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Effect of Replacing Dietary Fishmeal with Soy-Based Products on Production Performance of Near Commercial Size Cobia, *Rachycentron canadum*.

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EFFECT OF REPLACING DIETARY FISHMEAL WITH SOY-BASED
PRODUCTS ON PRODUCTION PERFORMANCE OF NEAR COMMERCIAL
SIZE COBIA, *RACHYCENTRON CANADUM*

By

Carlos E. Tudela

A THESIS

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

Coral Gables, Florida

December 2013

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Effect of Replacing Dietary Fishmeal with
Soy-Based Products on Production
Performance of Near Commercial Size Cobia,
Rachycentron Canadum.

(December 2013)

Abstract of a thesis at the University of Miami.

Thesis supervised by Dr. Jorge A. Suárez.

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Fish meal replacement experiment was conducted with cobia above 1.77 ± 0.3 kg. The fish were fed one control diet and three experimental diets, for duration of 91 days. All diets were formulated isonitrogenous and isoenergetic and supplemented with amino acid mix. The control diet was similar to commercial diet formulations. Two experimental diets were formulated in which 67% of protein from fishmeal was replaced by: a) a combination of dehulled SBM+ Soy Protein Concentrate (Solae Profine®) labeled as MXSB diet, and b) a combination of Soy Protein Concentrate (Solae Profine®) + Schillinger Navita™ labeled Navita™ diet. In the Navita™ diet, the Soybean meal was completely replaced by SG-Navita™. The final experimental diet was formulated to replace 80% of protein from fishmeal by a combination of Schillinger Navita™+ Soy Protein Concentrate (Solae Profine®) labeled Navita™ Extreme diet. Results showed no significant differences between all four diets in most performance criteria (FCR, PER, FE, MDI, GPI, and GEI). Data also showed no significant differences in MR, VSI, and HSI. These results concluded that no physiological alterations occurred when fish were feed high levels of soy-bean replacement diets. There existed significant differences in FIFO ratios between diets. Data showed that Navita™ extreme produced the lowest values, which reached as low

as 0.91 ± 0.16 . Our findings suggest that Navita™ has a high potential to serve as a fishmeal replacement in aquaculture feeds.

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Chapter 1. Introduction

Statement of Problem:

Aquaculture is the fastest growing food-producing sector in the world and one of the main sources of dietary protein for humans. Future aquaculture expansion necessitates the development of more environmentally viable and economically efficient feeds.

Analysts project that by 2030 global human fish consumption will reach 186 million tons or an annual average consumption of 22.5 kg per person (Pauly and Froese, 2012). Yet over the last couple of decades, global capture fisheries production has been static at approximately 90 million metric tons per year (Pauly and Froese, 2012). Seventy five percent of the commercial fisheries catch will not reach our tables. Instead, they are directed to reduction – fishmeal (FM) and fish oil (FO) production – which annually amounts to approximately 16.5 million metric tons (MMT) (Jackson, 2012). Traditionally, these two ingredients have been important sources of protein, essential fatty acids and energy for fish feeds. Presently, the annual global production of FM and FO is around 5 MMT and 1 MMT. In 1988, aquaculture utilized approximately 10% of the total FM (6.5 MMT) production for feeds (Tacon and Metian, 2009; Welch et al., 2010). More recently, in 2008 – 2009 aquaculture exploited 59% of the global FM production (5 MMT) and 80% of FO production (1 MMT) (Jackson, 2012). The rising cost of FM, US\$ 1,300/MT in 2012 (Pauly and Froese, 2012), stands to threaten the continuing expansion and prosperity of the global aquaculture industry. On the contrary, plant-based materials sell for a fraction of the price of FM (World Bank 2013) and are readily available worldwide. This accessibility, coupled with the lower cost of plant-based raw materials, make the substitution of FM with alternative protein and lipid sources attractive for aquaculture

nutritionists. The 2012 Food and Agriculture Organization of the United Nations – SOFIA report (Pauly and Froese, 2012) states “Although the discussion on the availability and use of aquafeed ingredients often focuses on FM and FO resources, considering the past trends and current predictions, the sustainability of the aquaculture sector will probably be closely linked with the sustained supply of terrestrial animal and plant proteins, oils and carbohydrates for aquafeeds.”

Cobia (*Rachycentron canadum*) is a pelagic species found in the tropical and subtropical waters worldwide, except for the eastern Pacific (George Vanderbilt Expedition, 1944; Briggs, 1960; Collette, 1999) and Mediterranean (Golani and Ben-Tuvia, 1986). With its fast growth rate and premium white meat, this species has been recognized as an excellent candidate for aquaculture for more than a decade (Benetti et al., 2007; Benetti et al., 2008). Even though cobia production has been increasing, there are major issues that need to be addressed to take this species to its full commercial potential. One of the paramount concerns for cobia farming, and the industry as a whole, is the economic and environmental sustainability of feeding carnivorous fish. Currently, top feed manufacturers produce diets with high inclusion levels of FM and FO resulting in a high fish in:fish out ratio (FIFO). Since feeds account for more than half of total operating costs in cage culture of cobia, the industry has shown great interest in maximizing FM replacement with suitable lower cost ingredients. The challenge for researchers is to achieve this with no detriment to growth performance, health, or flesh quality. Therefore, several studies have been conducted on the utilization of vegetable meals and oils as sources for FM and FO replacement in cobia diets.

FM and FO provide essential amino acids, fatty acids, and a combination of micronutrients necessary for growth and development that cannot be found in most

plant-based substitutes. For this reason, replacement of FM and FO is not easily accomplished and requires supplementation of specific nutrients, such as fatty acids (especially EPA, DHA, and omega-3) and essential amino acids (Morais et al., 2012). Lack of fatty acids not only impact fish health, but also the nutritional quality of the meat (Simopoulos, 2003). Plant-based ingredients contain several anti-nutritional factors which can negatively affect fish digestion and physiology (Francis et al., 2001). These drawbacks are typically addressed through different processing options designed to maximize vegetable raw materials. Soybean meal (SBM) is a plant protein source that has received significant attention because of its good amino acid profile. Nevertheless, its restricted methionine content and the presence of oligosaccharides limit possible inclusion levels in fish feeds (Biswas et al., 2007).

