Evaluation of organically certifiable alternate protein sources for production of the marine carnivore, cobia (*Rachycentron canadum*)

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ABSTRACT

Cobia represents one of the most attractive candidate species for aquaculture in the history of the industry. With rapid growth rate, high survival rates, and delectable flesh, cobia possess highly desirable characteristics for a cultured fish. Although interest in this species is high, issues pertaining to nutritional requirements must be resolved if this animal is to be produced sustainably. Cobia are high level marine carnivores and, as such, require relatively high dietary protein levels which usually are met through the use of fish meal. Fish meal supplies have become limited and costly, and alternate proteins must be utilized if future aquaculture production is to meet demand. Moreover, the movement towards organic aquaculture production presents additional challenges with respect to fish meal inclusion in aquafeeds designed for cobia. This thesis summarizes research pertaining to fish meal replacement in cobia aquafeeds with organically certifiable alternate protein sources.

Initial trials with an organically certifiable yeast-based protein source indicated that up to 25% of the fish meal could be replaced without detrimental impacts to growth rates, feed efficiency, or biological indices. Substitution levels above this resulted in decreased performance in all measured parameters. Based on these results and other research however, it is hypothesized that fish meal replacement levels could be increased to 40% without detrimental impacts upon production characteristics.

In a subsequent study, multiple organically certifiable alternate protein sources were investigated for their ability to replace fish meal in aquafeeds for juvenile cobia. A 25% inclusion level of yeast-based protein was used along with a 40% inclusion level. The remaining alternate proteins (soybean meal, soybean isolate, and hemp) also were included at 40% of dietary protein. Two additional diets were formulated to contain all four alternate proteins with or without 8% fish meal. Lack of fish meal resulted in poor survival, while the 8% inclusion of fish meal resulted in decreased overall performance compared to fish fed the fish meal control and the diets with up to 40% organic protein source. When included at 40% of fish meal

replacement, these alternate protein sources led to returned excellent weight gain, feed efficiencies, and other production characteristics when compared to the 100% fish meal control diet. I hypothesized that higher inclusion level of alternate protein sources could be achieved with specific amino acid supplementation.

Two additional trials involved the use of the yeast-based protein with supplementation by the amino acids methionine, tryptophan, and taurine. Diets containing 50 and 75% of the yeast-based protein were investigated with the addition of methionine (0.3%) and tryptophan (0.2%), with and without taurine (0.5%). Taurine significantly and dramatically increased production performance. A final trial re-evaluated that ability of the yeast-based protein to completely replace fish meal with supplemental taurine (0.5%). While growth at the 50% inclusion level equaled that of the control, at higher levels (75 and 100%), growth was reduced even with taurine supplementation, leading to the hypothesis that other essential amino acids may also have been limiting.

This thesis presents evidence that replacement of fish meal, as well as organic production of cobia, is feasible. However, these studies also illustrate the necessity of developing quantitative amino acid requirement data for cobia if these goals are to be fully realized.

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CHAPTER 1

General Introduction

Aquaculture has been the fastest growing food-producing sector in the world for more than two decades and was just recently surpassed by organic agriculture. Due to its rapid growth (approximately 10% per year since 1984; FAO, 2006), aquaculture production rather than harvest from wild fisheries may become the major source of future supplies of food fish (Watanabe, 2002). This increase in aquaculture is expected to continue for years to come; the consumption of fish globally has risen from approximately 40 million tonnes in 1970 to 86 million tonnes in 1998 (FAO, 2000) and is expected to increase to 110 million tonnes by 2010 (FAO, 1999). A more recent estimate (103 million tonnes in 2003) indicates that fish consumption has indeed increased worldwide (FAO, 2004). Commercial fisheries most likely will not be able to continue to meet this increasing demand for fish because their supplies have been relatively stable since the mid-1990s at 85-90 million tons per year (Goldburg et al., 2001; FAO, 2006). Not only are supplies from commercial fisheries peaking, but production from these sources can be variable and uncertain (Eagle et al., 2004). Since the aquaculture industry continues to rapidly expand, there is a greater need for larger supplies of industrial fishes to use as protein sources. Since this problem has arisen, many other protein sources have been evaluated as possible dietary replacements for fish meal (FM). Alternate protein sources have been investigated for carnivorous species such as cobia (*Rachycentron canadum*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*) and in omnivorous species such as channel catfish (*Ictalurus punctatus*) and tilapias (*Oreochromis sp.*). In order for protein sources to be considered effective replacements for FM, they must be economically competitive, capable of being produced in large quantities (Hardy, 2004), contain balanced amino acid profiles and proper crude protein levels, and not compromise the growth or health of the fish. It is also helpful if they are easily handled and stored and do not lead to environmental contamination from release of phosphorous and nitrogen. Furthermore, such protein sources must be commodity traded.

Fish meal

Fish meal (FM) is currently the major source of protein fed to aquacultured fish, especially carnivorous species. It is known for its high level of protein, good amino acid profile (especially the essential amino acids), well balanced lipid profile including essential omega-3 fatty acids, low carbohydrate level, high digestibility and low levels of antinutritional factors (Zhou et al., 2004). FM is made from species such as anchovy (*Anchoa mitchilli*) and capelin (*Mallotus villosus*) that are not typically consumed by humans (Hannesson, 2003). The production of fish meal involves cooking, pressing, drying and grinding in appropriate machinery designed for this purpose (FAO, 2001). During the cooking process, protein is coagulated and most of the water and oil runs off. Whatever does not run off is removed during the pressing process. The material that is removed from the pressing of the fish is typically called the press liquor. The press liquor is screened to remove coarse particles and then centrifuged to remove the oil. The leftover water portion from this process can be sold as condensed fish solubles or is more commonly added back to the fish meal. After pressing, the fish meal is dried by either direct or indirect methods at temperatures up to 500°C. Both the temperature and specific drying method used can affect the quality of the fish meal produced. The final process involved in making fish meal is grinding, which breaks down any lumps or portions of bone present in the meal (FAO, 2001). The production of FM has been steady over the past 15 years at about 7 million metric tons (mmt). The aquaculture industry often is accused of being too dependent upon FM in dietary formulations even though the majority of FM is consumed by terrestrial animals (Tidwell and Allan, 2001). About 25-35% of the FM produced globally is utilized in aquafeed formulations (Iowa Soybean Association; Craig and McLean, 2005). It is expected that FM and fish oil use by the aquaculture sector will increase by 30% by the year 2010 (Tacon, 2004). Marine fisheries cannot keep up with the increasing demand for FM, so due to public perception and more important, sustainability issues, research into alternative protein sources is rapidly increasing. Due to the decrease in landings of industrial fishes, it is likely that FM prices will increase in the foreseeable future. The increase in demand for FM and its corresponding increase in price is sometimes referred to as the "fish meal trap" (New and Wijkstrom, 2002). Researchers are hoping to find alternative ingredients to replace FM as the main protein source in aquafeeds to help reduce price, alleviate pressure on the dwindling industrial fish supply, and promote sustainability. Several studies have shown

promising results using plant-based protein sources (Gomes et al., 1995; McGoogan and Gatlin, 1997; Fagbenro and Davies, 2001; Tidwell and Allan, 2001; Forster, 2002; Pereira and Oliva-Teles, 2003; Chou et al., 2004). Some of the ingredients that have been examined as potential alternate protein sources include: soybean meal, corn gluten meal, lupin seed meal, rapeseed meal, pea seed meal, meat and bone meal, blood meal, poultry byproduct, feather meal, fisheries by-catch, and many more. Noteworthy is that fisheries by-catch represents about 25% of marine fisheries tonnage (Valdimarsson and James, 2001); rather than simply discarding the by-catch, it could be used to make more FM. Standardized, sound scientific research is necessary so that optimal inclusion levels of these potential protein sources can be determined for commercial aquafeeds.

Soybean Meal

Soybean meal (SBM) is probably the most promising and most studied alternate protein source to replace FM. Not only is it relatively high in protein content (approximately 48%) and has an excellent amino acid profile, except for methionine (El-Sayed, 1999), it also is produced in large quantities and represents a sustainably-produced feedstuff. Soybean meal is also reasonably priced and can allow cost-effective formulation of diets (Chou et al., 2004). It is consistent in quality, unlike FM, highly digestible by most fishes, and can be produced in a range of protein levels to suit the requirements of the fish (American Soybean Association, 2003). However, SBM does contain some antinutritional factors (ANF), such as protease (trypsin) inhibitors, phytohaemagglutinin (lectins), anti-vitamins, phytic acid, saponins, and phytoestrogens (El-Sayed, 1999; Francis et al., 2001). Phytates, protease inhibitors, lectins and anti-vitamins all can be removed from SBM by thermal processing, but phytoestrogens and saponins are heat-stable (Francis et al., 2001). There are many different processing methods for SBM that can affect the levels of ANF and crude protein. Generally, the hulls are removed and the beans are rolled into flakes, de-oiled by a solvent and toasted, which removes the trypsin inhibitors and other ANF affected by heat. This is the most common form, called dehulled soybean meal, and contains 48% crude protein (CP). If blended with the soybean hulls, then the protein content drops to approximately 44% CP. Full-fat soybean meal also can be produced by eliminating the fat extraction step. If aqueous alcohol extraction or isoelectric leaching is used, the resulting product contains even less ANF and the product is termed soy protein concentrate

