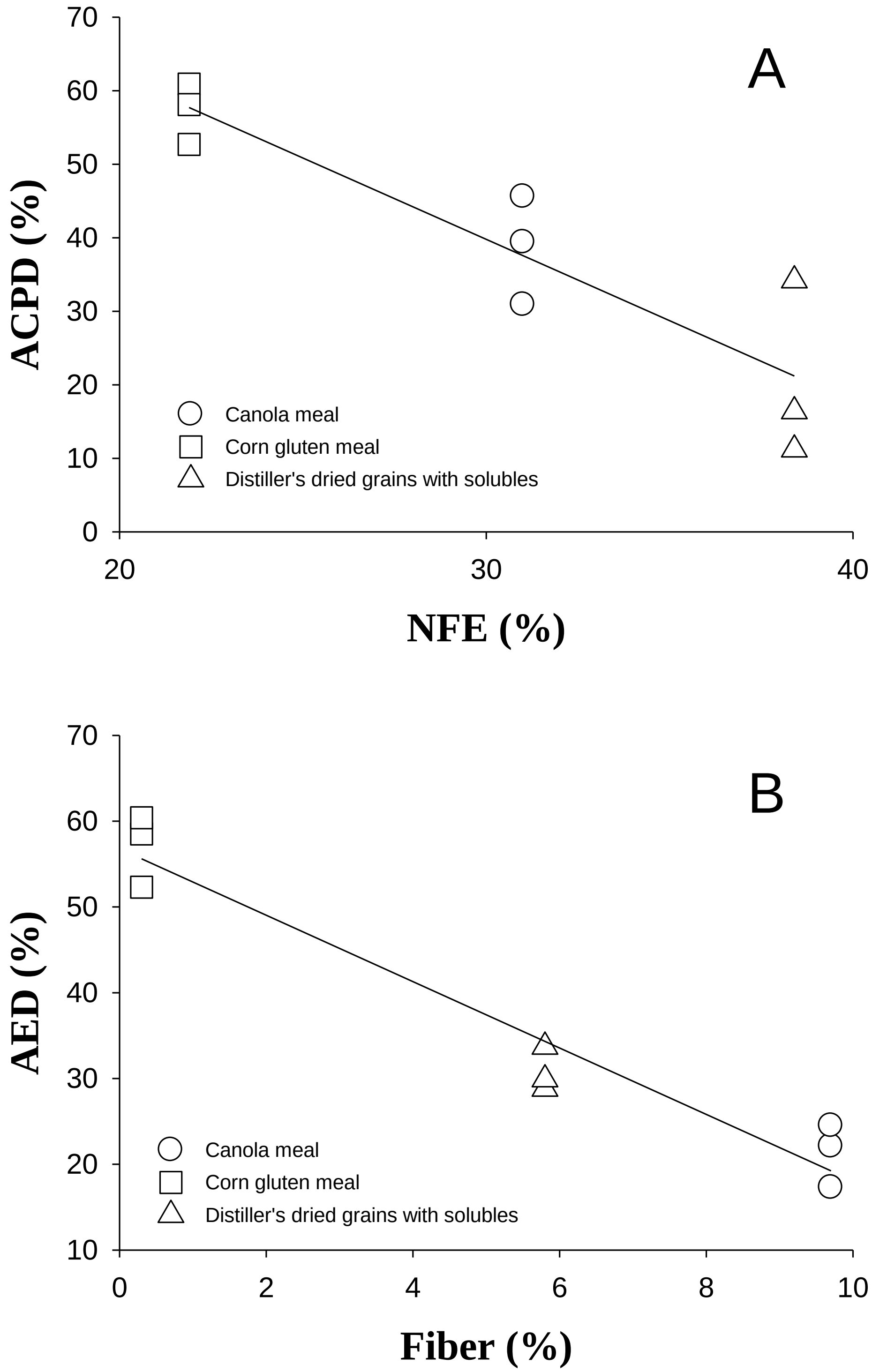

Figure 1. Regression of apparent crude protein digestibility (ACPD) versus nitrogen-free extract (NFE; Plot A; r = -0.91) and apparent energy digestibility (AED) versus fiber (Plot B; r = -0.97).

Florida pompano is short — less than one body length — which limits the gut-retention time to approximately three hours (personal observation). Carbohydrates, if present in sufficient quantity, can cause diarrhea in some fishes, further decreasing gut-retention time and reducing the time available for nutrient absorption. Carbohydrates also can affect digestibility by physically preventing digestive enzymes from contacting substrates in the intestinal lumen, thereby slowing the rate of food digestion and nutrient uptake. Fiber can have a similar physical effect on food digestion through interference with nutrient absorption and gut-retention time. However, the negative correlation of AED with fiber content in Figure 1 is a direct effect of fiber indigestibility. Fiber constitutes a portion of the gross energy of most diets but has no digestible energy value to mono-gastric species, including most fishes. Because the energy in fiber is unavailable, the AED of a diet typically decreases as fiber content increases. In addition, fiber, like carbohydrate, can affect gut-retention time and interfere with nutrient absorption (Krogdahl et al., 2010).

