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Apparent Digestible Protein, Energy, Phosphorus, and Amino Acid Availability of a Novel Strain of Soybean Meal for Juvenile Cobia, Rachycentron canadum

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APPARENT DIGESTIBLE PROTEIN, ENERGY, PHOSPHORUS, AND AMINO ACID AVAILABILITY OF A NOVEL STRAIN OF SOYBEAN MEAL FOR JUVENILE COBIA, *RACHYCENTRON CANADUM*

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Apparent Digestible Protein, Energy, Phosphorus, and Amino Acid Availability of a Novel Strain of Soybean Meal for Juvenile Cobia, *Rachycentron Canadum*. (August 2012)

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Apparent digestibility coefficient values of a novel strain of non-GMO soybean meal, Navita®, and an industry standard soybean meal (defatted soybean meal/roasted solvent-extracted), were evaluated for juvenile cobia, *Rachycentron canadum*. During the course of this 4-week feed trial, it was determined that Navita® has a high degree of protein and energy digestibility as well as amino acid availability, outperforming the regular soybean meal for nearly every analyzed component of the feed. Crude protein digestibility for the ingredients Navita® and regular soybean meal were calculated to be 81.83% and 68.51%, respectively, while energy digestibility values were found to be 62.65% and 38.83%, respectively. Amino acid availability for the Navita® ingredient ranged from 68.32-108.68 %, while amino acid availability for the RSBM ingredient was between 41.48-97.85%. The findings of this trial suggest that Navita® has high potential to serve as a fishmeal replacement in aquaculture feeds for cobia.

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CHAPTER 1: INTRODUCTION

BACKGROUND:

Cobia, *Rachycentron canadum*, is a tropical pelagic fish that has been recognized as a prime candidate for large-scale aquaculture production due to its relative ease of culture, rapid growth rates, and high-quality white meat. Although cobia aquaculture is leading the way for offshore aquaculture in the Americas (Benetti et al. 2007), a roadblock for the continued expansion of cobia aquaculture is being able to affordably and sustainably provide high-quality diets that fulfill the energetic and nutritional demands of these rapidly growing fish. Therefore, it is crucial to use an affordable, environmentally sustainable, high quality feed ingredients to ensure high aquaculture performance (growth and survival), without significant negative environmental impacts. Feeds account for the majority of production cost for most aquaculture operations, thus improving feed efficiency has become a priority for the aquaculture industry. Much research is currently being conducted to optimize aquaculture diets, balancing ingredient types, cost, and supply of ingredients.

At present, a major contentious issue in aquaculture involves the capture of small pelagic fishes for reduction into fishmeal (FM) and fish oil (FO). This process transforms small, low market value fish (most notably anchoveta, Chilean jack mackerel, Atlantic herring, chub mackerel, Japanese anchovy, round sardinella, Atlantic mackerel, and European anchovy) into high value products with agricultural and industrial applications (Naylor et al. 2000). FM is currently accepted as the best dietary protein source for aquaculture production of carnivorous fish because FM is

known for having high essential amino acid and fatty acid content, and low levels of carbohydrates and antinutritional 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). For these reasons, FM is presently the primary protein source in aquaculture feeds for carnivorous species.

A drawback to using FM for carnivorous fish aquaculture is that wild stocks of small pelagic fishes are straining under the pressure of widespread commercial fishing activities. In 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). And as the field of aquaculture continues to grow, so will the consumption of FM and FO, increasing demand and driving up prices (Naylor et al. 2009). Delgado et al. (2003) projected, under multiple possible scenarios, that the prices of FM and FO will rise significantly by the year 2020. This expanding consumption of small pelagic fishes for aquaculture has clearly resulted in detrimental effects to wild fish stocks. Over the past decade, capture of those small pelagic fishes that are exploited for reduction into FM and FO has leveled off and now has become essentially constant (see figure 1) and it has been estimated that these fisheries will remain static through the next decade (Pike and Barlow, 2003). In a review of FAO data detailing the state of major pelagic fish stocks, Tacon (2005) highlighted that there is no room for expansion of small pelagic fisheries as 52% of the worlds small pelagic stocks are fully exploited (at or very close to their maximum sustainable production limit), 17% are over exploited, 7% are depleted, and only 1% is recovering. This means that the worldwide small pelagic fishery is at a maximum level of exploitation and will not be able to

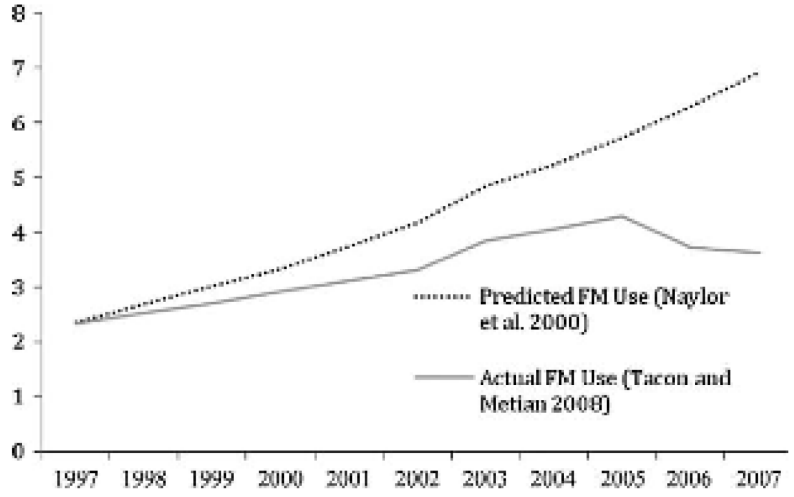

Figure 1: Predicted and actual global use of FM. (Welch et al. 2010; adapted from Kristofferson and Anderson, 2006)

provide higher quantities of FM in the future unless more stringent fishery management strategies are established that will allow stocks to recover. (Alder et al. 2008).