There have been several reported studies focused on replacing FM with a variety of plant-based alternatives in diets for juvenile cobia. For example, Chou et al. (2004) and Zhou et al. (2005) concluded that up to 40% of FM could be replaced with SBM (defatted or not). However, optimal replacement levels were only between 16-19%. Lunger et al. (2007) also attained a 40% FM replacement with various organically-certifiable alternate protein sources (SBM, soy protein isolate, hemp seed meal, and yeast). Salze et al. (2010) reported that juvenile cobia exhibited good growth performance when fed diets in which 75% of FM was replaced with alternative products. While these studies on juvenile fish are valuable, more nutritional research is necessary on larger size fish (up to harvest size) to more accurately describe the needs and responses of cobia throughout a normal commercial production cycle. The nutritional requirements may differ significantly between a 100 g juvenile and a 4 kg adult fish, typical harvest size for cobia. Information on near

harvestable size fish is extremely relevant because commercial farms utilized the largest amounts of feed at this stage of the production process.

Our study will be conducted to see the growth performance when replacing the maximum amount of fishmeal in near harvestable size cobia (between 1-5kg). The ability to replace high amounts of FM are indicative that the nutritional requirements of cobia may change with age and that developing more cost-effective and environmentally sustainable diets is possible without compromising health or growth rates. This study helps validate the need for additional research on cobia, not just at the juvenile stage, but at all stages of the commercial production.

In conclusion, rising costs and competition for FM among different user groups has prompted significant advances in FM replacement in formulated diets. This trend is promising, but more research is necessary to maximize FM substitutions with novel ingredients that may allow even greater replacements. A closer look at the nutritional requirements and digestive capacity of larger size fish is necessary to optimize growth and minimize waste in commercial cobia farms. This mammoth task will more rapidly attain its objectives under synergistic collaborations between all stakeholders in the productive chain from suppliers of raw materials and aquafeed manufacturers to commercial farmers and research institutions.

Background/Literature Review:

Due to cobia's increasing interest by the private sector, the demand for a more cost effective and ecologically efficient feed is currently on the forefront of aquaculture research. Due to the rapid growth of cobia, the nutrition requirement for these pelagic fish frequently changes. With these changes, the need for further advancements in the effects on growth and nutritional content of fish meal replacement, at all life stages (from larvae to adult), is key. Many research studies

have focused on cobia at the juvenile stage. For this reason, it has been recognized that the need for investigation on fish at a larger scale has been deemed necessary (Fraser and Davies, 2009). In this section, one will focus on the pros and cons of fishmeal replacements. One will focus on the growth and physiological effects soybean has in feeds.

Cobia is a fast growing, carnivorous fish that requires a high protein and lipid diet, which is necessary for growth and development (Benetti et al., 2008). Protein occupies the highest percentage and greatest cost expense in fish feeds (Watanabe, 2002). For this reason, it is important to have the precise amount of protein necessary for optimal growth. Not only does the diet need to have a high protein content, but the need for a balanced nutritional content is just as important.

Fishmeal provides a balanced nutritional content for various reasons. First, it's high protein source, which is around 20-60%, and has a great amino acid profile for fish, especially the ten essential amino acids (Watanabe, 2002). These ten essential amino acids are: lysine, methionine, arginine, threonine, tryptophan, histidine, isoleucine, leucine, valine and phenylalanine. Secondly, fishmeal contains the necessary lipid content (ex. docosahexaenoic acid and eicosapentaenoic acid) for optimal growth and development. Also, fishmeal is a highly digestible and palatable ingredient for fish. This allows for a reduction in waste and high nutrient uptake (De Silva, 1995). Moreover, fishmeal is low in antinutritional factors and carbohydrates (Zhou et al., 2004). Windsor (2001) states that fishmeal has a valuable source of vitamins (calcium, phosphorous, magnesium, potassium), minerals (B₁, B₂, B₆ and B₁₂), and of trace elements zinc (iodine, iron, copper, manganese, cobalt, selenium and fluorine). All these factors make fishmeal a valuable commodity for fish feed

manufactures. But, as described earlier, fishmeal is becoming ecologically unsustainable and economically expensive. For this reason, developing feeds that maximize fishmeal replacement is on the forefront of the industry.

The replacement of fishmeal with alternate protein sources has been investigated with various aquaculture species. Yet, in order for a fishmeal replacement ingredient to be functional at the commercial scale, several points need to be addressed. First, the product needs to be economically competitive. Due to the high demand of fishmeal and increase fishing pressures, the price of fishmeal has been dramatically increasing (Tacon and Metian, 2008). Secondly, the replacement product must be available in large quantities. Third, the replacement needs to contain good nutritional content. Since fishmeal has a good amino acid profile and high protein levels, the replacement will need to cover all the nutrients that the fishmeal delivered. Fourth, there can be no compromise to growth or health due to the replacement. Fifth, it should allow for easy storage and handling. Lastly, the product shouldn't allow for the leaching of contaminants. When utilizing alternate fishmeal replacements, phosphorus and nitrogen are common leaching pollutants. If the ingredient utilized for fishmeal replacement can obtain all the characteristics described above, then it has the potential to be employed in commercial diets (Lunger et al., 2007). Soybean is one plant based protein source that has the greatest chance for fishmeal replacement because it has all the characteristics described above (Lunger et al., 2007).