Mean ACPD and AED coefficients reported in this study were lower than those previously reported for Florida pompano and some other carnivorous fishes (Table 10). Riche and Williams (2010) reported protein and energy digestibility of CGM to be 82-83 percent and 77 percent, respectively, for Florida pompano cultured in brackish water (3 g/L or 28 g/L salinity). Williams (2008) reported protein and energy digestibility of DDGS to be 54-60 percent and 63-66 percent, respectively, for Florida pompano. Notwithstanding differences in the numerical values of coefficients determined in this study, and those of Riche and Williams (2010) and Williams (2008), results of these studies lean toward CGM having greater apparent digestibility for Florida pompano than DDGS.

Table 10. Apparent crude protein (ACPD) and energy (AED) digestibility coefficients for various fish species

Ingredient	Species	ACPD	AED	Source
CM				
	Florida pompano	38.6	21.3	Current study[3]
	Atlantic cod	76.0	60.6	Tibbetts et al., 2006[3]
	Atlantic salmon	74.1		Anderson et al., 1992
	Chinook salmon	84.5	64.5	Hajen et al., 1993
		79		NRC, 1993
	Cobia	89.0	83.1	Zhou et al., 2004[3]
	Haddock	83.0	60.1	Tibbetts et al., 2004[3]
	Rainbow trout	90.9	76.4	Burel et al., 2000
	Red seabream	83	44	Glencross et al., 2004[3]
	Silver perch	83.0	58.1	Allan et al., 2000a
	Turbot	82.9	69.3	Burel et al., 2000[3]
CGM				
	Florida pompano	57.2	57.1	Current study[3]
		81.9	77.4	Riche and Williams, 2010[1,3]
		83.4	77.4	Riche and Williams, 2010[2,3]
	Atlantic cod	86.3	82.7	Tibbetts et al., 2006[3]
	Atlantic salmon	83.1		Anderson et al., 1992
	Cobia	94.4	94.2	Zhou et al., 2004[3]
	Haddock	92.3	80.7	Tibbetts et al., 2004[3]
	Rainbow trout	87		NRC, 1993
		91.0		Halver and Hardy, 2002
		97.3		Sugiura et al. 1998
		87.4	80.0	Cheng and Hardy, 2003
	Rockfish	92	89	Lee, 2002[3]
	Silver perch	95.4	104.5	Allan et al., 2000a
DDGS				
	Florida pompano	20.6	30.7	Current study
		54.0	63.5	Williams, 2008[1]
		60.5	66.2	Williams, 2008[2]
	Rainbow trout	85	51	Williams, 2008
		72		Halver and Hardy, 2002
		90.4		Cheng and Hardy, 2004

[1]3 g/L salinity.
[2]28 g/L salinity.
[3]Studies used in Figure 2.

Burel and Kaushik (2008) reported that protein digestibility of CM is greater than 80 percent for fishes; however, energy digestibility can vary widely, from 21-83 percent. CM appears to be well digested by some fish but was not digested well by pompano in this study. Protein digestibility of CM, however, was similar to that of CGM and higher than protein digestibility of DDGS.

It is assumed in digestibility measurements that diet digestibility is the sum of the digestibility of individual diet ingredients. Thus, the ADC of a given nutrient in a diet should be calculable by summing the proportional ADCs for that nutrient in each of the individual ingredients composing the diet (Glencross et al., 2007). Based on the protein and energy ADCs of fish meal, soybean meal and soy protein concentrate established in previous feeding trials in this laboratory, the ACPD of the reference diet used in this study should have been approximately 90 percent. However, ACPD and AED of the reference diet were calculated to be 67 percent and 55 percent, respectively, indicating that ADCs for protein and energy in the reference diet ingredients were not additive in the current study. ADCs also are assumed to be constant, regardless of test ingredient inclusion level, and to be unaffected by the inclusion levels of other ingredients. However, in practice, interactions among diet ingredients do occur and the effects of such interactions on diet digestibility and nutrient availability can be difficult to predict. In the current study, apparent protein and energy digestibility of fish meal, soybean meal and soy protein concentrate were not as high, when combined in the proportions present in the reference diet, as has been shown possible for these ingredients in other studies with Florida pompano in this laboratory.

There are numerous factors that can affect digestibility measurements, including diet composition, feed intake, fish size and fecal collection method, among others (National Research

Council, 2011). It can be difficult to determine the reasons for variations in nutrient digestibility measurements among laboratories, or even within a laboratory during a period of time, although lack of methods standardization is a factor. For example, the 25 percentage-point difference between the protein digestibility of CGM obtained in the current study and that reported by Riche and Williams (2010) could be associated with the use of very dissimilar reference diets in the two studies, since culture methods were similar.

There is no universally accepted reference diet for digestibility trials. Reference diets vary in composition depending on many factors, including the experimental objectives of a research project. The reference diet used in this study contained a higher percentage of protein from plant products and a lower percentage of protein from animal products than reference diets previously used in digestibility studies with Florida pompano and some other marine species (Table 10). Figure 2 illustrates the relationship between dietary protein source (animal and plant), among reference diets used in several published digestibility studies with Florida pompano and other marine species (Table 10), and calculated protein digestibility of CM and CGM. Similar information for DDGS was not available in the literature.