(70% CP) and soy isolate (90%). The levels of ANF present in a given feed are most affected by duration of process, temperature and type of solvent (Forster, 2002). Numerous studies have been conducted with various types of soy protein as replacements for FM. In a study using African catfish, *Clarias gariepinus,* SBM could replace up to 50% FM and up to 75% when supplemented with methionine (Fagbenro and Davies, 2001). In diets for hybrid striped bass, *Morone saxatilis x M. chrysops,* SBM supplemented with methionine at levels of 25 and 75% inclusion yielded weight gains that were not significantly different from the FM control at the end of 12 weeks. Using larger fish, up to 75% SBM could replace FM (Gallagher, 1994). In diets for red drum, *Sciaenops ocellatus*, up to 90% of the FM could be replaced by SBM. When SBM was supplemented with glycine and fish solubles, weight gain increased (McGoogan and Gatlin, 1997). In another experiment involving red drum, SBM could replace only up to 50% FM (Reigh and Ellis, 1992). It was noted by McGoogan and Gatlin (1997) that this difference could be attributed to using SBM with 48% CP and formulated diets to contain 38% CP, whereas Reigh and Ellis (1992) used SBM with 44% CP and formulated diets to contain 35% CP. Soybean meal and lupin seed meal were included in diets for juvenile gilthead seabream, *Sparus aurata,* at 10, 20, and 30% replacement of FM. Mean feed intake, feed efficiency (FE), protein efficiency ratio, and weight increase were not affected by 20% SBM (Robaina et al., 1995). Lupin seed meal also could replace 20% FM and was more digestible than SBM. Soybean meal could replace up to 33% FM in diets for Atlantic salmon, where 25 and 33% inclusion levels were tested (Carter and Hauler, 2000). When SBM was included at a level of 29% with 29% meat and bone meal, and 10% distillers grain solubles, 100% of FM was replaced in diets for hybrid striped bass (Webster, 1999). In diets for rainbow trout, full-fat SBM (22.4%) was combined with other protein sources such as lupin seed meal (3%), faba beans (5.4%), corn gluten meal (CGM) (25%), and 20% FM. Up to 66% of FM could be replaced by this combination of vegetable proteins (Gomes et al., 1995). In diets for juvenile Japanese flounder (*Paralichthys olivaceous*), the best results were produced from a diet that contained 25% SBM in combination with 10% corn gluten meal and 5% blue mussel meat. This diet could replace 45% of FM protein (Kikuchi, 1999). In a study using Asian seabass (*Lates calcifer*), up to 15% of FM was replaced by either solvent-extracted SBM, extruded full-fat SBM, steamed full-fat SBM, or soaked raw full-fat SBM (Boonyaratpalin et al., 1998). The first three sources did not differ significantly in feed efficiency or survival. Fish fed the solvent-extracted SBM experienced

growth rates that were not significantly different from the control. Soaked raw full-fat SBM was deemed a poor protein source. Webster et al. (2000) fed sunshine bass (*Morone saxatilis x M. chrysops*) four different diets that contained SBM, meat and bone meal, poultry by-product meal, hempseed meal, and canola meal in different combinations. All diets supported good growth and the conclusion was made that all FM can be replaced in diets for sunshine bass. Fewer studies have investigated soy protein concentrate (SPC) as an alternate protein source. In diets for juvenile turbot (*Psetta maxima*), SPC could replace up to 25% FM (Day and Plascencia Gonzalez, 2000). For Atlantic halibut (*Hippoglossus hippoglossus*) between 600 and 900g, SPC could replace 45% of the total crude protein from FM. A squid coating was used as an attractant in some diets also, but this had no effect on feed intake, growth, or feed efficiency (FE) (Berge et al., 1999). Table 1.1 shows more detailed information about studies involving the replacement of FM with SBM.

Corn Gluten Meal

Corn gluten meal (CGM) is the product that remains after starch is extracted from corn. This product is commercially available, has a high protein content, low fiber, no ANF, and has a good amino acid profile except for lysine and arginine (Pereira and Oliva-Teles, 2003). When used in diets in combination with SBM, the two amino acid profiles complement each other and provide all essential amino acids. In a study by Kikuchi (1999), CGM could replace 40% of the FM in diets for juvenile Japanese flounder without affecting growth, FE, or protein efficiency ratio (PER). In gilthead seabream, up to 60% of the FM could be replaced by CGM without affecting growth or feed efficiency (Pereira and Oliva-Teles, 2003). This same study showed that CGM was highly digestible by seabream. Corn gluten meal could replace only 30% of the FM in diets for gilthead seabream in another study due to reductions of protein and lipid digestibility at higher levels (Robaina et al., 1997), although it was noticed that CGM was more digestible and readily accepted than meat and bone meal, which could only replace 20% of FM. When CGM was combined with soy protein concentrate and wheat gluten in diets for gilthead seabream, 100% of FM could be replaced (Kissil and Lupatsch, 2004). In fact, this combination diet was superior to the FM control diet in supporting growth and feed conversion ratio. Soy protein, wheat gluten and corn gluten meal all had superior protein digestibilities compared to FM as well. When CGM was blended with SBM at a 2:1 ratio, up to 50% of the FM could be

replaced in diets for Atlantic salmon (Mundheim et al., 2004). In diets for turbot, CGM could replace up to 20% of FM without affecting growth and feed utilization (Regost et al., 1999). Even though lysine and arginine were supplemented to these diets, no improvement in growth was observed. In a study by Morales et al. (1994) on rainbow trout, only one level of CGM was tested (40% inclusion). Even though CGM had better apparent digestibility for protein and fat, lupin seed meal produced better all-around results. When CGM was combined with wheat gluten, extruded wheat, rapeseed meal, SBM and only 5% FM, growth of European seabass (*Dicentrarchus labrax*) juveniles was still high. Apparent digestibility coefficients for protein, energy, dry matter, and phosphorous were comparable to all diets which contained less plant protein and more FM (Kaushik et al., 2004). Corn gluten meal in combination with soy protein concentrate, meat meal and SBM sustained growth of juvenile yellowtail (*Seriola quinqueradiata*) for 46 days even though palatability was poor and FE ratios were inferior to the FM control (Watanabe et al., 1998).

Single-cell Proteins

Other alternate protein sources that have been studied less extensively are the single cell proteins such as microalgae, bacteria, and yeast. Of these types of single-cell proteins, yeasts have been used the most frequently in aquafeed formulations (Oliva-Teles and Goncalves, 2001). Yeasts have a high nutritional value because they are a rich source of proteins, B-complex vitamins, complex carbohydrates, such as glucans, and nucleotides (Oliva-Teles and Goncalves, 2001; Olvera-Novoa et al., 2002; Li and Gatlin, 2006). They are also low in phosphorous, which will lead to less water and environmental contamination than fish meal and other plant-based alternate protein sources that contain high levels of phosphorous (Cheng et al., 2004). Yeasts represent a sustainable alternate protein source that is relatively cheap and easily produced on an industrial scale (Olvera-Novoa et al., 2002). Multiple studies have demonstrated the immunostimulating properties of yeasts, such as their ability to enhance non-specific immune activity (Olvera-Novoa et al., 2002; Li and Gatlin, 2004; Bagni et al., 2005). Yeasts have been used to replace FM in diets for numerous species with varying levels of success. For instance, Rumsey et al. (1991) incorporated brewer's dried yeast into diets for rainbow trout at 0, 25, 50, and 75%. Results of this study showed that beyond 25% inclusion of brewer's dried yeast, both growth and feed utilization declined significantly. Fish fed the 50 and 75% yeast diets would

take the feed into their mouth and then expel it, suggesting that palatability of diets was an issue. Perera et al. (1995) used a bacterial single-cell protein incorporated into diets for rainbow trout at 0, 25, 62.5, and 100%. Results showed that final mean weights tended to decrease with increasing content of bacterial single-cell protein. An inclusion level of 25% was suggested for rainbow trout because at this level, no deleterious effects on feed consumption, absorption efficiency, or growth rate were noticed. In a study using sea bass juveniles, brewers yeast was used to replace 10, 20, 30, and 50% of the FM protein (Oliva-Teles and Goncalves, 2001). Replacement of FM with up to 30% brewer's yeast did not have any effects on growth rate or feed intake, and significantly improved feed conversion. In an experiment involving hybrid striped bass, 1, 2, and 4% brewers yeast was added to diets to replace cellulose to evaluate its use as an immunostimulant (Li and Gatlin, 2003). Hybrid striped bass fed the experimental diets actually returned higher or equal weight gains of those fed the basal diet and the yeast positively influenced feed efficiency. Brewers yeast also was noticed to improve resistance to *S. iniae* infection. Olvera-Novoa et al. (2002) used torula yeast as a dietary protein source for tilapia fry and replaced meat meal by 25, 30, 35, 40, and 45%. Highest growth responses were observed for fish fed the 30% yeast protein diet. Feed acceptance and survival were not affected by dietary yeast content. Brewers yeast was included at 2% of the diet to evaluate growth, body composition, and health promoting affects on juvenile red drum, *Scioenops ocellatus* (Li et al., 2005). No significant differences were noticed for weight gain, feed efficiency ratio, HSI, intraperitoneal fat (IPF), or whole body composition among fish fed any of the diets. Brewers yeast also did not appear to have any health promoting benefits. Another benefit of the use of yeasts in diets for fish is their dietary nucleotide content. Although nucleotides are not required nutrients since they can be synthesized endogenously, under conditions of rapid growth, dietary nucleotides may be beneficial, as it limits the *de novo* synthesis of these molecules from their amino acid precursors (Carver, 1999). Studies involving tilapia larvae (Ramadan and Atef, 1991), juvenile rainbow trout (Adamek et al., 1996), and Atlantic salmon (Burrells et al., 2001) have shown improvements in weight gain with the inclusion of dietary nucleotides.

Miscellaneous Protein Sources

Many other protein sources have been tested in feeding trials for various species of fish. Some animal meals that have been tested are meat and bone meal (MBM), meat meal, blood meal, poultry by-product meal and shrimp by-catch. A 4:1 meat meal and blood meal mixture was fed to grouper (*Epinephelus coioides*) at various inclusion levels. There were no significant differences in growth performance when this mixture replaced up to 80% of FM (Millamena, 2002). In a study with Japanese flounder, meat meal could replace up to 60% of FM without affecting weight gain, FE or PER (Sato and Kikuchi, 1997). Kikuchi and Sakaguchi (1997) were able to replace 50% of FM with blue mussel meal in diets for Japanese flounder. Red drum juveniles were fed poultry by-product and MBM at various inclusion levels, and it was noticed that fish fed MBM diets had significantly lower weight gain, FE, and protein conversion efficiency along with lower food palatability and digestibility. Poultry by-product could effectively replace 67% of FM (Kureshy et al., 2000). Poultry by-product and feather meal were combined to replace either 50% or 100% of FM in diets for rainbow trout (Steffens, 1994). Even though some of the 100% FM replacement diets were supplemented with lysine and methionine, weight gain and feed utilization were reduced. Only 50% of FM could be replaced by this combination of protein sources. In Chinook salmon (*Oncorhynchus tshawytscha*), Fowler (1991) was able to replace 50% of FM with poultry by-product meal alone.

Various plant protein sources have been tested besides SBM and CGM. Some of these include rapeseed meal (or canola meal), pea seed meal, cottonseed meal, lupin seed meal, wheat gluten and extruded wheat. Rapeseed meal (RSM) has been used to replace FM in diets for juvenile channel catfish (*Ictalurus punctalus*), and up to 36% replacement of FM was recommended (Webster et al., 1997). Heat-treated RSM could replace up to 30% of FM in diets for turbot (Burel et al., 2000). In this same study, extruded lupin was included at a level of 50% and could replace up to 25% FM. In diets for tilapia, cottonseed meal replaced up to 50% FM without compromising growth (Mbahinzireki et al., 2001). Diets for rainbow trout that contained cottonseed meal had low energy digestibility and low digestive protein utilization, but lupin seed meal could replace up to 40% of FM (Morales et al., 1994). Pea seed meal was included in diets for juvenile European seabass and could replace up to 12% of FM (40% inclusion) (Gouveia and Davies, 1998). When pea seed meal was included at levels of 0, 10, 20, and 30%, no significant differences were noticed in apparent digestibility coefficients for protein, lipid, energy and

carbohydrate among diets (Gouveia and Davies, 2000). Pea seed meal also could replace up to 33% of FM in diets for Atlantic salmon (Carter and Hauler, 2000). Pereira and Oliva-Teles (2002) found that pea seed meal could replace up to 20% of FM in diets for juvenile gilthead seabream. Pea seed meal also replaced 20% of FM in diets for juvenile milkfish, *Chanos chanos* (Borlongan et al., 2003). Digestibility of protein and energy from extruded peas was low for both rainbow trout and turbot, while extruded lupin protein and energy was highly digestible for both species (Burel et al., 2000).