Soybean meal is an excellent candidate for fishmeal replacement in fish feeds. Yet, in order to properly replace fishmeal with soybean meal, one needs to understand and investigate the possible effects that may occur when replacing fishmeal in aquafeeds. These effects can be detrimental, not only to the health of the fish, but also

the nutrient content (Committee on the Nutrient Requirements of Fish and Shrimp; National Research Council, 2011). Below, one will discuss the growth and physiological effects that occur when replacing fishmeal in diets for marine fish.

Currently, problems facing the aquaculture industry is cost feasibility and the ecological impacts when utilizing high amounts of fishmeal in feeds. So scientists have been investigating the utilization of alternate protein sources as fishmeal replacements and the challenges faced when doing so. One of the primary challenges that has been encountered has been the effects on growth performance. Even though replacing large amounts of fishmeal has been a great challenge in marine fish, including salmonoids, there has been positive results where no detrimental effects occurred on growth performance (Espe et al., 2007; Espe et al., 2006; Lunger et al., 2007; Chou et al., 2004). There have even been studies done where 100% fishmeal replacement has performed equivalent to a standard fishmeal diet (Saadiah et al., 2011; Hernández et al., 2012; Barrows et al., 2007). On the contrary, there have been studies that have shown that growth and health has been effected. The effects can also vary depending on the choice in alternative protein sources and feed formulation being investigated. For plant-based proteins, soybean is considered one of the best choices because of high protein and good nutrient content (Watanabe, 2002). There is also health related issues associated with the application of soybean meal. One of the health related issues observed, in trout, salmon, cod, halibut, and carp has been enteritis (intestinal inflammation) (Sahlmann et al., 2013; Urán et al., 2008; P. a. Urán et al., 2008; Grisdale-Helland et al., 2002; Refstie et al., 2006; Bureau et al., 1998). Intestinal enteritis is a shortening of the mucosal fold causing a reduction in the ability of absorption of the enterocytes lining the epithelium (Urán, 2008). This leads to the depletion of health and ultimately an increase in mortality (Urán, 2008).

Another factor that can have an effect on the growth of a fish is the duration of feeding a substituted fishmeal diet. This can be one of the causes for mixed results in fishmeal replacement experiments. For example, de Francesco et al. (2004) found that when large rainbow trout (*Oncorhynchus mykiss*) were fed a 75% fishmeal replaced diet, with plant-based proteins and amino-acid supplements, for six months, there was significant effects on growth performance. Yet, significant growth reduction became apparent only after three months, from the initiation of the trial. Colburn et al. (2012) performed a 12 week trial with 100% fishmeal replacement with soybean meal and no enteritis was found. Whereas, Olsen et al. (2007) found that large Atlantic cod (*Gadus morhua*) acquired enteritis, in a 28 week trial where 100% fishmeal was substituted with soybean meal. The importance of this trial is that it was performed over 28 weeks unlike most growth performance studies which are administered for 3 months or less (Salze et al., 2010; Zhou et al., 2005; Saadiah et al., 2011; Chou et al., 2004; Colburn et al., 2012). The effects on growth and health by the substitution of fishmeal with soybean meal may be due to unknown nutrient deficiencies or depletion of stored nutrients in the fish (Committee on the Nutrient Requirements of Fish and Shrimp; National Research Council, 2011).

Feed intake is another cause for decrease in growth performance. Reduction in feed intake may be attributed to a variety of factors. Boonyaratpalin et al. (1998) found that Asian seabass, *Lates calcarifer*, feed intake was reduced due to palatability when fed various soybean product, causing growth to diminish. Another factor that can cause a lead to decrease in feed intake is the apparent digestibility of protein/amino acids found in the replacement ingredient. For example, soybean meal and other plant-based ingredients cannot complete the essential amino acid profiles for fish. Soybean is limiting in the sulfur-containing amino acid methionine (one of

the ten essential amino acids) and lysine (Ketola, 1982; Gatlin et al., 2007). It has been proven, that deficiencies in methionine causes cataracts and growth deficiencies in Atlantic salmon (*Salmo salar*), lake trout (*Salvelinus namaycush*), and rainbow trout (*Oncorhynchus mykiss*) (Ketola, 1982; Poston et al., 1982). Whereas, deficiency in lysine can cause dorsal and caudal fin erosion, which can lead to increased mortality in rainbow trout (*Oncorhynchus mykiss*) (Ketola, 1983). Lastly, feed intake can effect growth by antinutritonals factors found in plant-based proteins. These antinutritional factors include protease inhibitors, phytates, glucosinolates, saponins tannins, lectins, oligosaccharides and non-starch polysaccharides, phytoestrogens, alkaloids, antigenic compounds, gossypols, cyanogens, mimosine, cyclopropenoid fatty acids, canavanine, antivitamin, and phorbol esters. All these factors can lead negative growth/health impacts (Ketola, 1982; Sandholm et al., 1976; Espe et al., 2006; Rumsey and Ketola, 1975).

Fishmeal replacement quantity is another factor that plays a role on the growth performance of fish. In cobia, *Rachycentron canadum*, studies on fishmeal replacement with soybean has been done in juvenile fish, between 8 grams to 120 grams. The investigators found that the maximum fishmeal replacement with soybean is approximately 40%, yet optimal replacement is approximately 16 to 18 percent (Lunger et al., 2007; Chou et al., 2004; Zhou et al., 2005). Limited studies have been done on cobia in the later stage of commercial production (1 kilogram or larger). During this period, the majority of the feeds cost is used in commercial production (Fraser and Davies, 2009). Yet quantity isn't the only variable, size or age of the fish needs to be taken into consideration. For example, in Atlantic Cod, *Gadus morhua*, Colburn et al. (2012) found that in feeds, 50% of fishmeal can be replaced with soybean meal in juveniles (24 grams) and Olsen et al. (2007) discovered that 75%

replacement can be achieved in large fish (1.6 kg) without growth effects. As opposed to juveniles, adult, pelagic marine fish have the ability to digest and utilize feeds with higher fishmeal replacement because the nutritional requirements alter. Due to the onset of maturation, adult, marine pelagic fish require higher lipid and less protein, in their diet (Nguyen et al., 2010).