Another problem lies in the fact that aquaculture of carnivorous species is still perceived as a net fish consumer rather than producer. As a result of high FM and FO inclusion rates in aquaculture diets, many species require higher fish biomass inputs than what is generated at harvest (Naylor et al. 2000). The large quantities of FM and FO used for aquaculture and the state of current small pelagic fisheries has raised concerns about the long-term sustainability of the industry. In fact, it has been suggested that continued reliance on FM as a protein source in aquaculture feeds is both financially and environmentally unsustainable (Subasinghe & Phillips, 2007; Tacon & Nates, 2007).

Given the fact that aquaculture production is expected to grow significantly over the next decade and beyond, restrictions in the supply of FM and FO threaten to

limit the prospective viability, profitability, and expansion of the field. Therefore, to reap all of the potential benefits of aquaculture without causing a total collapse of the small pelagic fishery, many believe it is necessary to find a substitute source of high-quality protein for use in aquaculture feeds. This is accentuated by an escalating worldwide demand for safer and higher quality seafood products (FAO, 2006).

Reducing the inclusion of FM and FO in diets for aquaculture presents a significant obstacle for expansion of the field into the future, but various innovative animal- and plant-based protein and lipid sources with the potential to replace fish-based ingredients are being investigated now more than ever before. Specifically, the inclusion of plant-based substitutions to FM and FO is being extensively examined at this time, presenting notable advantages and drawbacks to their use. Benefits to using plant-based substitutions in aquaculture diets include high international availability at reasonable prices when compared to FM and FO, and the presence of nutritional properties that can satisfy the dietary requirements of many fish species (NRC, 2011). However, special care must be taken to ensure that inclusion of new ingredients into an aquaculture feed does not compromise the delivery of necessary nutritional components to the fish.

As described earlier, FM and FO provides essential amino acids and high levels of energy, which are necessary for healthy growth and development in fishes. In turn, reducing dietary levels of these ingredients would require supplementation of specific nutrients, such as fatty acids (especially EPA and DHA) and those essential amino acids that the plant-based substitutes do not provide, to ensure that the health of the fish is not compromised (Sargent et al. 2002). Also, the presence of

antinutritional factors in plant-based ingredients, such as toxins or antimetabolites, may result in adverse physiological effects on the fish that consume them (Francis et al. 2001). Moreover, changing the diet of an organism produced through aquaculture directly effects the nutritional quality of the final product sold to seafood consumers, so reducing in the inclusion of nutrients such as EPA, DHA, and Omega-3 would lower the beneficial nutritional results of seafood consumption for humans (Simopoulos, 2003). It is clear to see that many challenges and limitations to using plant-based substitutions to FM and FO in aquaculture feeds exist at this time.

One alternative to FM and FO that has gained popularity for use in aquaculture feeds is soybean meal (SBM) and soybean oil (SBO). SBM has been deemed to be among the highest quality plant-based ingredient because it is protein rich and contains an amino acid profile that 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 antinutritional factors, high levels of necessary fatty and amino acids. A novel strain of soybean that is hypothesized to exhibit these favorable traits, known as Navita® (produced by Schillinger genetics), is the focus of this digestibility trial.

PREVIOUS STUDIES:

Replacement of FM and FO with SBM and SBO in diets formulated for cobia aquaculture, as well as various other alternative protein sources, has been the subject of many recent scientific investigations. Chou et al. (2004) conducted a study to determine the maximum percentage of soybean meal (SBM) that could be used to replace FM in formulated aquaculture diets for cobia without diminishing production performance characteristics. Seven experimental diets were examined in this study with different SBM replacement percentages (0%, 10%, 20%, 30%, 40%, 50%, and 60%) and the cobia found all seven diets palatable. Detrimental effects on growth performance were clearly apparent when half of the FM protein in the diets was replaced by SBM. This study revealed a significant difference in fish weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (NPU) when the replacement level of SBM was increased from 40% to 50%, indicating that up to 40% of FM can be replaced by SBM without causing reduction in growth and protein utilization. However, when the collected data was analyzed by quadratic regression analysis, the optimal replacement level of FM with SBM was determined to be 16.9%. Also, FM replacement lead to changes is body composition characteristics, where lipid concentrations in the muscle increased significantly as dietary SBM increased.