Carnivores vs. Omnivores/Herbivores

Many studies with alternate proteins have been conducted with omnivorous and herbivorous fishes such as tilapia and channel catfish (El-Sayed, 1999; Fagbenro and Davies, 2001; Mbahinzireki et al., 2001; Webster et al., 1997) with promising results. The replacement of FM with alternate protein sources in diets for carnivores will be more difficult because they require higher levels of protein, there are issues of palatability, and the presence of ANF in some of these feedstuffs. Omnivores typically are fed lower protein diets (< 40%), while carnivores are fed high protein diets (> 40%) (Hardy, 2003). Carnivores such as cobia cannot digest many of the plant-based protein sources because they are rich in carbohydrates and fiber. This is because their intestinal tracts are much shorter and physiologically unable to digest carbohydrates, unlike omnivores and herbivores that have longer, more developed intestinal tracts. Clearly, diets for omnivores/herbivores that require less protein and can more efficiently utilize carbohydrates will be less expensive than diets for carnivores that require more protein and cannot utilize carbohydrates as efficiently (Hardy, 2003). Carnivores also do not readily accept the plant-based protein diets because of their taste or lack of attraction, whereas these issues are not a concern when dealing with omnivores and herbivores. The use of dehulled, solvent-extracted soybean meal was studied as a replacement for FM in African catfish by Fagbenro and Davies (2001). Since African catfish is omnivorous, they were able to replace FM up to 50% without methionine supplementation and up to 75% with methionine supplementation. In many studies involving tilapia, SBM could replace from 67-100% FM depending on fish size and species, dietary protein level, SBM source and processing methods (El-Sayed, 1999). It is apparent from these studies that larger amounts of FM could be replaced by plant protein sources, such as SBM, in omnivorous species.

Antinutritional Factors (ANFs)

Almost every plant-based alternate protein source has some sort of ANF present (Francis et al., 2001). These compounds are defined as substances that interfere in food utilization and affect the health and production of the animals. There are many different kinds of ANF, such as protease inhibitors, tannins, and lectins which affect protein utilization and digestion; phytates, gossypol, oxalates, and glucosinolates which affect mineral utilization; antivitamins and miscellaneous factors such as mycotoxins, cyanogens, alkaloids, mimosine, nitrate, saponins, photosensitizing agents and phytoestrogens (Francis et al., 2001). Another negative quality of these plant-based proteins is that they are usually low in palatability, especially when fed to carnivores (Hardy, 1996). Some examples of the plant-based protein sources that contain these ANF are SBM, rapeseed meal, lupin seed meal, pea seed meal, cottonseed meal, sunflower oil cake, leucaena leaf meal, alfalfa leaf meal, mustard oil cake and sesame meal (Francis et al., 2001). Some of these ANF can be eliminated through special treatment such as thermal processing. Heat-labile factors such as phytates, protease inhibitors, lectins, goitrogens and antivitamins can be destroyed by this process (Francis et al., 2001). Other antinutritional factors such as saponins, non-starch polysaccharides, antigenic proteins and estrogens are more heat stable and resistant to thermal processing. A list of plant-based protein sources and their corresponding antinutritional factors can be seen in Table 1.2.

Organic Certification

Just as sustainability is an important factor for alternate protein sources, it also is one of the main goals for organic food production (White et al., 2004). In the USA, currently there are organic certification standards for fruits and vegetables, grain, livestock, marine and freshwater algae, wild-harvested plants and honey (Mansfield, 2004), but the potential certification of fish is controversial. If aquaculture can break into the organic sector, new markets and economic opportunities will become available, although there is some confusion about the meaning of organic and the standards that should apply to fish. Most farmers see organic as different from natural in that the farmers are active agents in the production process because they have to address and control nutrition, health maintenance, and environmental challenges while relying upon the natural cycles of the crop (Mansfield, 2004). Their goal is to sustain the supply of their product while promoting ecological balance and conserving biodiversity (Mansfield, 2004). This

eliminates the majority of wild fish from ever becoming certified as organic because they cannot be managed and controlled. There are a few wild fish stocks that may be organically certified if they are also certified as sustainable. Another argument is that water cannot be controlled like soil because of its fluidity. Currents change and water moves, and it is difficult to control the different chemicals and pollution that may be in the water at any given time. It is very important in organic culture that no synthetic fertilizers, antibiotics, or other chemicals are used in production (White et al., 2004). This means that the only types of aquaculture systems that would be approved for organic certification are recirculating aquaculture systems (RAS) or closed systems in which water quality, inputs, and outputs can be tightly monitored. Since many types of aquaculture allow for control over the entire production process such as stocking, disease control, site selection and monitoring of pollutants, there is still hope for the organic certification of some fish (Mansfield, 2004). For certain fish species, organic certification will come more easily than others. All feed has to be organically certified and most likely the main source of protein in fish diets, FM, will not obtain organic certification because it is made from wild, non-organic fish. This means that omnivorous/herbivorous fishes such as catfishes, tilapias and carps that require lower protein levels most likely will achieve organic status before the more carnivorous, FM-dependent species such as cobia, salmon, trout, and seabream. Hence, the move towards organic production of fish is stimulating the search for alternate protein sources, as is the stagnant supply of FM and expansion of aquaculture. Currently, there are organic standards for aquaculture in place for the European Union (EU), Canada, Japan, New Zealand, Australia, Chile, and Ecuador, and there are many third party certifiers providing organic certification for aquaculture facilities outside the US. The National Organic Standards Board (NOSB) of the USDA has established an Aquatic Animal Task Force (AATF) to develop organic standards for aquacultured fish in the United States (Craig and McLean, 2005).

There are very few organic protein sources available at this time. The sources that are available are expensive and not very useful for cost-efficient diet formulation. This is because there is not a large demand for these products at this moment. This problem could hinder the advance toward organic aquaculture of fish. An example of an organically certified protein source is NuPro®, a yeast-based product manufactured by Alltech, Inc. (Nicholasville, Kentucky). It is made from the contents of yeast cells, which are a mixture of nucleotides, peptides and the contents of the cytoplasm. It also has relatively high crude protein (greater than

50%) and a good amino acid profile that is comparable to casein. Research conducted at the Virginia Tech Aquaculture Center in which NuPro® was completely substituted for FM and SBM as a protein source in tilapia diets did not impact weight gain (Craig and McLean, 2005). In fact, tilapia fed diets containing NuPro® had higher growth (319-458%) than those fed the commercial diet of FM and SBM (277%), except for the 100% NuPro® diet in which no difference in weight gain was observed. In the research reported here, a carnivorous species was used and the effect of replacement of FM with this source alone was observed. NuPro® also was used in combination with other alternative protein sources, such as SBM, to increase the amount of FM replacement in aquafeeds for this high level marine carnivore.

Background on Cobia

Cobia (*Rachycentron canadum)* is gaining popularity as a candidate species for intensive aquaculture, primarily in the United States and Asia. Currently, the culture of marine species in the United States is limited (Kaiser and Holt, 2004). At this time, red drum, *Sciaenops ocellatus*, is the only warm water marine species to be commercially cultured in the United States (Kaiser and Holt, 2004), but the production of cobia has already begun overseas in Taiwan (Liao, et al., 2004), Puerto Rico and the Philippines. Cobia is a migratory, pelagic fish distributed worldwide in tropical, subtropical, and warm temperate waters except for the eastern Pacific Ocean (Briggs, 1960; Shaffer and Nakamura, 1989; Kaiser and Holt, 2003). In the United States, they typically are found near the Carolinas or in the Gulf of Mexico during spring and summer months (Ditty and Shaw, 1992; Smith, 1995; Franks et al., 1996; Franks et al., 1999). Cobia are multiple batch spawners (Brown-Peterson et al., 2001), with activity typically occurring from April to October with peaks between May and June (Arnold et al., 2002). Sometime during the late fall, cobia migrate to their wintering grounds in Florida (Ditty and Shaw, 1992; Franks et al., 1996; Franks et al., 1999). Cobia is a highly prized recreational species that typically is caught in shallow waters (Ditty and Shaw, 1992; Kaiser and Holt, 2004). Even though there are no major commercial fisheries for cobia, US landings alone have averaged one million kg per year from 1984-1995, with 87% of this coming from recreational fishermen (Franks et al., 1999). Cobia typically are seen near the surface around objects such as buoys, boats, floating debris, and oil or gas platforms (Smith, 1995; Arnold et al., 2002; Kaiser and Holt, 2004).

Cobia is a promising candidate for aquaculture because of rapid growth rates, reaching up to 4-6kg in a year, hardiness, efficient feed conversion, excellent flesh quality, and comparatively low production costs (Franks et al., 1999; Chou et al., 2001; Kaiser and Holt, 2004; Liao et al., 2004; Wang et al., 2005). Higher-return markets are available that place high value on cobia (Liao et al., 2004). Cobia meat typically is eaten as sashimi (Chou et al., 2001) and fillets can be sold in Gulf and South Atlantic seafood markets for as much as eight dollars per kilogram, although supply is often limited (Rickards, 2001). Another advantage to the aquaculture production of cobia is that adults have been successfully spawned both naturally and through hormonal injections in captivity (Arnold et al., 2002, Franks et al., 2001). Cobia typically are cultured in near-shore sea cages (Liao et al., 2004), but conflicts in US coastal waters have stimulated research on the culture potential of cobia in land-based recirculating systems and ponds (Kaiser and Holt, 2004). As is typical of most candidate species for aquaculture, very little information is available on the nutritional requirements of cobia. If the culture of cobia is to reach its full potential, more research is needed to determine their nutritional requirements for protein, specific amino acids, carbohydrates, and lipid.