Nutrient content of fish is another factor that can be effected by fishmeal replacement. Changes in nutrient content of fish is when the nutrients from the feedstuff is absorbed in the muscle of the fish. This can lead to a change in the color, texture, flavor and quality of the fish's fillet. This is one topic that the commercial industry is skeptic when dealing with replacing fishmeal, especially with plant based proteins. Though there wide variety of literature describing the effects of fishmeal replacement, very little has been studied on the influence that plant based proteins has on fish flesh quality. Yet there are literature that investigates fillet quality. For example, Lunger et al. (2007) studied the effects of organic protein had on cobia, *Rachycentron canadum*, fillet quality parameters. The organic proteins utilized for the study were a yeast-based protein source, soybean meal (SBM), soybean isolate, hemp seed meal, and Menhaden fish meal (as the reference diet) as the sole protein sources in the diets. The texture analysis performed was fillet breaking force, rupture point, gel strength, and total energy. The results showed that fillet quality and muscle analysis varied between the reference diet and the alternate protein sources. To date, this is the only literature that tests cobia fillet quality. In contrast, several studies reported that fish fillet did not alter in quality when fishmeal was replaced with plant based proteins (Rosenlund et al., 2001; Committee on the Nutrient Requirements of Fish and Shrimp; National Research Council, 2011; De Francesco et al., 2007; Natasha D'Souza, 2006). Further investigation on this topic is

required to evaluate the impacts that fishmeal replacement might have on the final product, commercial fish sale. Formulating successful cost effective and environmental sustainable feeds requires a complete understanding of the nutritional requirements of the fish, at all life stages. Also knowledge of the digestibility and nutritional make-up of the ingredients that go into the developed feed is key to optimizing the health and growth. With these points in mind, one can go forward in developing a diet that is both economically efficient and ecologically sustainable for the commercial industry.

Objective:

The goal of this study is to maximize the replacement of fishmeal in cobia diets, at three different ages by optimizing the utilization of soy-based products in aquaculture feeds. The use of protein sources such as standard soybean meal and soy by selective breeding program, could greatly improve the profitability and image of the aquaculture industry. The main objective is to conduct a scientific experiment that will be beneficial for the commercial sector for the development of practical and cost-effective diets for cobia, *Rachycentron canadum*, in near harvestable size cobia, using soy-based products.

Chapter 2. Methods and Materials

The diets were formulated on an as-fed basis. The ingredients for the diets were purchased and sent to the University of Miami. After which time, the ingredients were sent to the Food Protein R& D Center at Texas A&M University for manufacturing.

Feed Formulation Strategy:

The four experimental diets were formulated based on current data and information about juvenile cobia, *Rachycentron canadum*, nutritional requirements (Chou et al., 2001; Chou et al., 2004; Craig et al., 2006; Romarheim et al., 2008; Salze et al., 2010; Salze et al., 2011; Sun et al., 2006; Zhou et al., 2005) All four diets were formulated isonitrogenous and isoenergetic, providing 47-50% crude protein and 9-11% total lipid, on a dry-matter basis, and were supplied with approximately 484-450 kcal gross energy 100g⁻¹ dry matter. The control diet contained the highest lipid level were due to the increased level of fishmeal. All diets were supplemented with an amino acid mix (methionine, lysine, glycine and taurine).

The first diet, labeled control diet (CD), formulation resembled a commercial cobia diet in which protein consisted of 26% fishmeal, 10.25% Soy Protein Concentrate (Solae Profine[®]), and 32% dehulled Soybean Meal. Two other experimental diets were formulated in which 67% of protein from fishmeal was replaced by: a) a combination of dehulled SBM+ Soy Protein Concentrate (Solae Profine[®]) labeled as MXSB diet, and b) a combination of Soy Protein Concentrate (Solae Profine[®]) + Schillinger Navita[™] labeled Navita[™] diet. In the Navita[™] diet, the Soybean meal was completely replaced by SG-Navita[™]. The final experimental diet was formulated to replace 80% of protein from fishmeal by a combination of

Schillinger Navita™ + Soy Protein Concentrate (Solae Profine®) labeled Navita™

Extreme diet.

Table 1: Formulation and proximate composition of the experimental diets (g/100 g of dry diet) were fed to adults, cobia *Rachycentron canadum*. MXSB= soybean-based diet in which the incorporation of soybean meal has been maximized; Navita™ Extreme= Navita™-based diet in which the incorporation of Navita™ meal has been maximized.

Experimental diet	Control diet	MXSB diet	Navita™ diet	Navita™ Extreme diet
Menhaden meal ¹	26.0	8.50	8.50	5.0
SPC ²	10.25	30.0	19.0	15.0
Soybean meal ³	32.0	28.0	0.0	0.0
SG-Navita™ ⁴	0.0	0.0	38.05	46.38
Wheat flour ⁵	16.0	16.0	15.9	15.0
Menhaden oil ⁶	5.86	8.06	8.30	8.93
Mineral-vitamin premix ⁷	6.0	6.0	6.0	6.0
Glycine ⁸	1.0	1.0	1.64	1.64
Taurine ⁹	1.0	1.0	1.0	1.50
Lysine HCl ¹⁰	0.0	0.19	0.0	0.0
DL-Methionine ¹¹	0.09	0.20	0.47	0.55
Cellufil ¹²	1.80	1.05	1.14	0.0
<i>Proximate analysis (n=3)</i>				
Crude protein (g/100 g DM)	47.3	49.3	48.7	50.2
Crude lipid (g/100 g DM)	11.2	10.3	10.5	9.8
Ash (g/100 g DM)	10.8	8.4	8.4	8.3
Moisture (g/100 g DM)	9.1	8.3	7.4	8.2
Calcium (g/100 g DM)	2.2	1.5	1.3	1.0
Phosphorus (g/100 g DM)	1.8	1.3	1.3	1.1
Energy (kcal/100 g DM)	488.8	489.9	487.6	484.3

¹Menhaden meal (Omega Protein Inc., Texas, U.S.A.)