Zhou et al. (2004) conducted a study comparing the apparent digestibility coefficients (ADCs) of various animal- and plant-based protein sources that are included in aquaculture diets for cobia. The protein sources tested this study were Peruvian FM, poultry meal, meat and bone meal, defatted SBM/roasted and solvent-

extracted, defatted SBM/solvent-extracted, peanut meal, rapeseed meal, and corn gluten meal, and the ADCs of dry matter (DM), crude protein (CP), crude lipid (CL), gross energy (GE), phosphorus, and amino acids were determined. Results of this study found that the ADCs of DM, CP, CL, and GE were higher for FM and corn gluten meal than the other ingredients tested. DM digestibility was higher for the animal-based ingredients (including FM) and corn gluten meal, ranging from 60.42-87.56%, than the SBMs and other plant-based ingredients, ranging from 58.52-70.51%. The ADC values of protein and lipid for both animal- and plant-based ingredients determined in this study were high, resulting in similar values (87.21-96.86% for animal-based ingredients and 88.97-96.93% for plant-based ingredients). Phosphorus ADC values were higher for the animal-based ingredients than the plant-based ingredients (62.36-71.22% and 56.32-69.76%, respectively). Generally, amino acid availability was higher for FM than all of the other ingredients tested.

Zhou et al. (2005) investigated the replacement potential of defatted SBM/roasted and solvent-extracted for reduction of FM inclusion in diets for juvenile cobia. This study, similar in design to that of Chou et al. (2004), determined that up to 40% of FM protein could be replaced by defatted SBM without causing a significant reduction in growth rate. At the replacement level of 20%, FCR was at a minimum and PER was at a maximum. For the seven diets tested in this study, there were no significant differences in the moisture, lipid, CP and ash content within the body composition and muscle of the cobia. However, there were significant differences in hemoglobin, hematocrit, red blood cell, plasma glucose and triglyceride concentration between fish fed diets with varying levels of SBM replacement. Additionally, this

study found that as the replacement level of SBM increased, so did the lipid content within the fish's liver. This is of particular concern because excess fat in the liver is a well-known problem for cobia produced through aquaculture, compromising the overall health of the fish. When the data collected in this trial was analyzed by quadratic regression analysis, the optimal replacement level of FM with SBM was determined to be 18.92%.

Lunger et al. (2007a) conducted trials examining the impacts of FM replacement with an organically certifiable yeast-based protein source with supplementation of methionine, tryptophan, and taurine to diets for juvenile cobia. For normal growth and metabolic functions, fish require methionine, an essential amino acid that many plant proteins lack. Taurine, an organic acid present in animal meals such as FM but absent from plant-based meals, may be required to improve weight gain and health when animal proteins are reduced. Therefore, this study aimed to determine if supplementation of these compounds would promote growth rates, feed efficiency, and survival for diets with increased levels of plant proteins. This was a follow-up study to Lunger et al. (2006), an investigation in which it was determined that the same yeast-based protein was able to replace 25% of FM without impacting production performance of cobia. When the yeast-based protein was supplemented with taurine, significantly higher weight gain and better feed efficiency was observed, but increasing amounts of the yeast-based protein led to decreased weight gains and feed efficiencies despite taurine supplementation. This study concluded that taurine supplementation does have a significant impact on growth and feed efficiency of juvenile cobia when they are fed diets containing high levels of plant-based proteins

as replacements for fishmeal, and that alternate proteins, especially those of plant and yeast-based origin can be incorporated at very high levels in diets for cobia with proper amino acid supplementation.

Another Lunger et al. (2007b) publication examined the impacts of various different organically certifiable alternate protein sources on growth, feed efficiency, biological indices, fillet proximate composition and fillet quality in juvenile cobia. Diets utilized in this study had 40% of the protein replaced with organically certified SBM, soybean isolate, hemp seed meal, or the same yeast-derived protein investigated in the previous study, as well as a diet containing 23% of each of the four plant-based ingredients with 8% FM and a diet containing 25% of each of the ingredients and no FM. This study found that all of the diets with 40% substitution of alternative proteins had similar weight increases regardless of the protein source used, as well as similar biological indices such as muscle ratio, visceral somatic index, packed cell volume, and fillet proximate composition for protein, lipid, dry matter, and ash. However, 40% substitution with the plant-proteins did affect fillet texture. The cobia fed the combined diet with 8% fishmeal exhibited significantly lower weight gain, specific growth rate, feed efficiency ratio values, muscle ratio, visceral somatic index, packed cell value, plasma protein, and a significantly higher visceral somatic index than all other fish. The fish fed the diet without any fishmeal did not survive to the end of the study. Results indicate that up to 40% fish meal protein can be replaced by any of the organically certifiable alternate proteins that were used in this study without detrimental impacts to weight gain, feed efficiency, biological indices, or fillet composition in juvenile cobia. Their results also suggest that alternate

proteins have differential effects upon final product quality, which may have implications in terms of cobia processing and development of industrial products.