Feeding Habits of Cobia in the Wild

Feeding habits of cobia in the wild have been evaluated with the help of recreational fishermen, with sampling events off the coast of North Carolina and in the Gulf of Mexico near Texas, Mississippi, and Alabama (Franks et al., 1996). Stomach contents were removed and analyzed for percent frequency of occurrence (%F), percent numeric abundance (%N), and percent total volume (%V). All of these values were used to determine an index of relative importance, or IRI (Smith, 1995; Franks et al., 1996). Contents that were not discernable were eliminated from the evaluation. These studies have demonstrated that cobia are opportunistic carnivores that feed primarily on benthic and epibenthic crustaceans and fish (Kaiser and Holt, 2004). In one study, prey items were divided into size classes and grouped into four categories (shrimp, crabs, teleost fishes, and elasmobranch fishes) to determine changes in food habits with growth (Smith, 1995). Of the 110 stomachs examined from cobia caught off the coast of North Carolina, 24 species of crustaceans, 16 species of fishes, and 1 cephalopod were identified (Smith, 1995). The species with the highest IRI was the blue crab (*Callinectes sapidus*), followed by the blackcheek tonguefish *(Symphurus plagiusa),* pipefishes (Family Syngnathidae),

and the smooth dogfish (*Mustelus canis)*. The largest amounts of crustaceans found included iridescent swimming crab (*Portunus gibbesii*), brown shrimp (*Penaeus aztecus*), mantis shrimp (Stomapotapods), coarsehand lady crab (*Ovalipes stephensoni*), blotched swimming crabs (*Portunus spinimanus*), and rock shrimp (*Sicyonia brevirostris*). Smaller cobia seemed to eat penaeid shrimps and teleost fishes, but as the cobia got larger, the consumption of decapod crab, and elasmobranchs such as smooth dogfish and *dasyatid* sting rays increased (Smith, 1995). Franks et al. (1996) examined the stomach contents of 49 juvenile cobia caught in the northern Gulf of Mexico and found that their diets consisted of mainly fishes, crustaceans, and cephalopods. Fish dominated the IRI and anchovies were predominant among them. Crustaceans ranked first in terms of numeric importance, with decapods being the most common. Only two species of cephalopods were found in cobia stomachs, slender inshore squid and longfin inshore squid. Smaller juvenile cobia tended to prefer crustaceans and fishes, while the larger juveniles preferred fishes. Meyer and Franks (1996) reported that adult cobia in the north-central Gulf of Mexico typically ate fishes, crustaceans, and cephalopods as well, with crustaceans being the primary food source. Knapp (1951), who examined cobia caught near Texas, found that cobia ate (in order of importance) fishes, stomatopods, penaeid shrimps, portunid crabs, and squids. Franks et al. (1996) also noticed that juveniles tended to feed during the daylight. Since cobia are very strong swimmers and aggressive feeders, they are very capable of catching moving prey objects (Franks et al. 1996), and the contents of their stomachs are evidence of this fact.

Larval Cobia Requirements

For marine fish larvae in general, it is very important to satisfy nutritional requirements, both quantitatively and qualitatively, due to their rapid growth rates (Sargent et al., 1997). Most researchers consider lipid nutrition to be of utmost importance when rearing marine larvae due to their high requirements for long-chain highly unsaturated fatty acids (HUFAs) such as eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and arachidonic acid (ARA, 20:4 n-6) which cannot be synthesized *de novo* in most marine finfishes. Therefore, fatty acid requirements for the larvae must be satisfied via maternal inputs and supplemented in the diet once exogenous feeding begins (Faulk and Holt, 2003). In the wild, marine larvae typically feed on wild zooplankton, such as copepod nauplii and adults, which contain very high

levels of these HUFAs (Sargent et al., 1997). These fatty acids are vital for maintaining cell membrane structure and function, proper development and function of neural and visual systems, and tolerance to stress (Rainuzzo et al., 1997; Sargent et al., 1997).

Traditionally, larval nutrient requirements have been determined through feeding trials which manipulate the levels of individual HUFAs, but it is now believed that this information can be determined by examination of eggs and yolksac larvae collected from the wild (Rainuzzo et al. 1997) due to the fact that nutritional requirements must be met endogenously for successful development prior to exogenous feeding (Faulk and Holt, 2003). With this in mind, Faulk and Holt (2003) decided to characterize the fatty acid profile of cobia eggs and larvae produced by a captive spawning broodstock, and to determine the effectiveness of four dietary treatments on growth and survival. The four diets included *Artemia* only, enriched rotifers (*Brachinous plicatilis*) on day 3 and microparticulate diet on days 3-13, enriched rotifers on days 3-5 and *Artemia* on days 3-13, and enriched rotifers on days 3-8 and *Artemia* on days 3-13. Rotifers and *Artemia* typically are fed to cobia larvae under culture conditions, but they are nutrient deficient and therefore, must be enriched via microalgae, lipid emulsions, fish oils, etc., in order to meet the nutrient requirements of larval marine finfishes (Rainuzzo et al., 1997; Sargent et al., 1997). The fatty acid profiles of eggs and yolksac larvae indicated no significant differences among individual fatty acids between these two stages. Cobia eggs in the tail-bud stage consisted of 31.4 ± 1.3 µg lipid/egg, but once the eggs hatched, lipid decreased significantly from 28.3 ± 0.3 to 23.2 ± 0.1 µg lipid/larvae during the yolk-sac stage. The fatty acids EPA, DHA, and ARA represented 80% of the total PUFAs present. There was very poor survival of larvae fed the *Artemia* diet and the 1-day rotifer diet, but there was no significant difference in survival among larvae fed rotifers for 3 or 6 days. Results from this study showed that cobia require rotifers for at least 4 days beyond the start of exogenous feeding. In another study by Faulk and Holt (2005), *Artemia* and rotifers were enriched with high levels of HUFAs to determine the impacts upon growth and survival of cobia. They also added live algae as a treatment to evaluate its efficacy. In the first trial, *Artemia* were enriched with Algamac 2000, a concentrated algae paste, Algamac 2000 supplemented with 10% Aquagrow arachidonic acid, Algamac 2000 supplemented with 20% Aquagrow arachidonic acid, or live *Nannochloropsis oculata*. One treatment also involved the use of rotifers grown on *N. oculata* followed by feeding of *Artemia* enriched with Algamac 2000. In the second trial, rotifers and *Artemia* were enriched with either

Algamac 2000, Algamac 3050, or *Isochyrsus galbana*, while two other treatments involved enrichment of rotifers and *Artemia* with Algamac 2000 and either *I. galbana* or *N. oculata* added to the rearing tanks. The results demonstrated that live algae and commercial enrichments significantly affected the fatty acid profile of live prey, but there were no significant differences in total lipid content. The Algamac 3050 treatment resulted in significantly higher levels of HUFAs, and a higher ratio of DHA/EPA in both rotifers and *Artemia*, while the 20% Aquagrow with Algamac 2000 significantly increased levels of ARA in both prey items. Prey items enriched with live algae had significantly lower levels of DHA, EPA, n-3 HUFAs, and DHA/EPA ratios. At 16 days post-hatch, the larvae fatty acid profiles closely resembled those of the live prey. Larval survival also was significantly higher when algae were present in the tank. The results of the study led the researchers to conclude that commercial enrichments resulted in higher levels of essential fatty acids than live algae enrichments, and that for optimal production of larval cobia, the diet should consist of rotifers and *Artemia* enriched with commercial preparations and that they should be reared in tanks with algae (Faulk and Holt, 2005). Reitan et al. (1997) also believed that rearing marine fish in tanks with live algae can enhance the appetite of the larvae, improve tank and gut microflora, and provide a food source for rotifers, thus stabilizing their nutritional value. Turner and Rooker (2005) conducted a study on larval and juvenile cobia to determine the rate of transfer of HUFAs from enriched *Artemia* and rotifers to the cobia. In as little as 24-72 hours, there was a significant increase in HUFAs in the lipid stores of larval and juvenile cobia. There also were significant changes in individual HUFAs found in the cobia when there was a corresponding dietary change, which proves that the lipid composition of marine fish reflects their diet (Hillestad and Johnsen, 1994; Peres and Oliva-Teles, 1999; Kim and Lee, 2005; Wang et al., 2005). Clearly, there are very few studies on larval cobia nutrition and in order to increase production and viability of cobia; further advancements in this area of research are needed.

Juvenile Cobia Requirements

Since cobia is quickly becoming an important species for aquaculture, it is essential to determine their optimal quantitative nutritional requirements. Juvenile cobia exhibit very rapid growth rates, and in order to maximize their true growth potential, protein, lipid, carbohydrate and energy requirements must be determined so that cost-effective feed formulations can be

developed. It also is necessary to determine feeding rates, frequencies and regimes in order to further improve feed conversion ratios.

Despite the intense global interest in cobia, relatively few studies have been conducted which focus on quantitative nutritional requirements. These studies have concentrated on optimal levels of dietary lipid and protein, digestibility of select feed ingredients, and the replacement of fish meal with alternative protein sources. The optimal dietary protein and lipid levels for juvenile cobia have been determined by varying the percentage of protein from 36-60 and the percentage of lipid from 3-18 (Chou et al., 2001). Cobia fed diets containing 44, 48, and 52 percent protein exhibited higher weight gain and feed conversion than cobia fed other diets, but weight gain showed a peak at 44.5% dietary protein, so this was determined to be the most suitable level for maximum growth (Chou et al., 2001). For lipid, weight gain tended to increase with dietary lipid content, but leveled off around 5.76%, so this is thought to be the optimal level of dietary lipid for cobia (Chou et al., 2001). A second study conducted by Her et al. (2001) used protein levels of 37, 41, and 45% along with lipid levels of 0, 5, 10, and 15% and dietary energy levels of 307, 347.6, 389.8, and 432.4 kcal per 100 g diet to determine the optimal protein/energy ratio. Results showed that cobia fed a diet containing 37% protein and 15% lipid exhibited statistically similar growth and feed conversion ratios to cobia fed diets containing 41 and 45% protein along with high energy levels. This study determined that the optimal dietary protein/energy ratio for cobia is 86-115 mg protein /kcal, and that a diet containing 37% protein and an energy level of 432 kcal supports optimal growth. This reduction in protein level, but increase in lipid level and energy shows the possible protein-sparing effects of lipid in cobia diets. However, Craig et al. (2006) did not observe protein sparing by lipid in a 3 x 2 factorial design with protein levels of 40 and 50% CP and lipid levels of 6, 12 and 18% total lipid (dry weight basis). There were no significant differences in weight gain or feed efficiency ratios in this study utilizing 44 and 12 g juvenile cobia. One other study has evaluated the impact of dietary lipid on growth, feed utilization, lipid deposition, and lipid metabolism of juvenile cobia (Wang et al., 2005). Diets consisted of 47% protein and lipid levels of either 5, 15, or 25%. Results showed that lipid levels higher than 15% had a negative effect on growth, and as lipid level increased, there was a decline in feed intake. Increasing the amount of dietary lipid also led to an increase in tissue and whole-body lipid levels. Cobia deposited fat in the muscle and viscera when fed 5 and 15% lipid, whereas cobia fed 25% lipid tended to deposit fat in adipose

tissue and muscle. N intake and N gain were significantly lower in the 25% lipid diet, but muscle protein was unaffected by dietary lipid level. Hence, lipid levels above 15% were not beneficial due to accumulation of fat and decreased growth, and there was no evidence that protein could be spared by dietary lipid (Wang et al., 2005).