ACPD of CM and CGM in this study — 39 percent and 57 percent, respectively — were considerably lower than ACPD coefficients previously reported for these ingredients fed to Florida pompano and other marine species (Figure 2). Reference diets reported by Burel et al. (2000), Lee (2002), Glencross et al. (2004), Tibbetts et al. (2004), Zhou et al. (2004), Tibbetts et al. (2006), Riche and Williams (2010) were formulated to contain high (greater than 60 percent) concentrations of dietary protein from animal sources and therefore low levels of crude protein from plant materials. In the current study, however, animal (fish meal) protein and plant (soybean and wheat) protein sources provided similar quantities of dietary protein. This suggests

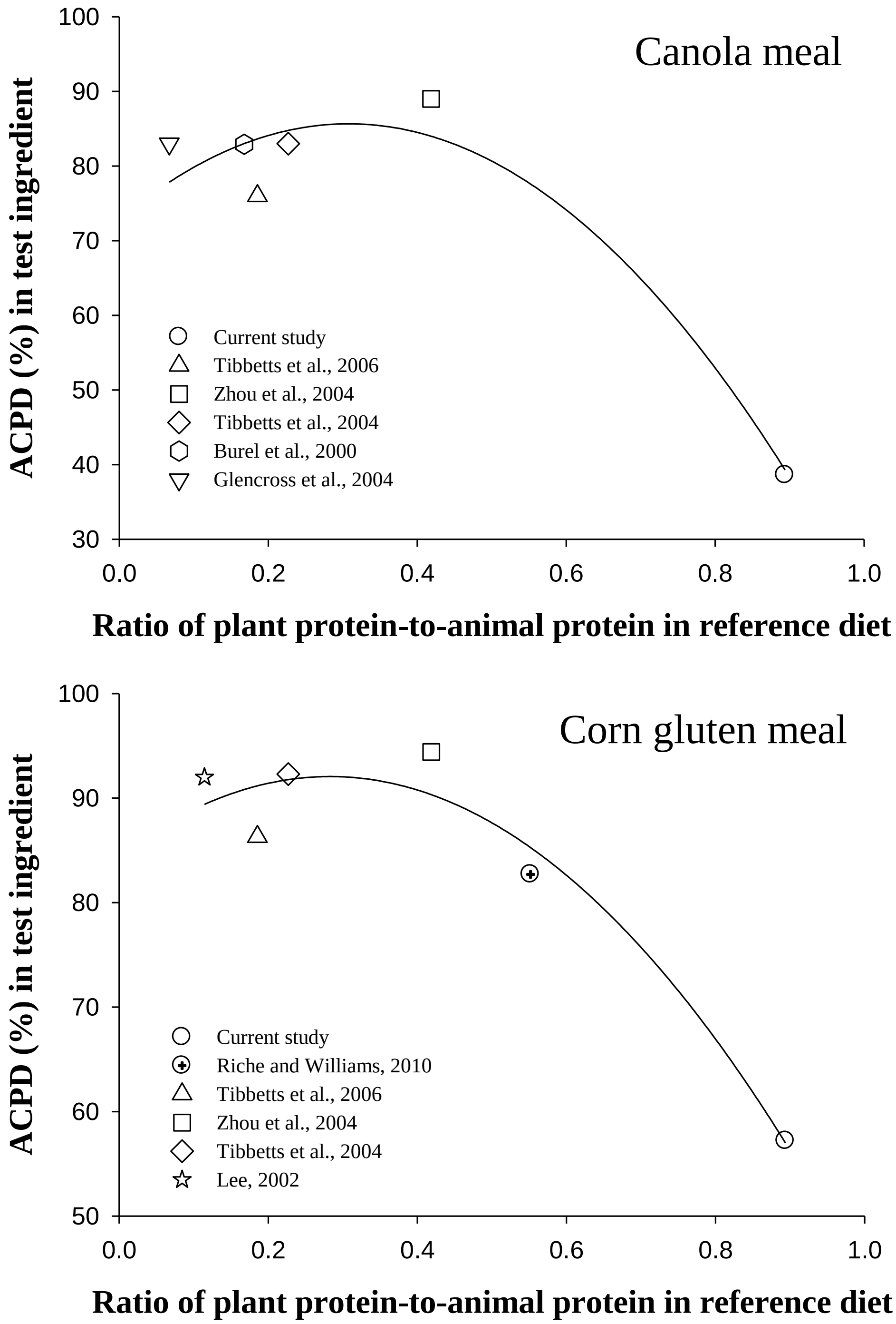

Figure 2. Plot of apparent crude protein digestibility (ACPD) versus ratio of dietary crude protein provided by plant and animal products in the reference diet of this study and several published studies (Table 10) with Florida pompano and other marine species fed canola meal (top) or corn gluten meal (bottom). Increasing quantity of plant protein in the reference diet is associated with reduced ACPD. Curves represent a second order polynomial.

that the quantity of plant-protein supplements in a reference diet may affect the apparent digestibility of protein in a test ingredient mixed with that diet in a 70/30 ratio. Thus, it is possible that reference diets containing high levels of crude protein from plant ingredients may yield lower ACPD coefficients for test ingredients combined with them than reference diets that contain high levels of animal protein products. It is not possible to prove this hypothesis with the data collected in the current study, but a strong relationship between reference diet composition and digestibility of protein in plant products fed to Florida pompano is indicated by the results of the current study.