In a study by Romarheim et al. (2008), the effect of SBM substitution on growth, feed conversion, and gastrointestinal (GI) tract development for juvenile cobia was examined. This research elaborated on the work of Baeverfjord and Krogdahl (1996) which examined SBM-induced enteritis in Atlantic Salmon, characterized by irregular folding of the intestines with increased connective tissue and loss of vacuolization in the absorptive cells in the intestinal epithelium, which leads to less efficient absorption of nutrients through the GI tract. The ingredients investigated in this study were a toasted defatted SBM, an untoasted defatted SBM, and a FM-based control. Feed intake of the FM and toasted defatted SBM diets were not significantly different, whereas the feed intake of cobias fed untoasted defatted SBM was significantly reduced. This study found that the specific growth rate and feed conversion ratio were not significantly different between cobias fed the FM and SBM diets, but significantly poorer results were obtained for the cobia fed the untoasted defatted SBM diet. This study also found no morphological differences in the GI tract could be attributed to the diets, and fish given SBM did not develop enteritis in the intestine.

A study conducted by Salze et al. (2010) attempted to formulate a diet for cobia using three inclusion levels of soy protein concentrate (50, 75, and 100% of dietary protein) and two levels of mannan oligosaccharide supplementation (0 and 0.3% of the total diet). Mannan oligosaccharides have been shown to improve intestinal development in cobia (Salze et al., 2008) and, therefore, may enhance

digestion and absorption of nutrients when dietary levels of FM are reduced. In this feed trial, the juvenile cobia performed well at levels of 75% FM replacement or less, while mannan oligosaccharide supplementation did not prove to be significantly beneficial for growth and health. In a second feed trial, FM was replaced by various combinations of alternative protein sources, with and without mannan oligosaccharide and amino acid supplementation. This trial attempted to formulate a diet with complete substitution of FM using soy protein concentrate, marine worm meal, and a yeast-based protein (identical to that used in Lunger et al., 2007) as well as mannan oligosaccharide. In this feed trial, the no-FM diet, formulated without amino acid supplementation, resulted in one of the best weight gains and feed efficiencies without elevated mortality or impacts on muscle or liver composition. However, it was determined that the ingredients in the no-FM diet would be too expensive to manufacture commercially, so it wouldn't be feasible to use at a production scale at this time. Nevertheless, this result illustrates the importance of the selection of high-quality ingredients when attempting to create aquaculture diets without the use of FM.

Saadiah et al. (2011) carried out a FM replacement trial in which poultry by-product meal was used for as a protein source substitution for juvenile cobia. Six diets were tested in this study with increasing levels of poultry by-product meal inclusion, ranging from 0-100% substitution. Upon comparison of growth performance, it was determined that weight gain, specific growth rate, and protein efficiency were not significantly affected by substitution of FM with any level of poultry by-product meal. The highest specific growth rate was observed for the diet with 60%

substitution of FM with the poultry by-product meal, outperforming the control diet that contained only FM as the protein source. Additionally, both of these diets resulted in similar FCR values. Data from this study proposes that the optimal level of FM replacement using poultry by-product meal is 60%, and that poultry by-product meal has the potential to be used for complete replace of FM in aquaculture diets for cobia.

Trushenski et al. (2011) conducted a soybean substitution trial evaluating the effects of replacing dietary FO with SBO for juvenile cobia. FO replacement has been particularly difficult to achieve for carnivorous marine species since FO provides long-chain polyunsaturated fatty acids, which promote growth performance as well as normal liver and cardiac function (Alexis 1997). In addition, FO inclusion serves the role of making a pelletized diet palatable enough for consumption sufficient to allow for healthy growth and development. This study found that production performance was generally unaffected by partial replacement of FO with SBO, but feed intake and final weight were significantly reduced when FO was completely excluded from the diet. Therefore, it was concluded that SBO can replace a substantial percentage of dietary FO, but juvenile cobia have a requirement for the long-chain polyunsaturated fatty acids found in FO. Alternative sources of essential fatty acids must be supplemented to the diet if levels of FO inclusion are greatly reduced.

OBJECTIVE:

The goal of this experiment is to investigate the nutrient digestibility of various aquaculture feed ingredients, including the meal of the soybean strain,

Navita® (Schillinger variety). This innovative soybean strain is characterized by particularly low levels of antinutritional factors (oligosacchaides, raffinose, and stachyose), which, when present, interfere with efficient metabolism and assimilation of nutrients (Francis et al. 2001; Glencross et al. 2007). This strain is also a natural hybrid (developed without genetic modification), which qualifies it to be usable in all of the world's fish consumer markets. Specifically, this project seeks to compare the apparent digestibility coefficients for crude protein, dry matter, energy (a measure of the combination of protein, lipids, and charbohydrates), and phosphorus as well as the nutrient availability for amino acids of Navita® SBM with an industry standard SBM (defatted and solvent-extracted containing residual amounts of anti-trypsin factor and phytates, cooked and typically used in poultry feeds), in addition to a reference diet containing Pollock FM. The results gathered in this experiment should have the potential to be used for formulating more biologically efficient and environmentally sustainable diets for the aquaculture production of cobia.

CHAPTER 2: MATERIALS AND METHODS

DIETS:

The diets examined in this experiment were formulated on an as-fed basis and are presented in Table 1.

Ingredient (g kg-1 dry diet)	Reference Diet	Test Diet
Pollock FM	612.6	428.8
Dextrin	120.0	84.0
Menhaden Oil	69.4	48.6
Mineral Premix[a]	50.0	35.0
Vitamin Premix[b]	40.0	28.0
Carboxymethyl cellulose	30.0	21.0
Celufill	73.0	49.6
Yttrium oxide	5.0	5.0
Test Ingredient		300.0

Table 1 Reference and test diet used to determine digestibility of crude protein, energy, and phosphorus, and the availability of amino acids from soybean meal (Navita, Schillinger variety), and regular soybean meal (defatted/roasted solvent-extracted) in juvenile cobia *Rachycentron canadum*.