Commercial production facilities currently utilize a wide range of dietary formulations, mainly due to the limited information on quantitative nutritional requirements for cobia. In the Asia/Pacific region, including Taiwan, China and Vietnam, cobia typically are fed a diet consisting of 42-45% crude protein and 15-16% lipid on a dry weight basis (Liao et al., 2004). These fish are usually fed once a day, six days a week at a feeding rate of 0.5-0.7% body weight (Liao et al., 2004). In North American facilities, current formulations utilized for grow out of juvenile cobia range from 50-59% crude protein and 14-17% total lipid (dry weight basis). While there are conflicting data concerning the ability of cobia to utilize lipid in lieu of protein, these higher lipid levels can contribute to a more desirable final product in terms of the sashimi market (Craig et al., 2006), but excessively high protein levels (> 50% CP dry matter basis) are not cost effective and should be lowered, not only for economic, but also for environmental reasons.

These studies clearly illustrate that like many other species of fish, body composition of cobia can be manipulated by dietary inputs (Peres and Oliva-Teles, 1999; Wang et al., 2005). One study conducted by Turner and Rooker (2005) involved the impact of four different diets on the fatty acid profile and growth of juvenile cobia. One diet, which served as their control diet, was a commercially produced feed by Rangen, while the other three consisted of wild-caught fish, shrimp, or squid. The results showed that juvenile cobia fed the control diet and the squid diet grew significantly larger than cobia fed the other diets. A significant difference in levels of all individual HUFAs was also noticed among the diets. Of all the diets, the squid diet seemed to exhibit the highest dietary fatty acid contribution, followed by the shrimp diet, and then the fish diet. Juveniles fed the different diets began to exhibit different HUFA signatures from day 49 to day 61. These data show a connection between growth, increase in biomass, and dietary HUFA composition because the cobia fed the squid diet grew the largest, had the greatest increase in relative biomass, and received the highest amount of dietary HUFAs. Although it was also noticed in this study that the cobia fed the shrimp diet exhibited the highest increase in relative biomass based on percent contribution of dietary fatty acids. This is currently the only study that dealt with growth and fatty acid profiles of juvenile cobia that are fed wild-caught diets.

Recently there has been great interest in the area of dietary fish meal replacement for cobia. Zhou et al. (2004) evaluated the ability of cobia to digest fish meal, defatted soybean meal/roasted and solvent-extracted, defatted soybean meal/solvent-extracted, poultry meal, meat and bone meal, peanut meal, rapeseed meal, and corn gluten meal. The digestibility of dry matter for all ingredients was generally poor, especially for plant-based ingredients, but cobia were capable of effectively digesting protein and lipid in almost all ingredients. Protein digestibility was worst for rapeseed meal and meat and bone meal. Energy digestibility for fish meal and poultry meal were significantly higher than for peanut meal, rapeseed meal, and solvent-extracted soybean meal, while phosphorous digestibility was significantly higher for fish meal and corn gluten meal than all other ingredients. The availability of amino acids was highest for corn gluten meal among the plant-based ingredients, and highest for fish meal and poultry meal among the animal ingredients. Due to the decreased digestibility of plant-based ingredients with high amounts of carbohydrate, they will not likely be good replacements for fish meal in cobia diets. The most promising alternate ingredients are poultry meal and corn gluten meal (Zhou et al., 2004). Chou et al. (2004) conducted a study in which soybean meal replaced fish meal in increments of 10, 20, 30, 40, 50, and 60%. A significant negative effect was noticed for weight gain, feed conversion, protein efficiency ratio, and net protein utilization when replacement of fish meal increased from 40 to 50%, indicating that soybean meal can replace up to 40% of fish meal without adversely affecting growth or protein utilization. Zhou et al. (2005) conducted a similar study with diets that would replace 0, 100, 200, 300, 400, 500, and 600 g kg^{-1} of fish meal with soybean meal. Protein content in the muscle decreased with increasing inclusion of soybean meal, while deposition of lipid in the liver tended to increase. Weight gain, protein efficiency ratio, and feed conversion ratio were negatively affected when soybean meal replaced greater than 400 g kg^{-1} of fish meal. These results agreed with those of Chou et al. (2004). Dietary requirements for specific amino acids need to be determined in order to replace larger amounts of FM. Only one study has been published in this area. Zhou et al. (2006) used six isonitrogenous and isoenergetic diets with varying levels of methionine between 0.61 and 1.68% dry matter in order to determine the optimal dietary methionine requirement for juvenile cobia. Dietary cystine was kept constant at 0.67%. Weight gain and SGR increased with increasing methionine level from 0.61% to 1.05% and then slightly decreased from 1.05 to 1.68%. There were no significant differences noticed among condition factor, HSI, VSI, or any

proximate analysis values for fish fed the various diets. Hence, in the presence of 0.67% cystine, juvenile cobia require 1.05% methionine for the best growth and feed utilization.

A more recent area of research that has raised considerable interest is the area of bioenergetics. The main goal of aquaculturists and nutritionists is to achieve maximum growth. Since growth seems to be the last in line for obtaining energy, it becomes very important to understand how a fish distributes its energy to functions other than growth (Smith, 1989). Two recent bio-energetic studies have also been conducted using juvenile cobia. Sun et al. (2006a) used a feed (*Anguilla* sp.) formulated for eel feed with five ration levels (starvation, 3%, 6%, and 9% of initial body weight and ad libitum) to determine the growth-ration relationship and energy budget for cobia. Faecal production significantly increased with increased ration. The optimal ration for juvenile cobia is 9% because specific growth rate also increased with increased ration, but there was no significant difference observed between the 9% and ad libitum ration. Also, feed conversion efficiency increased with ration up to 9% and then decreased at ad libitum. The proportion of energy intake that was retained as growth ranged from 6.5-22.1% and was the highest at 9% ration per day. Another experiment conducted by Sun et al. (2006b) used three different feed types (natural sardine fish (NSF), commercial eel formulated feed (CEFF), and commercial marine fish formulated feed (CMFF)) and four ration levels varying between starvation and ad libitum to investigate growth, faecal production, nitrogenous excretion, and energy budget of cobia. The chemical composition of the fish was affected by both feed and ration. As ration increased, so did protein, lipid, and energy content of the fish. Protein, lipid and energy contents were highest for fish fed the NSF- and CEFF-feeds and lowest for the CMFF feed. Both faecal production and nitrogenous excretion increased with increased ration for each feed type. Faecal production was highest for fish fed CMFF and lowest for the NSF feed, and nitrogenous excretion was the opposite. In general, specific growth rates (SGR) and feed efficiencies (FE) for the NSF and CEFF fed cobia were superior to the cobia fed the CMFF feed. Their results indicated that the NSF feed was preferred by juvenile cobia, and that ration levels between 70 and 100% of satiation appeared suitable.

Broodstock Nutrition

A requirement for true aquaculture is total enclosure and control over the complete life cycle of the species of interest. A critical factor for marine-based aquaculture operations is the

timely supply of high quality, fully weaned animals. Often, disappointing results from weaning trials have been explained by an insufficient understanding of a species' environmental needs, inadequate knowledge of broodstock nutrition, poor gamete quality, and other factors (Chong et al., 2004). Captive broodstocks are often considered to represent the major investment of commercial aquaculture operations and it is not surprising that considerable efforts are taken to ensure the wellbeing of brood animals. That being said, there is surprisingly little information available on broodstock nutrition for cobia or any other species for that matter. Information on adult cobia feeding habits in the wild has been documented, but no research has been conducted on adult cobia in captivity. As might be anticipated, broodstock nutrition has significant effects on gonadal growth, fecundity, fertilization, hatch rate, and viability of eggs (Rainuzzo et al., 1997). Lipids are the nutrient in broodstock diets that is specifically known to directly affect the quality of the eggs (Izquierdo et al., 2001). In this respect, it is important in terms of egg and larvae production and survival, that adult cobia nutrition requirements are met as well. Dietary deficiency in omega-3 fatty acids and protein negatively impact gamete viability and larval survival (Kah et al., 1994; Cerdà et al., 1995), and inappropriate dietary ratios of polyunsaturated fatty acids (PUFA) affect circulating levels of androgens in sea bass with the outcome of asynchrony of maturation between males and females (Cerdà et al., 1997). Dietary enhancements, for example, with vitamin C, perhaps due to its antioxidant capacity, improves sperm motility, concentration, and fertility (Mangor-Jensen et al., 1994; Ciereszko and Dabrowski, 1995; Dabrowski and Ciereszko, 2001), while PUFA-enrichment of feed increases reproductive performance in terms of egg quality and larval development in marine species (Astuarino et al., 2001; Mazorra et al., 2003; Ma et al., 2005). The importance of arachidonic acid and its metabolites in eicosanoid production and the latter's involvement in a range of reproductive functions and egg development is becoming more widely established (Bruce et al., 1999; Mazorra et al., 2003). For cobia no information is available upon optimized brood diets and this clearly represents an important production limitation. Broodstock cobias are generally maintained upon diets of frozen fish and squid, for instance Faulk and Holt (2003) fed their broodstock 80% marine fish supplemented with 20% squid and shrimp. Although this differs slightly from cobia feeding habits in the wild, it seemed to be sufficient since egg hatch rates were approximately 80%. However, to date, larval survival has been relatively poor at about 3 larvae per liter, which is below what would be considered economical (Carvahlo et al., 2005).

This poor survival is likely due to a variety of factors including insufficient broodstock nutrition. In order for future commercial production of cobia to flourish, all areas of nutrition should be examined especially broodstock nutrition. Without healthy broods, there will be poor egg quality and most likely poor larval survival which will negatively affect the potential of the industry.

Summary

Currently, there is very little information available on nutrient requirements of cobia at various life stages. Cobia feeding preferences have been observed in the wild, which indicate that their diets consist primarily of fishes, crustaceans and some cephalopods. Since they are carnivorous fish by nature, their diets under culture conditions typically contain large amounts of fish meal. For larval cobia, lipid nutrition is extremely important for proper growth and development since they cannot synthesize polyunsaturated fatty acids *de novo*. Larval cobia in the wild typically feed on zooplankton, and in culture conditions they are fed rotifers and artemia and are gradually weaned onto commercial pelleted diets. The rotifers and *Artemia* are nutrient deficient for larval cobia and, therefore, must be enriched with PUFAs before feeding. Juvenile cobia typically are fed pelleted diets with high levels of fish meal. The optimal dietary protein and lipid values for juvenile cobia were determined to be 44.5% and 5.76%, respectively (Chou et al., 2001). Due to the decrease in the fish meal supply worldwide, research has aimed at replacing fish meal with nutrient rich, sustainable protein sources, such as soybean meal. Cobia have been able to tolerate a 40% replacement of fish meal with soybean meal without negatively impacting growth, feed conversion ratios, or biological indices (Chou et al., 2004, Zhou et al., 2005). Broodstock nutritional information is clearly lacking and represents an untapped area of research. Cobia exhibit rapid growth, good feed conversion values, and great flesh quality. In order to take full advantage of these characteristics, more nutritional information at all life stages is needed. This will ensure the future development of the cobia industry and secure its position within commercial aquaculture production.

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Table 1.1 Replacement of fish meal with soybean meal in various studies.