The current study was conducted over a five-week period to allow adequate collection of fecal sample. The duration of the experiment could have contributed to the low ADCs reported in the current study. Negative effects of plant products are likely magnified as exposure time is increased. ADCs only represent a snapshot of digestibility when conducted over a short period. Animals along with gut micro-flora communities are able to adapt to diets increasing digestive efficiency, however chronic exposure to plant products could decrease digestive efficiency.

It should be remembered that there is no single, “true” digestibility value for any nutrient in a feedstuff. ADCs are variable and will fluctuate with multiple environmental and physiological factors. However, ADCs determined for the same species of fish fed the same feedstuff should be relatively consistent when the animal is cultured under similar conditions.

Results of the current study, when compared with work done with Florida pompano at other laboratories, indicate that nutrient availability of feedstuffs can vary considerably among studies, even when culture conditions, fish size, and experimental methods are similar. This suggests that efforts in recent years to improve the accuracy and precision of mathematical equations for digestibility calculations may address only part of the standardization problem.

Information on effects of ingredient combinations and ingredient-inclusion levels on the digestibility of individual feedstuffs also is needed to produce realistic nutrient-digestibility coefficients for use in practical diet formulation.

Published data on nutrient availability in feedstuffs is not only species-specific, but also diet-specific. Digestibility/nutrient availability is a function not only of the chemical composition of a feedstuff itself, but also of the chemical and physical composition of the larger diet of which it is a part. Thus, reference diet composition may be yet another significant factor that researchers should consider more closely when measuring nutrient digestibility/availability in feedstuffs.

Reference diets can be designed so that the digestibility data generated can be reliably applied in the formulation of practical diets with similar ingredient composition for fish production. As aquaculture continues to develop sustainable solutions to feeding fish more cost-effectively, knowledge of nutrient availability in feedstuffs will become increasingly important.

CHAPTER 4. SUMMARY AND CONCLUSIONS

Rising diet costs due, in part, to increasing fish meal prices has led to expanded research to identify less expensive alternatives for fish meal in aquafeeds. Inclusion levels of fish meal in aquatic animal diets are now at historic lows and declining (Hardy, 2010). However, growth performance of some fishes can be negatively affected when fish meal is entirely excluded from the diet. Plant products can provide suitable alternatives to fish meal if they possess an appropriate nutrient profile, are digestible, and are palatable in prepared diets. Identifying feedstuffs that meet all of these requirements can be difficult. Generalizations regarding the nutritional value of particular plant products in fish diets are problematic due to wide variation in food habits, digestive capabilities, and tolerance of plant-derived feedstuffs among fishes. Thus, identification of suitable replacements for dietary fish meal is species-specific research.

The goal of this research was to provide nutritional information to advance the development of all-plant diets for Florida pompano, an attractive aquaculture species in the United States that consistently demands a high market price. However, high costs associated with pompano culture have constrained commercial expansion for decades and United States aquaculture production of Florida pompano has not developed. All-plant diets for pompano could contribute to increased profitability and sustainability of pompano aquaculture, and make successful production of this popular species more economically feasible.

Soybean proteins are the most promising plant-based alternatives to fish meal based on nutritional profile. In the first experiment of this study, pompano achieved equivalent growth when fed all-plant-protein, soy-based diets or a similar diet containing 10 percent menhaden fish meal. Under the culture conditions of this study, equivalent growth with all-plant-protein, fish-meal-free (FMF) diets was achieved; however, limits to the inclusion level of soybean meal

(SBM) and soy protein concentrate (SPC) were evident. Florida pompano fed a zero percent SBM (59 percent SPC) diet experienced inferior growth compared with fish fed diets that contained a combination of SBM and SPC. Furthermore, diets containing 25-30 percent SBM (39-43 percent SPC) were superior to those containing 20 percent or 35 percent SBM (46 or 36 percent SPC). Differences in growth performance among fish fed these diets were attributed to reduced diet palatability, which is a problem with all-plant-protein diets (Kissil et al., 2000; Chatzifotis et al., 2008), and resultant differences in feed consumption.

Increasing the palatability of all-plant-protein diets is complicated by the fact that ingredients with high feeding-attractant properties are primarily animal by-products derived from fisheries, poultry, and livestock processing. Crystalline amino acids (CAA), typically produced by industrial fermentation techniques, can be added to diets as potential feeding attractants but the use of CAA must be cost-effective, and effective combinations of CAA vary among species (J. Caprio, personal communication). Several (unpublished) trials were conducted in our laboratory to evaluate the effectiveness of CAA as pompano feeding attractants. In each trial, a CAA mixture was top-sprayed on the M35/C36 diet (used in Chapter two of this volume) which was devoid of taurine and phytase supplementation (M35/C36-TP). Fish fed the M35/C36-TP diet achieved zero weight gain and minimal feed intake. It was observed that fish rejected the M35/C36-TP diet immediately after taking it into the mouth. Fish fed the M35/C36-TP diet plus CAA attractant had higher ($P < 0.05$) feed intake than fish fed the M35/C36-TP diet, but lower ($P < 0.05$) feed intake than fish fed the commercial control diet (unpublished data). In the final trial, increasing the concentration of CAA attractant and adding taurine produced better results; feed intake was not different ($P > 0.05$) between the control diet and the CAA-taurine supplemented M35/C36-TP diet.