[a] 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.

[b] 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-mix[c], 10.0; Cellulose, 874.1

[c] 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

The three diets investigated in this experiment were a Pollock FM diet (reference diet), and test diets containing regular SBM and Navita®. The reference diet was prepared to fulfill the basic dietary requirements of the experimental fish. The test diets were composed of 70% of the reference diet formulation and 30% of the test ingredients. Each of the experimental diets also contained 0.5% of an yttrium oxide (Y_2O_3) indigestible marker, necessary for digestibility analysis. The experimental feeds were prepared by mixing the dry ingredients in a Hobart mixer for 10 minutes. Then, the liquid ingredients were added and mixed for an additional 10

minutes. The ingredient mash was run through a meat grinder attachment to create strands, which were dried and broken by hand to the appropriate size. The experimental diets were vacuum-sealed in plastic bags, and stored in an air-conditioned room until they were fed. Chemical contents of the ingredients and experimental diets were obtained and are given in Table 2.

	Test Ingredients		Diets		
Proximate components	Navita® [1]	RSBM [2]	Ref. diet	Navita® diet	RSBM diet
Dry Matter	93.38	92.53	94.22	90.90	88.35
Crude Protein	52.01	49.61	42.90	44.69	41.88
Crude Lipid	1.51	1.84	11.25	7.86	7.69
Ash	5.56	6.19	13.51	10.57	10.59
Crude Fiber	5.96	3.80	7.15	5.03	3.95
Calcium	0.34	0.59	3.86	2.60	2.55
Phosphorus	0.79	0.86	2.31	1.78	1.75
Essential Amino Acids					
Arginine	3.36	3.28	3.08	3.21	2.72
Histidine	1.44	1.46	0.85	0.96	0.84
Isoleucine	1.85	1.66	1.25	1.56	1.58
Leucine	2.77	2.56	2.02	2.48	2.52
Lysine	2.78	2.50	1.89	2.81	2.88
Methionine	0.03	0.03	0.90	0.84	0.86
Phenylalanine	8.10	7.39	3.74	4.05	4.52
Threonine	1.57	1.51	2.23	2.00	1.79
Tryptophan	0.00	0.00	0.00	0.00	0.00
Valine	2.20	1.94	1.74	1.91	1.82
Non-Essential Amino Acids					
Alanine	3.66	3.50	2.16	2,82	2.48
Aspartic acid [4]	5.33	5.43	3.87	4.39	3.72
Cysteine	0.90	0.98	0.91	0.49	0.46
Glutamic acid [5]	6.94	7.01	4.96	5.86	4.82
Glycine	1.96	1.86	4.35	3.37	3.03
Proline	2.28	2.03	2.20	2.21	1.92
Serine	2.17	2.16	1.90	2.01	1.71
Tyrosine	1.52	1.37	1.06	1.23	1.18
Taurine	0.02	0.00	0.58	0.37	0.34

Table 2 Analysed composition (%) of test ingredients and experimental diet fed to cobia *Rachycentron canadum* (values expressed on an as fed basis)

[1] Navita®, Schillinger variety
[2] Regular soybean meal. Defatted/ roasted solvent-extracted
[3] Aspartic acid + asparagine
[4] Glutamic acid + glutamine

ANALYTICAL PROCEDURE:

Dry matter, ash, crude protein, crude lipid, and crude fiber levels in the diets and feeds were determined according to the Association of Analytical Communities official methods (AOAC 2000). Dry Matter was measured gravimetrically after drying for 16 hours at 105^{o}C in a Thermo Scientific Precision oven (model 6522, Thermo-Fisher Scientific, Marietta, OH USA). Ash content was determined by combusting samples for 6 hours at 600^{o}C in a Thermolyne Benchtop Muffle furnace (model FA8050, Thermo Scientific, Ashville, NC USA) and measured gravimetrically. Crude Protein (N x 6.25) was measured on a LECO elemental nitrogen analyzer (model FP-528, LECO Corp., Lakeview, MI USA). Crude Lipid was determined by petroleum ether extraction with an Accelerated Solvent Extractor (model 200, Dionex Corporation, Bannockburn, IL USA). Crude Fiber was measured gravimetrically. Phosphorus and calcium was analyzed by inductively coupled plasma atomic emission spectroscopy using a Model Atomscan 16 radial configuration instrument (Thermo Jarrel Ash, TJA Solutions, Franklin, MA, USA). Amino acid profiles of ingredient and diets were analyzed using an Agilent HPLC (model 1200 Series, Agilent Technologies, Inc, Santa Clara, CA USA) as detailed in Ju et al (2008).