Study (Reference)	Ingredient	Species	Amount of Fish meal replaced	CP %	Inclusion Rate (%)	Biological Effects
Chou et al., 2004	Soybean meal	Cobia	Up to 40%	48	10, 20, 30, 40, 50, and 60	All diets readily accepted Significant decrease in weight gain, FCR , PER, and net protein utilization at 50 and 60%
Fagbenro and Davies 2001	Soybean flour methionine supplemented at 75%	African catfish	50% best up to 75 with methionine supplement		25, 50, and 75	Protein & energy digestibilities high (>80%) Similar growth and feed utilization as 100% fish meal except 75% w/out met. Supp.
Boonyara-tpalin et al., 1998	Solvent-extracted SBM Extruded full-fat SBM Steamed full-fat SBM Soaked raw full-fat SBM	Asian seabass juveniles	15% 15% 15% 15%	40	21 27 28.5 27.5	Growth not sign. different from control FE & survival did not differ between first 3 diets Lower growth than control Lower growth than control Sign. Lower weight gain, FE, protein efficiency, and survival. Poor protein source
Gallagher 1994	SBM supp. with methionine	Hybrid striped bass	25 & 75% up to 75% with larger fish	35	25, 50 or 75	FE not sign. different between diets 6 wks.-growth same for 25% and control 12 wks.-no sign. difference in weight gain for 25, 75, and control With larger fish-no sign. difference in weight gain at 25, 50, 75% or control after 8 wks.
Webster et al., 2000	1-SBM&MBM 2-SBM, MBM & hempseed meal 3-SBM&PBM 4-SBM, MBM & canola meal	Sunshine bass	100% except control control= 30% SBM and 30% FM		35 & 35 27, 27 & 20 30 & 30 27, 27 & 20	Diet 1 had sign. higher weight gain compared to 3 and 4, but not 2 and the control Specific growth rate for diet 1 sign. Higher than diet 3 but not others FCR's of diet 3 & 4 sign. Higher compared to others Percentage of fillet weight & HSI not sign. different among treatments

Reigh, and Ellis, 1992	SBM	Red drum	50%	34	0, 25, 50, 75, 100, and 100 with methionine supp.	100% soy diet with and without supplement were poorly consumed 0, 25, and 50% diets all had higher growth rates than 75% Highest FE from 50% diet, but lower feed consumption than 75, 25, and 0% soy diets
McGoogan, and Gatlin, 1997	SBM	Red drum	90%	38	0, 90, 90+ glycine, 90+ fish solubles, 95, 100	No growth reduction up to 90% replacement; 95 and 100% inclusions led to sign. decrease in weight gain; Glycine and fish solubles increased weight gain compared to unsupplemented 90% SBM diet
Kikuchi, 1999	SBM SBM, blood meal SBM, CGM SBM, BM, and blue mussel meat SBM, CGM, and blue mussel meat	Japanese flounder juveniles	45%	48 49 51 48 49 51 49	40 40&10 30&10 40&10 30&10 25,10,&5 25,10,&5	 Weight gain comparable to control, but FE & protein efficiency ratio sign. lower Inferior growth and feed utilization Best growth and feed utilization seen in last 2 diets Final body weight, weight gain and protein efficiency ratio all higher than control
Berge et al., 1999	SPC SPC with squidmeal coating	Atlantic halibut	45% for 600-900g fish	48	28 28	No effect of dietary treatment on specific growth Squid had no effect on feed intake, growth, or FE FE sign. lower for both diets compared to 100% FM
Day and Gonzalez 2000	SPC	Turbot juveniles	Up to 25%	50	0, 25, 50, 75, 100	FCR increased with increasing SPC, but no sign. difference b/t diets 1, 2, & 3; ACPD were not affected by levels of SPC; No sign. difference in PER of diets 1 and 2; no differences in growth b/t diets 1 & 2

Robaina et al., 1995	SBM LSM	Gilthead seabream juveniles	Up to 20%	55	10, 20, or 30%	All diets accepted by fish; mean feed intake, FE, PER, and weight increase not affected by type or amount of protein PER sign. higher for SBM20% than LSM20% Protein retention lower for SBM20% and LSM10% Highest weight increase from SBM10%, sign. higher than LSM30%
Kissil et al., 2000	SPC RPC	Gilthead seabream juveniles		62 59	30, 60, and 100%	100%RPC and 60 and 100%SPC had lower body lipid, and energy compared to control Increase in plant protein led to decreased feed intake Except for RPC30%, weight gain was sign. lower for RPC and SPC diets
Kissil and Lupatsch, 2004	SPC WG CGM	Gilthead seabream	Combination diet can partially or completely replace FM	45	Mixture of plant proteins 25, 50, 75, and 100% 4 diets of 100%SPC,WG,CGM, and FM control	Growth on 100% WG, and 25-100% combination diets were superior to fish meal control; combination diets also had lower FCR's
Carter and Hauler 2000	SBM Narrow-leafed Lupin Field Peas	Atlantic Salmon	33% for SBM and peas	66 or 38	25 and 33% compared to nutritionally balanced control, and commercial salmon diet	No sign. difference between control and diets with plant proteins in weight gain Feed consumption sign. higher for lupin at 33% FER and PPV highest for peas and SBM (not affected by inclusion), lowest for lupin at 33%

[1]Abbreviations:

SBM – Soybean meal
SPC – Soy protein concentrate
CGM – Corn gluten meal
LSM – Lupin seed meal
MBM – Meat and bone meal
PBM – Poultry by-product meal
WG – Wheat gluten
RPC – Rapeseed protein concentrate
FM – Fish meal
FCR – Feed conversion ratio
FE – Feed efficiency
PER – Protein efficiency ration
ACPD – Apparent crude protein digestibility
PPV – Productive protein value
HSI – Hepatosomatic Index

Table 1.2 Alternative protein ingredients and their corresponding antinutritional factors

Alternative Protein Ingredient	Antinutritional Factor
Soybean meal	Low palatability, protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens, low in methionine
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins, high fiber
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, antivitamins, cyclopropenoic acid
Lupin seed meal	Protease inhibitors, saponins, phytoestrogens, alkaloids
Corn Gluten meal	High fiber, presence of cartenoids can turn the flesh of fish to a yellowish color
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins
Wheat Gluten	Expensive
Poultry by-product meal	Variable quality, high ash content

CHAPTER 2

Replacement of fish meal in cobia diets using a yeast-based organically certified protein

Abstract

A six-week feeding trial was conducted to evaluate the use of a yeast-based, certified organic protein source as a replacement for fish meal in diets for cobia (*Rachycentron canadum*). Five experimental diets were formulated to provide 40% crude protein and 11% dietary lipid (dry matter basis), with the yeast-based protein source replacing Special Select® menhaden fish meal at 25, 50, 75 and 100% of dietary protein. Ten juvenile cobia (initial weight, 11.5 g/fish) were randomly stocked in triplicate 300 l circular fiberglass tanks (n=30 treatment^{-1}) and hand-fed the diets based upon total tank biomass twice daily at 0900 and 1400 h. Fish were group weighed weekly to monitor performance and adjust feeding rations. Water temperature and salinity were maintained at 27 C and 15 ‰, respectively. At the end of the feeding trial, weight gain, ranging from 86 to 512% , and feed efficiency ratio values, ranging from 0.17 to 0.53, were significantly affected by the inclusion of the yeast-based protein source, with decreasing values as inclusion levels of the yeast-based protein source rose above 50% of dietary protein. Cobia fed the diet containing 25% of dietary protein from the yeast-based protein source had equal weight gain and feed conversion ratio values as fish fed the control diet composed of 100% fish meal (503 vs 512 and 1.9 vs 1.9, respectively). Biological indices including hepatosomatic index, visceral somatic index and muscle ratio, were all similarly affected by inclusion of the yeast-based protein source, with significant impacts when inclusion levels rose above 50% of dietary protein. As with the weight gain and feed efficiency ratio values, fish fed the diet containing 25% of protein from the yeast-based source had values similar to those observed in the control animals. This study represents the first attempt to utilize an organically certified protein source as a replacement for fish meal in diets for juvenile cobia. Although levels of inclusion of the yeast-based protein source above 50% of dietary protein resulted in detrimental effects on production characteristics, the data clearly suggest that, at a minimum, 25% of dietary protein can be provided by this yeast-based protein in diets for cobia.

Key words: yeast, recirculating aquaculture, quality, composition, growth, cobia, *Rachycentron canadum*

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1. Introduction

Fish meal is generally considered to represent the "gold standard" dietary protein source for carnivorous fishes. However, even though the animal feedstuffs and competing industries have increased demands for fish meal, global production of this commodity has remained relatively stable over the last decade, and supplies are unlikely to increase (FAO, 2004). Indeed, the increasing scarcity of suitable protein sources for human consumption may result in the use of industrial fish for the plate, resulting in a further weakening in supplies (Craig and McLean, 2005). Already, aquafeeds account for $\geq$ 50% of variable operating costs of intensive aquaculture operations, with protein representing the most costly feed ingredient (Bassompierre et al., 1997). If aquaculture is to continue to expand to meet global demands for seafood products, development of cost-effective and sustainable dietary formulations will be mandatory (Catacutan and Pagador, 2004). This only can occur through significant reductions in the dependence of the aquafeed industry upon fish meal supplies.

Because fish meal represents a finite resource and as it has become more expensive over time (FAO, 2004), it is not surprising to find that the aquafeed industry has sought out alternative, less expensive, protein sources. For alternative or supplemental proteins to be useful, however, they must possess certain characteristics. Alternative proteins must be competitively priced relative to fish meal on a unit protein basis. They cannot negatively impact fish performance (digestibility, growth, disease resistance, etc.) or product quality and must be commodities (*i.e.*, traded internationally) (Hardy and Tacon, 2002). As well, alternative proteins must not be environmentally degrading with respect to nitrogen and phosphorus discharge and should be easily handled, stored and amenable to pelleting. Because of these restrictions, there presently exists, only a limited number of potential candidates. These include the pulses, oilseeds, grains, rendered animal meals, processing discards and fishery by-catch. Soybean meal, in particular, represents one of the most widely-used alternate protein sources employed by aquaculture, due to its global distribution, cost, relatively high digestibility, good amino acid profile and high protein content (Storebackken et al., 2000). Nevertheless, soybean and other alternative protein meals each contain a variety of anti-nutritional factors that negatively impact production performance of cultured fish (Francis et al., 2001).