Another cause for differences among diets, unrelated to their nutritional value, can be disrupted digestive enzyme activity and nutrient uptake. SBM, for example, contains chemical compounds that can induce enteritis (inflammation of the intestine), which reduces nutrient uptake due to deformed mucosal-cell membranes and impaired enzyme activity (Francis et al., 2001). SPC is devoid of enteritis-inducing compounds and therefore can usually be included in diets at higher levels than SBM (Walker et al., 2010). Results suggest that enteritis effects likely were minimal in this study, because growth did occur among pompano fed all diets that contained SBM compared with fish fed the one diet devoid of SBM.

Canola meal (CM), corn gluten meal (CGM), and distiller's dried grains with solubles (DDGS) are promising protein replacements for fish meal in compounded diets for various fishes. One method of evaluating the quality of an ingredient is to determine apparent digestibility coefficients (ADCs) of nutrients it contains. The second experiment in the current study showed apparent crude protein digestibility (ACPD) was higher ($P < 0.05$) in CGM than in DDGS, while protein digestibility of CM did not differ ($P > 0.05$) from ACPD of either CGM or DDGS. Apparent energy digestibility (AED) was highest in CGM, lowest in CM, and intermediate in DDGS ($P < 0.05$). Apparent amino acid availability (AAAA) also was determined for each ingredient. No apparent associations in AAAA were observed among ingredients with regard to individual amino acids or types of amino acids (acidic, basic, etc).

ADCs for two of the three ingredients evaluated in the current study were lower than values reported for those ingredients in previous digestibility studies with Florida pompano. This is not necessarily surprising considering ADCs can vary, depending on multiple factors. One source of variation could be differences in the reference diet formulation used in different experiments. Results of digestibility trials conducted in different laboratories could be made more comparable if a species-specific reference diet and/or a standardized "reference ingredient" were used. This might be accomplished by including an ingredient of "known" digestibility, such as a high quality fish meal, as one of the test ingredients. Incorporating an ingredient of known digestibility might allow results to be compared more effectively by providing a correction factor that could be applied to ADCs. However, selection of an appropriate reference ingredient with known digestibility for a particular species, under a specific set of culture conditions, could be difficult in practice. Also, agreement among laboratories on the standardization of a single reference diet for Florida pompano digestibility research is unlikely to occur soon. Nonetheless, improved standardization of research methods is needed to produce maximum benefits from digestibility and nutrient availability research with pompano and other species of aquacultural importance.

A major cause of decreased ADCs in plant-based reference diets and test ingredients could be carbohydrate levels. Feed production techniques can decrease the negative effects of carbohydrates in fish feeds. Heat treatment affects carbohydrate composition by breaking down complex (long chain) carbohydrates into smaller saccharides. Burel et al. (2000) determined heat-treated CM to have increased ACPD and AED compared to untreated CM when fed to turbot. Heat treatment also occurs when feeds are extruded. Unlike the pelletized feeds used in the current study, extruded feeds are manufactured at much higher temperatures. Cheng and Hardy (2003) determined extruded CGM to have increased AED compared to non-extruded CGM when fed to rainbow trout. Carbohydrate levels also can be reduced with ingredient processing. Like soybean meal, CM can be further processed into protein concentrates from which soluble carbohydrates are removed. Canola protein concentrates may be more suitable protein sources for Florida pompano diets than less-processed canola meal.

Meal size has been reported to negatively affect ADCs (Glencross et al., 2007). In the current study fish were fed to apparent satiation, as determined by observation, with multiple small feedings during a time period of less than one hour. All fish were allowed to achieve apparent satiety. Large meal sizes are thought to reduce feed efficiency by decreasing gut-transit time, which also can be negatively affected by carbohydrate and fiber in the diet. Effects of meal size may be magnified when feeding diets that contain high levels of plant proteins, which typically are accompanied by other, less-digestible compounds in practical feedstuffs. Digestibility may be improved when plant-based diets are fed in small quantities over extended periods of time; for example, with automatic feeders.

In addition to ADCs, knowledge of the nutritional requirements of the target species is necessary for effective least-cost diet formulation. While the database of ADCs for Florida pompano is growing, quantification of nutritional requirements is needed. Protein is the most expensive nutrient in fish feeds, which makes knowledge of minimal inclusion levels beneficial to fish producers and diet formulators. However, there is no requirement for a specific level of dietary protein, but rather requirements for individual amino acids that dietary protein provides.