EXPERIMENTAL CONDITIONS:

The juvenile cobia used in this experiment were spawned and reared at the University of Miami Experimental Hatchery (UMEH). The cobia (av. wt 286.75 grams at the beginning of the experiment) were randomly distributed into a flow-through system consisting of three 4,500-l cylindrical fiberglass tanks, each tank

containing two air stones and an oxygen stone, at a stocking rate of 80 fish per tank. Each tank was fed 2% of total biomass per day, divided into equal morning and afternoon rations. Sand filtered well water and seawater was pumped into each tank with an exchange rate of 1600% per day. The tanks were siphoned daily and the system was backwashed every other day. Throughout the 4-week experimental period, water temperature values were 24.4 ± 0.3°C, salinity was 34-38 ppt, and dissolved oxygen was maintained above 6.3 mg/L.

FECAL COLLECTION:

Prior to the onset of the fecal collection period, the fish were fed their respective diets for a week as an acclimatization period. After that week, feces were collected by means of manual stripping technique, deemed as the best fecal collection method for digestibility studies (Rawles et al. 2010; Glencross et al. 2007; Vandenberg and de la Noue 2001). The fish were fed approximately 3.5 hours prior to fecal collection (the optimal amount of time for feed digestion as determined by a preliminary fecal collection trial). Collection of feces from approximately twenty fish per tank provided sufficient digested material for the analyses.

The fish were individually netted out of the tanks and ~5 mL of a 4 ppt MS-222 solution was sprayed into their gills. The gills and mouth cavity were held closed until the fish were sedated and the muscles around the abdomen were relaxed. Then, the gills were rinsed with a gentle stream of seawater for approximately 30 seconds to rid the gills of residual anesthetic. To extract feces, gentle pressure was applied down the lower abdomen of the fish with the thumb and forefinger, extracting feces from its

distal intestinal tract. Care was taken to exclude urine, mucus, and other contaminants from the collection vessel. In a recovery tank, the fish were held over oxygen bubbles until they regained consciousness and were able to swim unassisted before they were returned to their respective tanks.

The collective fecal samples from each of the experimental diets was then pooled, placed in a drying dish, heated in an oven at 60^{o}C until the samples were dry, and then sent away to the Texas A&M University for analysis. Once sufficient digestant had been collected for laboratory analysis, the feed type given to each tank was rotated. This rotation was repeated three times so that digestant from each of the diets was collected from every tank.

DIGESTIBILITY DETERMINATION:

Apparent Digestibility Coefficients (ADCs) in experimental diets were calculated according to the following formulas:

$$ADC_{DM} \text{ of diet (\%)} = 100 * [1 - (\% \text{ marker in diet}/\% \text{marker in feces})] \quad (1)$$

$$ADC_{nutrient} \text{ of diet (\%)} = 100 * [1 - (\% \text{ marker in diet}/\% \text{ marker in feces})] * (\text{nutrient concentrations in feces/nutrient concentration in diet}) \quad (2)$$

$$ADC_{energy} \text{ of diet (\%)} = 100 * [1-(\% \text{ marker in diet}/ \% \text{ marker in feces})] * (\text{energy concentration in feces/energy concentration in diet}) \quad (3)$$

ADC's for ingredients were obtained with a combination of a reference diet and a test diet; the composition of each is given in table 1. To calculate the ADC of ingredients, the following formula was used according to NRC (2011).

$$ADC_{test\ ingredient} = ADC_{test\ diet} + ((ADC_{test\ diet} - ADC_{ref.\ diet}) * (0.7 * D_{ref}/0.3 * D_{ingredient})) \quad (4)$$

where D_{ref} equals the nutrient percentage of the reference diet, and $D_{ingredient}$ is the nutrient percentage of the test ingredient.

CHAPTER 3: RESULTS

RESULTS:

All of the experimental diets tested in this study were readily accepted (highly palatable) by the juvenile cobia. The ADC values and amino acid availability coefficients determined for the experimental diets examined in this trial are presented in Table 3.

ADC of Experimental Diets

Component	Navita® diet[1]	RSBM diet[2]	Ref. diet
Protein	75.70 ± 1.27	71.26 ± 3.21	72.60 ± 2.82
Energy	61.49 ± 0.15	54.28 ± 3.44	61.01 ± 0.66
Phosphorus	-39.75±13.18	36.52 ± 3.10	-92.76±11.85
Essential Amino Acids			
Arginine	96.48 ± 0.25	94.43 ± 0.54	92.60 ± 4.19
Histidine	80.13 ± 1.63	77.52 ± 1.55	76.39 ± 3.91
Isoleucine	80.48 ± 2.81	75.12 ± 0.75	83.33 ± 10.38
Leucine	82.48 ± 2.24	80.16 ± 0.75	86.30 ± 6.99
Lysine	81.53 ± 0.72	81.63 ± 0.51	88.31 ± 6.11
Methionine	79.98 ± 1.56	81.98 ± 1.36	81.49 ± 3.06
Phenylalanine	79.72 ± 1.48	76.54 ± 3.40	83.44 ± 4.47
Threonine	73.44 ± 0.57	64.49 ± 1.00	73.09 ± 8.95
Valine	78.37 ± 2.44	73.62 ± 0.57	80.58 ± 9.32
Non-Essential Amino Acids			
Alanine	85.00 ± 2.43	82.51 ± 0.42	83.87 ± 6.89
Aspartic acid [3]	76.27 ± 0.25	74.27 ± 2.47	63.52 ± 6.15
Cysteine	75.56 ± 3.14	65.96 ± 2.52	70.24 ± 11.79
Glutamic acid [4]	84.43 ± 1.28	81.20 ± 0.89	82.85 ± 5.42
Glycine	73.01 ± 1.30	72.31 ± 2.45	67.65 ± 8.79
Proline	77.15 ± 2.88	74.81 ± 1.25	75.71 ± 9.77
Serine	87.87 ± 0.42	77.20 ± 1.25	78.83 ± 10.32
Tyrosine	90.37 ± 6.89	87.57 ± 2.07	90.76 ± 2.53

Table 3 ADC values (%) of experimental diets. Data represents mean ± SD. Values in the same row with different superscripts are significantly different ($P<0.05$).