An issue of more recent concern relates to that of biosecurity and food safety. Western consumers have, due to enhanced education and increased access to scientific and media

services, become more sophisticated in their purchasing decisions. In an age of bioterrorist threat, outbreak of unusual zöonoses (*e.g.*, transmissible bovine spongiform encephalitis, severe acute respiratory syndrome), increasing health concerns related to chemical contaminants (Hites et al., 2004) and the advent of genetically modified organisms, more attention than ever before is being given to food quality and safety (Reid et al., 2004). This shift in consumer eating patterns has stimulated production of organic foods. As of the early 1980s, aquaculture represented the world's fastest-growing food production sector. However, since 1999, for many countries organic agriculture has supplanted aquaculture as the fastest-growing food production sector (FAO, 1999; El-Hage Scialabba and Hattam, 2002). This trend continues on a global basis and includes a growing organic aquaculture segment. Interest in organic aquaculture is based primarily upon the potential profitability of the organic sector (Craig and McLean, 2005). Although no official statistics are available with respect to organic aquaculture production, estimates suggest that in 2000 it did not exceed 5000 tons, which represents 0.01% of global aquaculture output (Bergleiter, 2001). This negligible production of certified aquaproduce underscores the difficulties inherent in achieving organic aquaculture standards. The principal problem encountered relates to sourcing organic feed and nutrient resources (Tacon and Pruder, 2001). Based on current estimates of certified organic aquaculture production and anticipated growth of the industry, it has been predicted that organic aquaculture harvests will achieve 1.2 million tons by 2030 (El-Hage Scialabba and Hattam, 2002). If such an increase is to be realized, however, new sources of certifiable feeds must be found. The search for organically certified alternate proteins, especially for carnivorous species, represents a greater challenge than securing alternative proteins alone. The present study was initiated with this challenge in mind. The carnivorous cobia was used as an experimental animal due to the hardy nature of this fish and the increasing interest associated with this species. It is also believed that if fish meal can be completely eliminated from a high level marine carnivore diet, then it should be possible to remove all fish meal from diets for all other species as well. The organically certified yeast-based protein was employed as the alternative protein source in this study due to its relatively high crude protein levels and satisfactory amino acid profile.

2. Materials and Methods

2.1 System and husbandry

The feeding trial was conducted at the Virginia Tech Aquaculture Center in Blacksburg, Virginia in a custom-designed, recirculating aquaculture system (RAS; Fig. 2.1). The RAS was comprised of twenty-four 300 l circular fiberglass tanks, a bubble-bead filter (BBF-2, Aquaculture Technologies Inc., Metaire, LA, USA) to remove suspended solids, a UV light sterilizer (Emperor Aquatics, Pottstown, PA, USA), a KMT fluidized bed with media (Kaldnes Inc., Providence, RI, USA) for biological filtration, and a side-looped protein skimmer (R&B Aquatic Distribution, Waring, TX, USA) to remove smaller solids and decrease turbidity. A thermostatically-controlled heater, placed in the biofilter sump, was employed to maintain water temperature at 27 degrees C. Water salinity was maintained at 15 ‰ with the addition of synthetic sea salts (Marine Enterprises International, Baltimore, MD, USA). Fish were exposed to a 12:12 light:dark cycle through fluorescent lighting positioned 8 m above the culture system. Water quality parameters during the feeding trial were as follows: dissolved oxygen, 6.10 $\pm$ 0.24 mg/L; total ammonia nitrogen, 0.40 $\pm$ 0.07mg/L; nitrite, 0.32 $\pm$ 0.06 mg/L, nitrate, 8.78 $\pm$ 3.33 mg/L; and pH, 7.57 $\pm$ 0.19.

Juvenile cobias (*Rachycentron canadum*) were purchased from the Aquaculture Center of the Florida Keys and acclimated in four 1000 l tanks for 2 weeks. After the acclimation period, ten juvenile cobia, (average initial weight, 11.5 g/fish), were placed into each of 15 experimental tanks. Fish were hand-fed twice per day, at 09.00 and 16.00 h. The ration was divided equally between the two feedings, based upon total body weight, initially starting at 8% body weight per day, and decreased to 7% during the final week of the feeding trial which maintained a level of apparent satiation without overfeeding. Fish in tanks were group weighed weekly to adjust the feeding rates and monitor growth performance.

2.2 Diets

Menhaden fish meal (Special Select®, Omega Protein, Hammond, LA, USA) and a yeast-based product were the two protein sources utilized in this study. NuPro® is a certified organic yeast-based protein source comprising a mixture of nucleotides, peptides, and the contents of the cytoplasm. NuPro® was obtained from Alltech, Inc. (Nicholasville, KY, USA) and served as a replacement for fish meal in the experimental diets. The five experimental diets were isonitrogenous and consisted of a control diet (100% fish meal) and four other diets in

which NuPro® replaced a proportion of fish meal (25, 50, 75, and 100% of dietary protein). The diets were formulated to provide 40% crude protein and 11% lipid on a dry weight basis, supplying 1243 kJ available energy /100 g dry diet, except for Diet 5 (0 fish meal/100% NuPro®) which was formulated to provide 1142 kJ available energy/100 g dry diet to meet the constraint of maintaining the diets as isonitrogenous (Table 2.1). Menhaden fish oil was used as the lipid source (Omega Oils, Reedville, VA USA) and dextrin was included in the diets as the carbohydrate source. Calcium phosphate was added to Diets 4 (25/75) and 5 (0/100) which contained higher inclusion levels of NuPro®, to balance dietary phosphorous levels.

2.3 Analyses

At the end of the feeding trial, three fish from each tank (N=9 treatment^{-1}) were euthanized by an overdose of clove oil (Sigma-Aldrich, St. Louis, MO, USA) and bled via caudal venipuncture for measurement of packed cell volume (PCV) and plasma protein levels. Fish were measured for length and weight and weight gain, feed efficiency ratio values (FE = g gain / g of weight gained), survival, visceral somatic index (VSI = visceral mass weight *100 / total body weight), hepatosomatic index (HSI = liver weight *100 / total body weight), and muscle ratio (MR = fillet weight *100 / total body weight) were calculated. Muscle and liver samples also were collected for proximate analysis, including crude protein, total lipid, dry matter and ash (AOAC, 1994). Liver samples were analyzed only for lipid due to sample size.

2.4 Statistical analyses

All data were subjected to analysis of variance utilizing SAS 9.1 (SAS, Cary, NC, USA). Where appropriate, data also were subjected to Duncan's multiple range test for means separation. Differences were considered significant at $\alpha < 0.05$.

3. Results

Weight gain ranged from 86 to 512% (Table 2.2) and was significantly affected by inclusion of the yeast-based protein source. There was a noted decrease ($P < 0.0001$) in weight gain with increasing inclusion of the yeast-based protein source, except for Diet 1 and Diet 2 which had similar weight gains of approximately 500%. Feed efficiency ratio values (FE) ranged from 0.17 (Diet 5) to 0.53 (Diets 1 and 2) with the FE decreasing ($P < 0.0001$) as inclusion rate of the yeast-based protein source increased (Table 2.2). Once again, there was no

difference in growth between Diet 1 and Diet 2, and these two diets produced the highest feed efficiency ratio values during the feeding trial. Survival also was affected significantly by dietary treatment as the fish fed Diet 5 had lower survival (63%) compared to an overall survival rate of 99% among cobia fed the remaining diets (Table 2.2). Muscle protein also tended to decrease as inclusion of the yeast-based protein source increased, with a range of 17.8-19.7% (wet weight basis : Table 2.3).

Muscle protein in fish fed the control diet (Diet 1) and the Diet 2 did not differ. Muscle lipid ranged from 0.5-1.8% (wet weight basis), with the highest lipid level being observed in fish fed the diet containing 25% of the yeast-based protein source, and the lowest lipid level in fish fed Diet 5 (Table 2.3). Dry matter and ash ranged from 20.3-23.7% and 6.0-9.9%, respectively, with inclusion rate of the yeast-based protein source having significant impacts ($P < 0.05$). Liver lipid concentration also was significantly impacted by the presence of the yeast-based protein source, ranging from 4.2-24.6% (wet weight basis), with fish fed Diets 4 and 5 having lower hepatic lipid levels ($P < 0.0001$) compared with fish fed the remaining diets.

Muscle ratios ranged from 10.1-25.3% and decreased ($P < 0.001$) with increasing levels of yeast-based protein inclusion (Table 2.4), although differences were only noted in fish fed the diets with the highest inclusion levels (Diets 4 and 5, respectively). Visceral somatic index (VSI) increased as inclusion rate of the yeast-based protein source increased. The range of VSI was 10.8-16.4% with the lowest VSI from the control diet (Diet 1) and the highest VSI in fish fed the diet containing 100% of dietary protein from the yeast-based protein source (Diet 5; Table 2.4). Hepatosomatic index (HSI) ranged from 2.2-4.6% and was significantly impacted by dietary treatment. Smallest livers were observed in fish fed the diet containing 100% of dietary protein from the yeast-based protein source (Diet 5) whereas the largest HSI was recorded in fish fed the control diet (Diet 1; Table 2.4). Packed cell volume (hematocrit) measurements ranged from 32-51% and were significantly affected by inclusion of the yeast-based protein source. The lowest value was reported for fish fed the 100% yeast-based diet and may indicate that the fish were anemic. Fish fed the control diet (Diet 1), Diet 2, Diet 3, and Diet 4 did not differ in hematocrit values and were higher ($P < 0.0001$) than that observed in fish fed the diet with 100% yeast-based protein (Table 2.4). Plasma protein concentrations followed an identical trend as that observed for packed cell volume, ranging from 2.4-4.6% with fish fed Diets 4 and 5 having significantly lower plasma protein levels compared to fish fed the remaining three diets. Fish fed

Diet 2 had the highest plasma protein concentrations ($P < 0.0001$) of any fish in the feeding trial (Table 2.4).

4. Discussion

The lack of availability of organically certified alternate protein sources represents the major impediment to the development of the organic aquaculture sector (Craig and McLean, 2005). Debate surrounds the certifiability of by-catch from commercial fisheries, and of by-products and processing wastes from aquaculture, fish and meat processing industries as organic aquafeed ingredients. Moreover, questions remain regarding the palatability and amino acid availability of such products (Li et al., 2004a). Challenges also are presented when considering vegetable protein sources, especially for use in feeds for higher level carnivores such as cobia. Most plant proteins harbor anti-nutritional factors and have low dietary value due to essential amino acid deficiencies and/or imbalances and poor digestibility (Hardy, 1996; Francis et al., 2001). These issues may be amplified of organically certified plant proteins, where delayed field operations, poor soil moisture, competition by weeds, and reduced mineralization of organically-certified manures throughout a growing season, may each severely impact crop production and quality. Moreover, the risks of contamination of organic crops, especially for grains and pulses, by traditional and genetically modified harvests, are of serious concern (Hanson et al., 2004). The use of fermentation technologies for the production of single cell-based products surmounts all the preceding problems, besides providing a totally biosecure production environment.