The amino acid requirements of Florida pompano have not been determined, so in the current study the amino acid composition of all diets was designed to reflect as closely as possible the whole-body amino acid composition of Florida pompano. This approach appears acceptable even with dietary crude protein levels of 40 percent, as weight gain and feed conversion ratio (FCR) of Florida pompano in this study were improved compared to results reported by Lazo et al. (1998) for similar-size fish. Lazo et al. (1998) estimated that Florida pompano require at least 45 percent protein in the diet. Riche (2009) determined the minimal digestible protein in diets for Florida pompano to be 37 percent. Estimates of dietary protein “requirements” may be further reduced as amino acid requirements are determined and improved diet formulations with higher nutrient availability are developed.

Protein synthesis and other metabolic processes require adequate energy to function properly. If dietary energy from non-protein sources is inadequate, protein will be catabolized to meet energy needs. In fish diets, energy is commonly provided by lipids, often from fish oil, which is expensive. Plant proteins contain carbohydrates, a relatively inexpensive energy source if it is digestible. Accurately accounting for the AED of plant products allows diet formulators to reduce dietary lipid levels, which can reduce diet cost and provide ancillary benefits in the feed production process, such as improved pellet quality.

Least-cost feed formulation is most powerful when ADCs for a variety of ingredients are available. Digestibility coefficient databases should be updated and expanded as new information on useful feedstuffs becomes available, to maximize options as ingredient prices and availability fluctuate. A wide range of ingredient choices allows diet formulators to choose the most economical mix of available feedstuffs.

No plant product is capable of replacing fish meal in a 1:1 ratio. Therefore, a combination of plant ingredients and nutrient supplements will be necessary for all-plant diets to match the growth potential of fish-meal-based diets. Information from the current study provides encouraging results for development of all-plant, nutritionally complete diets for Florida pompano. Continued research on sustainable, economical diets for Florida pompano will assist the development of pompano aquaculture as a viable industry in the United States.

REFERENCES

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J., Rebecca, W., 2000a. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture 186, 293-310.

Allan, G.L., Rowland, S.J., Mifsud, C., Glendenning, D., Stone, D.A.J., Ford, A., 2000b. Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus* V. Least-cost formulation of practical diets. Aquaculture 186, 327-340.

Anderson, S.J., Lall, S.P., Anderson, D.M., Chandrasoma, J., 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in sea water. Aquaculture 108, 111-124.

AOAC (Association of Official Analytical Chemists), 2002. Official Methods of Analysis of AOAC International, 17th edition, March 2002 revision, (Horwitz, W., editor). AOAC International, Gaithersburg, Maryland.

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 13, 265-272.

Biswas, A.K., Kaku H., Ji, S.C., Seoka M., Takii K., 2007. Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. Aquaculture 267, 284-291.

Bonaldo, A., Roem, A.J., Pecchini, A., Grilli, E., Gatta P.P., 2006. Influence of dietary soybean meal levels on growth, feed utilization and gut histology of Egyptian sole (*Solea aegyptiaca*) juveniles. Aquaculture 261, 580-586.

Burel, C., Boujard, T., Tulli, F., Kaushik, S.J., 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

Burel, C., Kaushik, S.J., 2008. Use of rapeseed/canola in diets of aquaculture species. *In* Alternative protein sources in aquaculture diets. (Lim, C., Webster, C.D., Lee, C., editors). New York: The Haworth Press, Taylor & Francis Group. p343-403.

Chatzifotis, S., Polemitou, I., Divanach, P., Antonopouiou, E., 2008. Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet. Aquaculture 275, 201-208.

Cheng, Z.J., Hardy, R.W., 2003. Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture Nutrition. 9, 77-83.

Cheng, Z.J., Hardy, R.W., 2004. Nutritional value of diets containing distiller's dried grains with solubles for rainbow trout, *Oncorhynchus mykiss*. Journal of Applied Aquaculture 15, 101-113.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry and Physiology 73B, 25-41.

Deng, J.M., Mai, K.S., Ai, Q.H., Zhang, W.B., Wang, X.J., Xu, W., Liufu, Z.G., 2006. Effects of replacing fish meal with soy protein concentrate on feed intake and gorwth of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture 258, 503-513.

Denstadli, V., Skrede, A., Krogdahl, A., Sahstrom, S., Storebakken, T., 2006. Feed intake, growth, feed conversion, digestibility, enzyme activities and intestinal structure in Atlantic salmon (*Salmo salar* L.) fed graded levels of phytic acid. Aquaculture 256, 365-376.

Erdogan, F., Olmez, M., 2010. Digestibility and utilization of canola meal in angel fish (*P. scalare* Lichtenstein 1823) feeds. Journal of Animal and Veterinary Advances 9, 831-836.

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.

Forster, I., 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquaculture Nutrition 5, 143-145.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G.S., Krogdahl, A., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., Wurtele, E., 2007. Expanding utilization of sustainable plant products in aquafeeds: a review. Aquaculture Research 38, 551-579.

Glencross, B., Hawkins, W., Curnow, J., 2004. Nutritional assessment of Australian canola meals. I. Evaluation of canola oil extraction method and meal processing conditions on the digestible value of canola meals fed to the red seabream (*Pagrus auratus*, Paulin). Aquaculture Research 35, 15-24.