[1] Navita, Schillinger variety
[2] Regular soybean meal. Defatted/ roasted solvent-extracted
[3] Aspartic acid + asparagine
[4] Glutamic acid + glutamine

The ADC values and amino acid availability coefficients determined for the test ingredients examined in this trial are presented in Table 4.

	ADC of Ingredients	
Component	Navita® [1]	RSBM[2]
Protein	81.83 ± 3.79	68.51 ± 9.80
Energy	62.65 ± 0.50	38.83 ± 11.34
Phosphorus	90.12 ± 45.46	388.0 ± 11.5
Essential Amino Acids		
Arginine	102.61 ± 0.64	97.85 ± 1.56
Histidine	86.01 ± 4.21	79.16 ± 3.80
Isoleucine	76.24 ± 7.00	58.09 ± 2.32
Leucine	76.29 ± 5.87	64.78 ± 2.63
Lysine	68.32 ± 2.13	64.98 ± 1.77
Methionine	76.64 ± 5.02	82.02 ± 1.49
Phenylalanine	74.51 ± 3.55	73.12 ± 5.08
Threonine	74.19 ± 1.76	41.48 ± 3.68
Valine	74.53 ± 6.68	53.39 ± 1.82
Non-Essential Amino Acids		
Alanine	88.31 ± 9.50	80.12 ± 1.15
Aspartic acid[3]	90.96 ± 0.54	87.64 ± 5.54
Cysteine	77.24 ± 4.14	64.92 ± 3.13
Glutamic acid[4]	86.08 ± 2.62	78.84 ± 2.16
Glysine	100.54 ± 8.00	78.90 ± 5.93
Proline	80.14 ± 8.83	72.51 ± 4.49
Serine	108.68 ± 1.38	73.31 ± 4.22
Tyrosine	89.94 ± 14.37	82.79 ± 5.17

Table 4 ADC values (%) of test ingredients. Data represents mean ± SD. Values in the same row with different superscripts are significantly different (P<0.05).

[1] Navita, Schillinger variety
[2] Regular soybean meal. Defatted/ roasted solvent-extracted
[3] Aspartic acid + asparagine
[4] Glutamic acid + glutamine

Some anomalous ADC values were attained in this trial. For one, phosphorus digestibility figures gathered from our fecal samples are erroneous. While using the fecal stripping technique, it was very difficult to exclude secretions other than feces from the collection vessel. These unusual phosphorus values are likely due to the

excessive inclusion of urine, mucus, semen, and other impurities into the fecal samples, leading to interference with proper laboratory analysis. Therefore, conclusions about the digestibility of these dietary components cannot be drawn from the data collected in this trial.

DISCUSSION:

The data collected in this study indicated that the Navita® diet exhibited very high digestibility of protein, energy, and amino acids, out performing the regular SBM for nearly every component of the feed. The two SBM diets differed in regard to protein digestibility, with more highly digestible protein observed in the Navita® diet than in the regular SBM diet; in fact, protein digestibility was higher for the Navita® diet than the reference diet, which contained only Pollock FM, as well as the RSBM diet. Hence, incorporation of the Navita® ingredient into experimental feed increased the diets crude protein level as well as protein digestibility. Amino acid availability was also higher in the Navita® diet than the regular SBM diet, with at least marginal increases in all amino acids except for lysine and methionine. Amino acid availability increases of 5% or more between the Navita® and regular SBM diets were observed in isoleucine, threonine, valine, cystein, and serine.

Ingredient digestibility coefficients for protein followed a similar trend to those of the diets. Navita® had higher ADC values for protein than the regular SBM. Amino acid availability of the test ingredients was also higher for Navita® than regular SBM for all amino acids but methionine. Amino acid availability increases of 10% or more between the Navita® ingredient and regular SBM ingredient were

observed in isoleucine, leucine, threonine, valine, alanine, cystein, glycine, proline, and serine. The amino acid availability of the Navita® ingredient was significantly higher ($P<0.05$) than that of the regular SBM for eight of the 17 individual amino acids.