²Soybean Protein Concentrate (The Solae Company, St. Louis, MO)

³Soybean meal

⁴SG-Navita™ (Schillinger Genetics, Iowa, U.S.A.)

⁵Wheat flour (Dawn Food Products, Inc., Florida, and U.S.A.)

⁶Menhaden oil (Omega Protein Inc., Texas, U.S.A.)

⁷Mineral Premix composition (g/kg): Ca(H₂PO₄)₂ · H₂O, 136.00; Ca(C₆H₁₀O₆) · 5H₂O, 348.553; FeSO₄ · 7H₂O, 5.00; MgSO₄ · 7H₂O, 132.00; K₂HPO₄, 240.00; NaH₂PO₄ · H₂O, 88.00; NaCl, 45.00; AlCl₃ · 6H₂O, 0.084; KI, 0.15; CuSO₄ · 5H₂O, 0.50; MnSO₄ · H₂O, 0.70; CoCl₂ · 6H₂O, 1.00; ZnSO₄ · 7H₂O, 3.00; NaSeO₃, 0.0127.

Vitamin Premix composition (g/kg): Ascorbic acid, 50; dl-calcium pantothenate, 5.0; Choline chloride, 36.2; Inositol, 5.0; Menadione sodium bisulfite, 2.0; Niacin, 5.0; Pyridoxine HCl, 1.0; Riboflavin, 3.0; Thiamine mononitrate, 0.5; dl-alpha-tocopherol acetate (250 IU/g), 8.0; Vitamin A palmitate (500,000 IU/g), 0.2; Vitamin micro-mix^c, 10.0; Cellulose, 874.1 Vitamin Micro-mix composition (g/100g): Biotin, 0.50; Folic acid, 1.8; Vitamin B12, 0.02; Cholecalciferol (40 IU/ug), 0.02; Cellulose, 97.66

⁸Glycine (Affymetrix, Inc., California, U.S.A.)

⁹Taurine (Affymetrix, Inc., California, U.S.A.)

¹⁰Lysine HCl (Affymetrix, Inc., California, U.S.A.)

¹¹DL-Methionine (Affymetrix, Inc., California, U.S.A.)

¹²Cellulfil (Sigma-Aldrich, Inc., Missouri, U.S.A.)

Analytical Procedure:

All diets were sent for full amino acid profile analysis by the Department of Wildlife and Fisheries Sciences, at Texas A&M University. The analyzed content of all four experimental diets are shown in table 2.

Experimental Conditions:

The enclosure utilized during the experiment was a twelve tank, flow-through system. Each tank was 4,000 liter cylindrical fiberglass tanks containing one air stone and an oxygen stone. Well water will be pumped with a 2.0hp centrifugal pump (HaywardTM, Hayward industries, <http://www.haywardnet.com/aboveground/products/pumps/>) and mechanically filtrated with a sand filtered housing filled with crushed coral media (Triton II, Pentair Pool Ltd., Minneapolis, MN, USA) into each tank at an exchange rate of 1600% per day. Each tank will be siphoned three times per week and the system will be backwashed every other day. Throughout the 13-week experimental period, a YSI Professional Plus Meter (YSI, Inc., 1700/1725 Brannum Lane, Yellow Springs, Ohio, 45387) will be utilized to record water temperature, pH, salinity, and dissolved oxygen. The parameters for the experiment will be maintained and recorded

as follows: water temperature values between 24-25 °C, pH 7.3-8.0, salinity 33-34 ppt., and dissolved oxygen will be between 7-9 mg L⁻¹.

The cobia used in this experiment will be spawned, reared, and raised at the University of Miami Experimental Hatchery (UMEH). The initial cobia weight, at the beginning of the experiment, will be approximately 1.0 kg. Each fish will be distributed at random into the twelve tanks. At the start of the experiment, each tank will be stocked at a weight biomass of 19-20 kg or approximately 5.0 kg m⁻³. The diet for each tank will be randomly selected and fed throughout the entire trial. All four experimental diets will have three replicates. Diets will be administered by hand, ad libitum, once per daily, at 0900 h for 91 days. The total amount of diet consumed per tank will be recorded daily.

Harvest, Sample Collection and Production Performance:

One week before the start of the experiment, numeric T-bar tags will be inserted into each fish's musculature, just below the dorsal fin with the tag barb anchored through the boney fin ray supports. These numeric tags will be used for fish identification to track individual growth performance at three different harvest periods. Before the start of the experiment, five fish will be randomly selected and euthanized using tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA). Standard procedures will be used for determining proximate components (AOAC, 2000). The fish will then be minced, in a meat grinder, for whole-body proximate analysis and stored in -80°C for three day; after which time, the fish will be lyophilized and sent to for lipid, protein, energy, and ash analysis. At each harvest, fish will be anesthetized with 12 ppm 100% clove bud oil (Spice USA, Inc., Hialeah, FL, USA) and individual fish length and weight will be recorded for

growth performance. Also during the harvest, all tanks will be disinfected and fish given a two to five minute freshwater bath before returning into their designated tank, to ensure optimal health. The harvests will be performed as follows: 29 days, 60 days, and 91 days after the start of the experiment. At the end of the experiment, three fish, from each tank, will be euthanized with a concentration of 50ppm tricaine methanesulfonate (MS-222; Western Chemical, Inc., Ferndale, WA, USA). The fish will be dissected for VSI, HIS, and MR. After which time, the three fish, from each tank, will be placed in a meat grinder and homogenized for whole-body proximate analysis. Two samples from each pool will be taken. The samples will be stored in -80°C for three days followed by lyophilization. The samples will later be sent for protein in dry matter analysis.