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.

Gonzalez-Felix, M.L., Davis, D.A., Rossi, W., Perez-Velazquez, M., 2010. Evaluation of apparent digestibility coefficient of energy of various vegetable feed ingredients in Florida pompano, *Trachinotus carolinus*. Aquaculture 310, 240-243.

Gothreaux, C.T., Reigh, R.C., Williams, M.B., Chesney, E.J., 2010. Amino acid availability of soybean meal for Florida pompano. North American Journal of Aquaculture 72, 309-313.

Hajen, W.E., Higgs, D.A., Beames, R.M., Dosanjh, B.S., 1993. Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 2. Measurement of digestibility. Aquaculture 112, 333-348.

Halver, J.E., Hardy, R.W., 2002. Appendix Table A.6. *In* Fish nutrition. Halver J.E., Hardy, R.W., editors. Third edition. San Diego: Academic Press. p780-783.

Hansen, A.C., Rosenlund, G., Karlsen, O., Koppe, W., Hemre, G.I., 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I – Effects on growth and protein retention. Aquaculture 272, 599-611.

Henken, A.M., Kleingeld, D.W., Tijssen, P.A.T., 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell, 1822). Aquaculture 51, 1-11.

Kader, M.A., Koshio, S., Ishikawa, M., Yokoyama, S., Bulbul, M., 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. Aquaculture 308, 136-144.

Khan, M.A., Jafri, A.K., Chadha, N.K., Usmani, N., 2003. Growth and body composition of rohu (*Labeo rohita*) fed diets containing oilseed meals: parital or total replacement of fish meal with soybean meal. Aquaculture Nutrition 9, 391-396.

Kissil, G.W., Lupatsch, I., Higgs, D.A., Hardy, R.W., 2000. Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream *Sparus aurata* L. Aquaculture Research 31, 595-601.

Krogdahl, A., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquaculture Research 41, 333-344.

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

Lee, S., 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture 207, 79-95.

Matsunari, H., Furuita, H., Yamamoto, T., Kim, S.K., Sakakura, Y., Takeuchi, T., 2008. Effect of dietary taurine and cystine on growth performance of juvenile red sea bream *Pagrus major*. Aquaculture 274, 142-147.

McGoogan, B.B., Reigh, R.C., 1996. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. Aquaculture 141, 233-244.

Muirhead, S., 2011. Ingredient Market. Feedstuffs. April, 2011.

National Research Council (NRC), 1993. Nutrient Requirements of Fish. National Academy Press, Washington DC. 114pp.

National Research Council (NRC), 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington D.C. 376 pp.

Park, G.S., Takeuchi, T., Yokoyama, M., and Seikai, T., 2002. Optimal dietary taurine level for growth of juvenile Japanese flounder *Paralichthys olivaceus*. Fisheries Science 68, 824-829.

Pereira, T.G., Olivia-Teles, A., 2003. Evaluation of corn gluten meal as a protein source in diets for gilthead sea bream (*Sparus aurata* L.) juveniles. Aquaculture Research 34, 1111-1117.

Peres, H., Lim, C., 2008. Utilization of soybean products in diets of nonsalmonid marine finfish. *In* Alternative protein sources in aquaculture diets. (Lim, C., Webster, C.D., Lee, C., editors). New York: The Haworth Press, Taylor & Francis Group. p281-312.

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., Williams, T.N., 2010. 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.

Sajjadi, M., Carter, C.G., 2004. Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar* L.) fed a canola-meal-based diet. Aquaculture 240, 417-431.

Salze, G., McLean, E., Battle, P.R., Schwarz, M.H., Craig, S.R., 2010. Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Pachycentron canadum*. Aquaculture 298, 294-299.

Smith, R.R., Peterson, M.C., Allred, A.C., 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. Progressive Fish-Culturist 42, 195-199.

Sugiura, S.H., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159, 177-202.

Sullivan, J.A., Reigh, R.C., 1995. Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone saxatilis ♀ x Morone chrysops ♂*). Aquaculture 138, 313-322.

Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H., Ukawa, M., 2008. Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. Aquaculture 280, 198-205.

Takagi, S., Murata, H., Goto, T., Hatate, H., Endo, M., Yamashita, H., Miyatake, H., Ukawa, M., 2010. Necessity of dietary taurine supplementation for preventing green liver symptom and improving growth performance in yearling red sea bream *Pagrus major* fed nonfishmeal diets based on soy protein concentrate. Fisheries Science 76, 119-130.

Tibbetts, S.M., Lall, S.P., Milley, J.E., 2004. Apparent digestibility of common feed ingredients by juvenile haddock, *Melanogrammus aeglefinus* L. Aquaculture Research 35, 643-651.

Tibbetts, S.M., Milley, J.E., Lall, S.P., 2006. Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua* (Linnaeus, 1758). Aquaculture 261, 1314-1327.

Walker, A.B., Sidor, I.F., O'Keefe, T., Cremer, M., Berlinsky, D.L., 2010. Partial replacement of fish meal with soy protein concentrate in diets of Atlantic cod. North American Journal of Aquaculture. 72, 343-353.