Across the board, the digestibility coefficients obtained in this study are lower than those described in a previous cobia digestibility experiment (Zhou et al. 2004). It was determined that these lower coefficients resulted from the use of a different fecal collection method. Glencross et al. (2007) contains an extensive discussion of fecal collection methods, including advantages as well as shortcomings of each technique. The three principal methods of collection that were described in this publication were collection by dissection (killing the fish to remove excrement from intestines), collection by manual stripping (applying gentle pressure down the lower abdomen of the fish causing it to expel its fecal contents), and collection of voided feces directly from the experimental tank (installing a fecal settling and removal apparatus to the bottom of the tank or siphoning excreted feces). When feces are collected by dissection or stripping, there is the potential to underestimate digestibility because of incomplete digestion and potential contamination of digested matter with foreign material. In contrast, when feces are collected from the water column or following settlement, there is the potential to overestimate digestibility due to leaching of organic matter into the surrounding environment. Glencross et al. (2007) identified stripping as the preferred fecal collection method for plant protein digestibility trials.

A study by Vandenberg and de la Noue (2001) compared the influences of these three fecal collection methods on digestibility determination. The findings of

that study suggested that collection of voided feces results in significantly higher diet digestibilities than those determined from fecal stripping techniques. Fecal stripping provides a more conservative estimate of both diet and ingredient digestibilities than that provided using settlement techniques. With this information in mind, we decided to use the fecal stripping technique due to ease of collection and to ensure that digestibilities are not overestimated.

Zhou et al. (2004) collected feces using settling columns (collection of voided feces from the water column), while we employed manual fecal stripping technique. It is stated in Glencross et al. (2005) that feed ingredients containing high carbohydrate levels, including SBM, tend to result in diffuse fecal pellets, making collection from the water column difficult and inaccurate. According to NRC (2011), digestibility coefficients determined from indirect methods of feces collection are expected to be 5-9% higher than those determined from direct feces collection methods due to leaching of soluble nutrients into the water.

CHAPTER 4: CONCLUSIONS

Our findings suggest that Navita® has high potential to serve as a non-genetically modified fishmeal replacement in aquaculture feeds. More in-depth studies investigating the practical usage of this ingredient could provide a large forward step on the path to sustainable aquaculture production. Digestibility information, such as that gathered in this study, could effectively promote the use of ingredient substitutions in least-cost formulated diets for cobia.

It is undeniable that conducting investigations to optimize the replacement of FM with more sustainable protein options is necessary for the development of the field of aquaculture as there is clearly a need to reduce the dependence of the industry on wild fish stocks. However, a recent study found that reducing the inclusion of FM in diets for cobia could have detrimental effects on the health of the fish. Schock et al. (2012) used nuclear magnetic resonance (NMR) spectroscopy to analyze how specific nutrients from a diet are metabolized, giving insight on how components of a diet are utilized by the fish. This study found that cobia fed diets with increased inclusion of SBM as a protein source were metabolically different from those fed diets with higher inclusion levels of FM. Specifically, it was determined that fish fed reduced FM diets had higher levels of tyrosine and betadine, metabolites linked to physical stress, and lower levels of glucose, a primary energy source necessary for biological functions. This suggests that cobia consuming diets with increased inclusion of SBM do not receive the necessary nutritional requirements to support healthy growth. Additionally, the NMR spectroscopy revealed that the cobia fed a diet with only FM as the protein source contained significantly higher levels of lactate compared to the

cobia fed diets that included SBM. Lactate is the byproduct of bacteria in the gut metabolizing carbohydrates, so it can be surmised that a high FM-protein diet enhances gut microfauna activity in cobia. The researchers deduced that this increased gut microfauna activity might be one of the necessary conditions for optimal cobia growth. Reinforcing this conclusion, the cobia in this study that were fed the diet containing FM as the only protein source exhibited more rapid growth when compared to the fish fed diets in which FM was replaced with SBM.

Along the same lines as the previously mentioned study, some experts in the field believe that increasing the inclusion of SBM in aquaculture diets is not the correct solution for increasing sustainability and developing production for cobia. Formulation of diets incorporating the highest quality FM possible would lead to lower FCRs across the board, which would cause an increase in aquaculture production efficiency, a decrease in fish consumption of anti-nutritional factors, a reduction in the release of effluent wastes into the environment, and a more nutritious final product for human consumption. The scientific community is in need of detailed studies to determine if optimization of the FM included in aquaculture diets would be a more precise method of attaining sustainability than taking measures to reduce FM inclusion with substances the fish would not normally consume.

Essentially, recent innovations in aquaculture feed composition, including increased inclusion of plant based meals and oils, are helping to reduce the reliance of aquaculture on wild fish stocks, which will allow the field to continue expanding into the future. Although replacement of FM in diets for cobia with alternative protein sources has become a hot topic in the field of aquaculture nutrition, further research

in some areas needs to be conducted to fully understand the implications of such replacement. First, dietary trials over a longer part of the production cycle and under varying environmental conditions may be required to clarify the effects of the inclusion of SBM in diets for cobia. At this point, the vast majority of FM replacement studies have been conducted using juvenile cobia, which have different nutritional and energetic demands than adult fish. This is important to note because the feed intake of adult cobia is much higher than that of juveniles, so deficiencies that result from poor nutritional input may be exaggerated in larger fish. Also, the biological mechanisms involved in metabolizing plant-based proteins for carnivorous marine fish is largely unknown and should be investigated further. Through understanding the effects of FM replacement on a cellular level, proper supplementation of feed components not provided by novel feed ingredients could be supplied, leading to the most efficient levels of fish health and growth as well as overall aquaculture production.

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