The following variables will be determined for growth performance:

ADG = average daily gain; SRG = specific growth rate; MDI = mean daily intake; GEI = gross energy intake; GPI = gross protein intake; FE = feed efficiency; PER = protein efficiency ratio; FCR = feed conversion ratio; FIFO = fish in:fish out; MR= muscle ratio; VSI= visceral somatic index; HSI= hepatosomatic index

1. ADG (g/d)
2. SGR = $100 (\ln \text{ average final weight} - \ln \text{ average initial weight}) / \text{numbers of days}$
3. MDI = (g/fish/day)
4. GEI = (kcal/fish/day)
5. GPI = (g/fish/day)
6. FE = $(\text{weigh final} - \text{weight initial}) / \text{total feed intake}$
7. PER = $\text{weight gain in g} / \text{protein intake}$
8. FCR = $\text{feed intake} / \text{wet weight gain}$

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

Yield of fishmeal from wild fish = 22.5 (Jackson, 2009).

Yield of fish oil from wild fish = 5.0 (Jackson, 2009).

10. MR= muscle weight * 100/body weight

11. VSI= VSI weight * 100/body weight

12. HSI= HSI weight * 100/body weight (AOAC, 2000)

Statistical Methods:

Analysis of variance and Duncan's multiple range tests will be performed using the SAS[®] 2002–2005 statistical software program to first determine whether significant differences existed among the dietary treatments and then to identify where they occurred. Results will be considered statistically significant at $P < 0.05$.

Chapter 3. Results

Effect of Replacing Dietary Fish Meal with Soy-Based Products:

The four experimental diets utilized were formulated and manufactured isonitrogenous and isoenergetic. Nevertheless, the control diet had the highest ash, calcium, and phosphorus level compared with the other experimental diets. This was due to the heightened levels of FM included in the Control Diet (Table 1).

In regards to the amino acids analyzed, the methionine concentration in the Navita™ diet and Navita™ Extreme diet was of 0.9% and 1.0%, respectively, whereas in the Control and MXSB diets, concentrations were 0.7% and 0.6%, respectively. The fluctuation in concentration of methionine among were due to the Navita™ diet and Navita™ Extreme diets being supplemented with higher levels of DL-Methionine (Table 2).

Table 2: Amino acid composition of experimental diets (g/100 g of dry diet)

Proximate components	Control diet	MXSB diet	Navita™ diet	Navita™ Extreme diet
<i>Indispensable Amino Acids</i>				
Arginine	2.3	2.2	2.9	3.1
Histidine	1.0	1.0	1.2	1.3
Isoleucine	1.9	1.7	2.1	2.1
Leucine	3.1	2.8	3.5	3.7
Lysine	2.5	1.7	2.6	2.6
Methionine	0.7	0.6	0.9	1.0
Phenylalanine	2.2	2.4	2.6	2.7
Threonine	1.7	1.4	1.8	1.9
Valine	2.1	1.8	2.3	2.4
<i>Dispensable Amino Acids</i>				
Alanine	2.2	1.6	2.0	2.1
Aspartic acid ¹	2.9	2.4	3.4	3.5
Cysteine	0.1	0.1	0.1	0.2
Glutamic acid ²	4.8	4.1	5.7	6.0
Glycine	3.6	3.4	4.1	4.4
Proline	2.3	2.1	2.6	2.7
Serine	1.8	1.7	2.2	2.4
Tyrosine	1.3	1.6	1.5	1.6

¹ Aspartic acid + asparagine

² Glutamic acid + glutamine

Water quality physico-chemical parameters were maintained within the suitable levels for Cobia, *Rachycentron canadum*. No mortalities occurred during the experiments. For both harvests at 29 days and 60 days, the cobia fed the MXSB diet showed significantly lower specific growth rate (SGR) compared to other treatments. No significant difference in weight gain, average daily gain, mean daily intake, gross energy intake, gross protein intake, feed efficiency, protein efficiency ratio and feed conversion ratio was observed among the MXSB, Navita™, Navita™ Extreme, and Control diet (Table 3).

Table 3: Performance of adults Cobia *Rachycentron canadum* in Harvest I (29 days), Harvest II (60 days) and Harvest III (91 days)