Webster, C.D., Thompson, K.R., Metts, L.S., Muzinic, L.A., 2008. Use of distiller's grains with solubles and brewery by-products in fish and crustacean diets. *In* Alternative protein source in aquaculture diets. (Lim, C., Webster, C.D., Lee, C., editors). New York: The Haworth Press, Taylor & Francis Group. p475-499.

Weirich, C.R., Groat, D.R., Reigh, R.C., Chesney, E.J., Malone, R.F., 2006. Effect of feeding strategies on production characteristics and body composition of Florida pompano reared in marine recirculating systems. North American Journal of Aquaculture 68, 330-338.

Williams, T.N., 2008. An assessment of alternative feed ingredients in practical diets for Florida pompano (*Trachinotus carolinus*) held in low salinity recirculating systems. Master's thesis. University of Maine, Orono, Maine, USA.

Zhao, H., Jiang, R., Xue, M., Xie, S., Wu, X., Guo, L., 2009. Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) less than two grams. Aquaculture Nutrition 16, 648-653.

Zhou, P., Zhang, W., Davis, D.A., Lim, C., 2010. Growth response and feed utilization of juvenile hydrid catfish fed diets containing distiller's dried grains with solubles to replace a combination of soybean meal and corn meal. North American Journal of Aquaculture 72, 298-303.

Zhou, Q., Tan, B.P., Mai, K.S., Lui, Y., 2004. Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum*. Aquaculture 241, 441-451.

APPENDIX A: QUARANTINE PROTOCOL

Wild Florida pompano, *Trachinotus carolinus*, were collected with a beach seine at Holly Beach and Grand Isle, Louisiana. After capture, fish > 1-cm, were transported to the Aquaculture Research Station at the Louisiana State University Agricultural Center, Baton Rouge, Louisiana. Upon arrival, fish were slowly acclimated to the quarantine system water. Water exchanges were achieved by draining 25-50 percent of the hauler water and replacing it with system water. This was repeated every hour, over at least six-hours to allow for proper acclimation. A slow acclimation was necessary to avoid stress related mortality which was observed in previous collections. After acclimation, fish were treated in a separate tank with a three-hour potassium permanganate bath (2 mg/L) to destroy any external opportunistic bacteria. After the bath, fish were roughly sorted and placed in separate floating cages in the quarantine tank. The quarantine system consisted of one, 10,000-L, circular tank, filled to 4,500-L; a biological bead filter; and a 120-watt ultraviolet-light sterilizer. Salinity was maintained at 12 g/L with synthetic sea salt. The temperature was maintained with outside ambient heat. Cutrine was added to the system at 0.25 mg Cu^{2+}/L and maintained for 30 days to remove monogenetic trematodes and parasites. Each cage was given an initial and weekly, one-hour, formalin bath (150 mg/L) in a separate tank. Cutrine addition ceased after 30 days and Cu^{2+} dissipated within two days. On day-33, the water level was reduced by half and a 24-hour praziquantal bath (2.5 mg/L) was given to remove gastric parasites and worms. The water level was returned and on day-35, fish were transported into the experimental and grow-out culture systems. During quarantine, fish were fed a marine starter diet (Aquaxcel, Cargill, Franklinton, Louisiana) twice daily and water exchanges of approximately five percent of system volume were performed daily.

APPENDIX B: DIGESTIBILITY EQUATIONS

Equation (1):

$$ADC\ (\%) = 100 – [((\%\ indicator_{diet} / \%\ indicator_{feces}) \times (\%\ nutrient_{feces} / \%\ nutrient_{diet})) \times 100]$$

Equation (2):

$$ADC\ (\%) = (100/30) \times [test – (70/100 \times reference)]$$

where *test* = the ADC of the nutrient of interest in the test diet; *reference* = the ADC of the same nutrient in the reference diet.

Equation (3):

$$ADNC_{ingr} = [(a+b) \times ADCN_{com} – (a) \times ADCN_{ref}] / b$$

where *ADCN* = apparent digestibility of the nutrient of interest; *ingr* = ingredient of interest; *(a+b)* = level of nutrient in the combined (test) diet; *com* = combined diet (ref + test ingr); *a* = nutrient contribution of the reference diet to nutrient content of the combined diet [calculated as: (level of nutrient in reference diet) x (100 – level of test ingredient in combined diet)]; *ref* = reference diet; *b* = nutrient contribution of the test ingredient to nutrient content of the combined diet [calculated as: (level of nutrient in test ingredient) x (level of test ingredient in combined diet)].

VITA

Gregory P. Lech was born on September 8, 1986, in Pennsylvania. In east Philadelphia Gregory was born and raised and fishing is where he spent most of his days. He attended Mansfield University of Pennsylvania and earned a Bachelor of Science degree in fisheries biology and a minor in chemistry. While attending Mansfield University, Gregory interned with Rutgers University Shellfish Research Laboratory and the New Jersey Department of Agriculture. Gregory began his graduate studies at Louisiana State University, Baton Rouge, Louisiana, in January 2009 under the tutelage of Dr. Robert Reigh. Gregory is currently a candidate for a Master of Science degree in fisheries at Louisiana State University.