Treatment		Performance										FIFO ratio ⁹
		Final mean wt (kg)	Weight gain wt (kg)	ADG ¹	SGR ²	MDI ³	GEI ⁴	GPI ⁵	FE ⁶	PER ⁷	FCR ⁸	
Harvest I. IW= 1.86±0.3 kg Final biomass 6.5 ±0.5 kg/m ³	Control diet	2.38 ±0.4 ^a	0.59±0.1 ^a	20.5 ±5.4 ^a	1.02±0.4 ^a	35.7±3.4 ^a	174.8±17 ^a	16.9±1.6 ^a	0.56±0.1 ^a	1.2±0.2 ^a	1.8±0.3 ^a	2.08±0.4 ^c
	MXSB diet	2.58 ±0.5 ^a	0.51±0.06 ^a	17.6 ±2.0 ^a	0.74±0.2 ^b	34.3±1.3 ^a	168.1±7.0 ^a	16.9±0.7 ^a	0.51±0.04 ^a	1.0±0.08 ^a	1.95 ±0.2 ^a	1.17±0.1 ^b
	Navita™ diet	2.50 ±0.5 ^a	0.64±0.06 ^a	22.1 ±2.2 ^a	1.02±0.4 ^a	39.5±5.6 ^a	192.9±27 ^a	19.2±2.7 ^a	0.57±0.13 ^a	1.17±0.3 ^a	1.81 ±0.4 ^a	1.10±0.3 ^b
	Navita™ Extreme diet	2.38 ±0.4 ^a	0.57±0.15 ^a	19.9 ±5.2 ^a	0.97±0.3 ^a	34.1±5.5 ^a	165±27 ^a	17.1±2.7 ^a	0.57±0.06 ^a	1.14±0.1 ^a	1.74 ±0.2 ^a	0.91±0.16 ^a
Harvest II. Final biomass 7.6 ±0.4 kg/m ³	Control diet	2.8±0.5 ^a	1.0±0.15 ^a	16.9 ±2.5 ^a	0.76±0.2 ^a	34.3±2.2 ^a	167.7±11 ^a	16.2±1.0 ^a	0.49±0.04 ^a	1.0±0.09 ^a	2.0±0.19 ^a	2.3±0.22 ^c
	MXSB diet	3.0±0.6 ^a	0.95±0.08 ^a	15.8 ±1.3 ^a	0.61±0.16 ^b	33.5±2.0 ^a	164.5±10 ^a	16.5±1.0 ^a	0.47±0.02 ^a	0.95±0.05 ^a	2.1±0.12 ^a	1.27±0.07 ^b
	Navita™ diet	2.84 ±0.7 ^a	0.98±0.04 ^a	16.4 ±0.6 ^a	0.69±0.22 ^a	35.3±1.1 ^a	172.2±5.5 ^a	17.2±0.5 ^a	0.46±0.02 ^a	0.95±0.05 ^a	2.1±0.13 ^a	1.31±0.08 ^b
	Navita™ Extreme diet	2.81 ±0.5 ^a	0.98±0.19 ^a	16.4 ±3.2 ^a	0.71±0.19 ^a	33.2±4.7 ^a	160.7±23 ^a	16.6±2.3 ^a	0.49±0.03 ^a	0.98±0.06 ^a	2.0±0.13 ^a	1.0±0.06 ^a
Harvest III.	Control diet	3.15 ±0.6 ^a	1.36±0.14 ^a	15.0 ±1.5 ^a	0.62±0.2 ^a	35.9±1.5 ^a	175.6±7.4 ^a	16.9±0.7 ^a	0.41±0.026 ^a	0.88±0.05 ^a	2.4±0.15 ^a	2.8±0.18 ^b

Final biomass 8.7 ±0.54 kg/m ³	MXSB diet	3.3± 0.7a	1.27± 0.16a	13.9 ±1.7 a	0.53± 0.14a	34.4± 2.4a	168. 7±12 a	17.0± 1.2a	0.40± 0.02a	0.82± 0.05a	2.5± 0.16 a	1.5±0 .09a
	Navita™ diet	3.27 ±1.0 a	1.41± 0.04a	15.4 ±0.5 a	0.59± 0.21a	36.1± 0.25a	176± 1.2a	17.5± 0.12a	0.43± 0.01a	0.88± 0.02a	2.3± 0.07 a	1.4±0 .04a
	Navita™ Extreme diet	3.1± 0.6a	1.29± 0.23a	14.2 ±2.6 a	0.58± 0.15a	35.9± 5.5a	173. 9±27 a	18.0± 2.7a	0.39± 0.01a	0.78± 0.03a	2.5± 0.09 a	1.3±0 .04a

Values with different superscripts within the same column were significantly different ($P < 0.05$).

ADG = average daily gain; SRG = specific growth rate; MDI = mean daily intake; GEI = gross energy intake; GPI = gross protein intake; FE = feed efficiency; PER = protein efficiency ratio; FCR = feed conversion ratio; FIFO = fish in-fish out

After 91 days of culture, most production performance criteria were unaffected by dietary protein sources. Although no statistically significant differences were found among any of the treatments and productive parameters evaluated, cobia the Navita™ diet numerically showed the highest performance for most parameters evaluated (Table 3 and Figure 1).

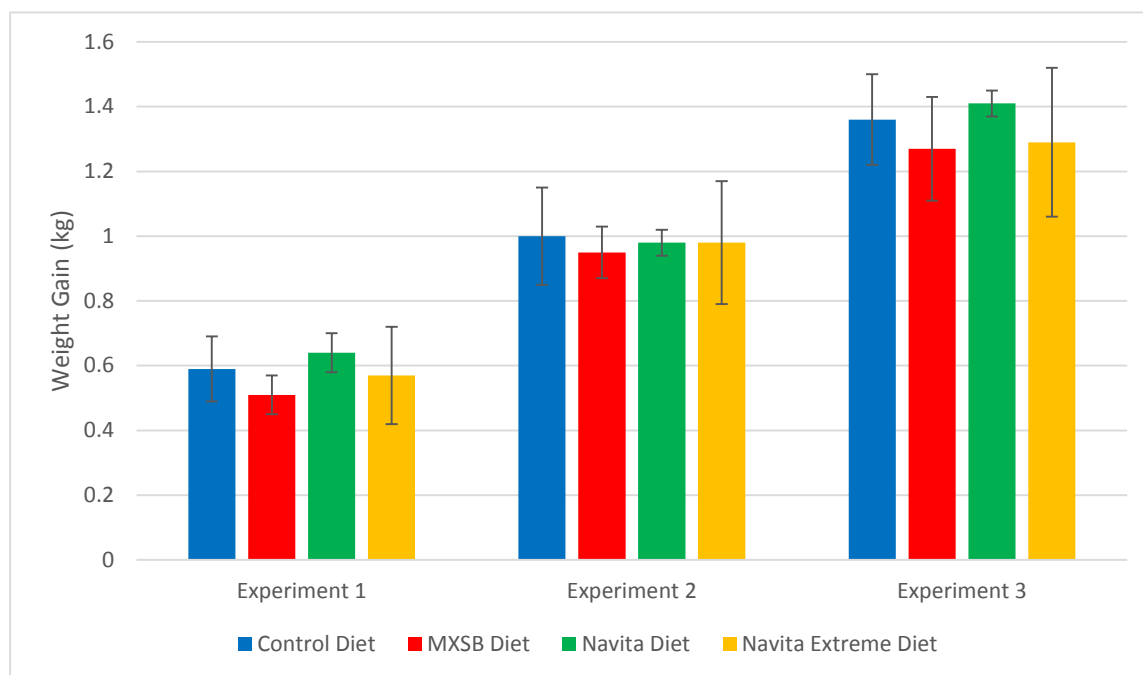


Figure 1: Mean Weight Gain (kg) between control diet and three experimental diets. Data points represent mean weight gain (kg) from Tables 3 – 5. Experiment 1 representing average weight gain from 1 – 29 days, Experiment 2 from 1 – 60 days and Experiment 3 from 1 – 91 days.

Fish in-fish out (FIFO ratio) values from this study ranged between 1.3 (Navita™ Extreme diet) to 2.8 (Control diet). Cobia fed the Control diet had a significantly high FIFO ratio (2.8 ± 0.18) compared to the other dietary treatments. The lowest value for FIFO ratio was obtained with the Navita™ Extreme diet (Table 3). The Navita™ Extreme diet with 5% FM, corresponded to the maximum level of FM substitution by plant protein meals without affecting weight gain, specific growth rate, average daily gain, mean daily intake, gross energy intake, gross protein intake, feed efficiency, protein efficiency ratio and feed conversion ratio.

Table 4: Biological indices including the muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI) in adults Cobia, *Rachycentron canadum* fed experimental diets (means of three replicates, means \pm std).

Experimental Diets	MR ¹	VSI ²	HSI ³
Control diet	48.3 \pm 4.3 ^a	9.2 \pm 1.1 ^a	2.1 \pm 0.45 ^a
MXSB diet	46.2 \pm 1.9 ^a	10.7 \pm 0.9 ^a	1.8 \pm 0.09 ^a
Navita™ diet	46.4 \pm 4.2 ^a	9.5 \pm 0.5 ^a	1.99 \pm 0.25 ^a
Navita™ Extreme diet	43.4 \pm 2.9 ^a	9.0 \pm 0.8 ^a	1.96 \pm 0.47 ^a

Values with different superscripts within the same column were significantly different ($P < 0.05$).

¹ MR= muscle weight * 100/body weight

² VSI= VSI weight * 100/body weight

³ HSI= HSI weight * 100/body weight

No significant differences in muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI) were observed among the MXSB, Navita™, Navita™ Extreme, and control diet (Table 4). This may indicate that the cobia's physiology is not altered when fed diets that have a high level of soy-based products. These results are contrary to those found by Salze et al (2006), where biological indexes were all significantly impacted by dietary treatments. The difference in the results in both studies is probably due to the size of the cobia used. In the Salze et al (2006) study they utilized cobia weighing 300 g while in our study, we used cobia with weights of

3,200 g, indicating that cobia of greater size are less sensitive to diets with high levels of inclusion of soy-based products.

Chapter 4. Discussion

For the first time, a study of FM replacement in Cobia was performed on fish close to commercial harvestable size, providing very valuable information to fish farmers and feed companies at the growth stage when the greatest consumption of feed occurs.

The results of this study indicate that the combination of soy-based products is an appropriate substitute for FM in diets for large cobia, *Rachycentron canadum*, at an 80% substitution level. These results are in agreement with a previous report indicating that a 75% FM replacement by soy-based products did not affect weight gain of juvenile cobia (Salze et al., 2010).

The high survival (100%) and good overall health condition of the fish during the entire trial suggest the absence of any nutrient deficiency. Cobia were harvested when they reached commercial sizes, averaging 3.2 ± 0.7 kg. This is relevant because most nutritional studies are conducted with smaller fish for short periods of time.

Growth results and survival confirmed that Cobia, *Rachycentron canadum*, can be fed a mixture of soy-based product (46% Navita™ meal–15% SPC) reducing FM inclusion level from 26 to 5 g per 100 g dry weight. For this level of replacement, it was found necessary to include an amino acid mix (DL-Methionine 0.5%, Glycine 1.6% and Taurine 1.5%).

Our findings clearly indicate that Navita™ has a high potential to serve as a non-genetically modified fishmeal replacement in aquaculture feeds for cobia. Digestibility information, such as that gathered in this study, could effectively promote the use of ingredient substitutions in least-cost formulated diets for cobia. This innovative soybean strain is characterized by particularly low levels of antinutritional factors (oligosaccharides, 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. Results from these trials show that Navita™ has the potential to be used for formulating more biologically efficient, environmentally sustainable, and economically viable commercial diets for cobia and potentially other marine fish species.

One of the most contentious issues in aquaculture is the use of fishmeal and fish oil in feeds and the amount of wild fish it takes to produce farmed fish. The debate has centered primarily around the use of fish oil and fishmeal in salmon diets, and a wide range of different values have been quoted for the number of tons of wild fish it takes to produce a ton of farmed salmon (FIFO ratio) (Jackson, 2009). Currently, aquaculture converts 65% of the wild fish reduced into fishmeal at a FIFO ratio between 0.66 (Jackson, 2009; Kaushik and Troell, 2010) and 0.7 (Tacon and Metian, 2008). The FIFO ratio for salmon and marine fish was 2.2 and 1.9, respectively (Jackson, 2012). In the current study with cobia, a FIFO ratio of 1.3 was obtained, with a 5.0% inclusion of FM and 8.9% of FO, after 91 days (Table 3).

Results from this study of FM replacement in diets of commercial size cobia demonstrate that, during the stage when there is a higher consumption of feed, diets can be formulated (and fed) with high levels of soy-based products without affecting performance in growth, survival and feed efficiency.

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 the 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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