The present investigation demonstrates that, at a minimum, 25% of the fish meal component in cobia diets can effectively be replaced by the yeast-based protein source utilized in the present study without negative consequences to animal performance. Concurrently, these studies represent the first demonstration of fish meal replacement with an organically certified alternate protein in cobia feeds. It is highly likely that the level of this yeast-based protein source could be increased substantially, since cobia production characteristics began to decrease only following 50% inclusion rate of the yeast-based protein source. These results are thereby similar to observations for other species of juvenile carnivorous fishes in which fish meal was replaced using yeast-based products to 30-50% without negative impact (Beck et al., 1976; Rumsey et al., 1990; Oliva-Teles and Goncalves, 2001). Only two other studies have examined fish meal replacement in cobia diets; both used traditional soybean meal and were able to achieve 40%

substitution without detrimental effects upon cobia weight gain and feed conversion (Chou et al., 2004: Wang et al., 2005). Juvenile cobias fed the diet containing 100% of dietary protein from the yeast-based protein source were severely compromised with respect to all production and biological parameters examined, exhibiting low weight gain response, reduced FE ratio values and poor survival. One reason underlying the decreased performance of animals fed the 100% yeast-based diet was most likely product palatability. It was observed that there was a significant amount of feed remaining in the tanks receiving to the 100% yeast-based diet after feeding. Although feed intake was not directly measured, this observation indicates that palatability was poor in this diet. Likewise, the significantly reduced biological indices (higher VSI, lower HSI, muscle and liver lipid) in fish fed this diet can be attributed to their extremely low growth rates. It appears that for cobia and other marine carnivores, a blend of alternate protein sources will be required if fish meal is to be effectively replaced without negative impact upon production performance (Craig and McLean, 2005).

Another feature of dietary yeasts and yeast products are their immunostimulating properties. A wide variety of studies, with a broad range of species, have illustrated enhanced non-specific immune activity, particularly under conditions of immuno-depression and environmental stress (Lara-Flores et al., 2002; Olvera-Novoa et al., 2002; Li and Gatlin, 2004; Li et al., 2004b; McLean and Craig, 2004; Bagni et al., 2005; Choudhury et al., 2005). While immune response was not specifically tested in the present study, the significantly heightened plasma protein concentrations observed in cobia fed the diet containing 25% of dietary protein from the yeast-based protein source may indicate a beneficial immunological impact of inclusion of this product in aquafeeds for cobia. It is noteworthy that this species demonstrates elevated hematocrit levels, an indicator of the high metabolic activity, and thus rapid growth, observed in cobia.

Differences in muscle and hepatic lipid levels observed in fish fed the diets containing 25 and 50% of dietary protein from the yeast-based product may indicate impacts from nucleotide inclusion on energy partitioning. The availability of pre-formed peptides, oligopeptides and nucleotides in the yeast-based diets may have decreased overall energy demands, leaving more energy for potential storage, which was diverted to these tissues (Burrells et al., 2001). Clearly, this aspect of alternative protein research is worthy of more thorough investigation.

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Table 2.1 Composition of the experimental diets (g/100g dry weight).

Ingredient Fish meal: Yeast-based protein	Diet 1 100/0	Diet 2 75/25	Diet 3 50/50	Diet 4 25/75	Diet 5 0/100
Extracted fish meal[1]	54.4	40.8	27.2	13.6	0
NuPro®[2]	0	19.5	39.0	58.5	78
Dextrin[3]	9.5	9.5	9.5	9.5	3.6
Menhaden fish oil[4]	9.6	9.6	9.5	9.4	9.4
Mineral mix[5]	4.0	4.0	4.0	4.0	4.0
Vitamin mix[6]	3.0	3.0	3.0	3.0	3.0
CMC[3]	1.0	1.0	1.0	1.0	1.0
CaPO4[7]	0	0	0	1.0	1.0
Cellufil[3]	18.5	12.6	6.8	0	0
Crude protein[8]	40	40	40	40	40
Crude lipid[8]	11	11	11	11	11
Available energy (kJ/100 g diet)[8]	1243	1243	1243	1243	1142

[1] Omega Proteins, Hammond, LA.
[2] Alltech Incorporated, Nicholasville, VA.
[3] US Biochemical Corporation, Aurora, IL.
[4] Omega Oils, Reedville, VA.
[5] ICN Corporation, Costa Mesa, CA.
[6] See Moon and Gatlin (1991).
[7] Sigma-Aldrich, St. Louis, MO.
[8] Calculated.

Table 2.2 Weight gain, feed conversion efficiency values (FCE) and survival in juvenile cobia fed experimental diets in which fish meal was incrementally replaced by the yeast-based protein source.[1]

Diet (Fish meal : yeast-based protein source)	Weight gain[2]	FCE[3]	Survival (%)
100/0	512[a]	1.9[c]	100[a]
75/25	503[a]	1.9[c]	100[a]
50/50	432[b]	2.4[bc]	97[a]
25/75	238[c]	3[b]	100[a]
0/100	86[d]	5.8[a]	63[b]
Pooled SE	15.56	0.14	7.60
P < *F*	0.0001	0.0001	0.0248

[1] Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] Weight gain = (final tank weight – initial weight)/initial tank weight x 100.
[3] FE = g gained/g fed.

Table 2.3 Muscle protein, muscle lipid, muscle dry matter and ash and liver lipid in juvenile cobia fed experimental diets in which fish meal was incrementally replaced by the yeast-based protein source[1].

Diet Fish meal:yeast-based protein source	Muscle protein	Muscle lipid	Muscle dry matter	Muscle ash	Liver lipid
100/0	19.3^{ab}	0.93^{bc}	22.07^{ab}	6.20^{b}	16.16^{c}
75/25	19.7^{a}	1.80^{a}	23.70^{a}	5.97^{b}	24.64^{a}
50/50	19.0^{b}	1.30^{b}	22.40^{a}	6.40^{b}	20.87^{b}
25/75	18.3^{c}	0.93^{bc}	21.70^{ab}	6.10^{b}	13.37^{c}
0/100	17.8^{d}	0.50^{c}	20.30^{b}	9.80^{a}	4.19^{d}
Pooled SE	1.10	0.25	0.50	0.44	1.90
$P < F$	0.0001	0.0001	0.0536	0.0215	0.0001

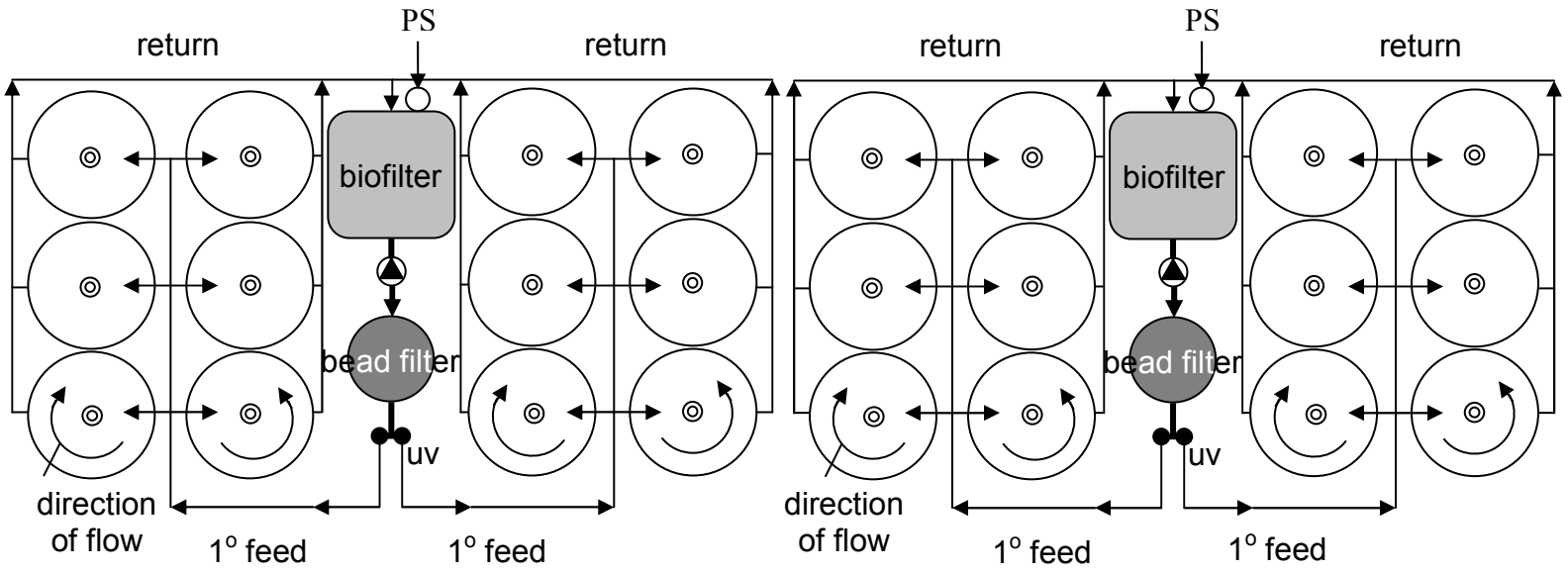
[1] Means of 3 fish per tank ($N = 9$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$).

Table 2.4 Biological indices including the muscle ratio (MR), visceral somatic index (VSI), hepatosomatic index (HSI), packed cell volume (hematocrit) and plasma protein concentrations in juvenile cobia fed experimental diets in which fish meal was incrementally replaced by the yeast-based protein source[1].

Diet Fish meal:yeast-based protein source	MR[2]	VSI[3]	HSI[4]	Packed cell volume (%)	Plasma protein (%)
100/0	25.3^{a}	10.8^{c}	2.1^{e}	40^{ab}	3.8^{b}
75/25	25.9^{a}	12.0^{bc}	3.3^{c}	49^{a}	4.6^{a}
50/50	15.4^{b}	12.9^{b}	3.9^{b}	51^{a}	4.2^{ab}
25/75	16.7^{b}	15.4^{a}	4.6^{a}	48^{a}	3.8^{b}
0/100	10.1^{c}	16.4^{a}	2.7^{d}	32^{b}	2.4^{c}
Pooled SE	2.62	0.72	0.33	6.0	0.44
$P < F$	0.0001	0.0001	0.0001	0.0019	0.0001

[1] Means of 3 fish per tank ($N = 9$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] MR = muscle weight*100/body weight
[3] VSI = VSI weight*100/body weight
[4] HSI= HSI weight*100/body weight

Figure 2.1 Diagram of recirculating life support system employed during the present investigations. The system comprised two independent 12-tank units serviced by a biological filter, side-looped protein skimmer (PS), bead filter and UV light. Temperature was maintained using a thermostatically-controlled heater positioned within the biofilter sump.



CHAPTER 3

The effects of organic protein supplementation upon growth, feed conversion, and quality parameters of juvenile cobia

Abstract

An eight-week feeding trial was conducted to examine the impacts of organically certifiable alternate protein sources on growth, feed efficiency, biological indices, fillet proximate composition and fillet quality of juvenile cobia, *Rachycentron canadum*. Diets were formulated to be isonitrogenous and isocaloric. The control diet provided 45% crude protein from Special Select® menhaden fish meal and 10% total lipid (dry weight basis). The remaining diets were formulated with 25 and 40% inclusion of NuPro® (an organically certifiable yeast-derived protein source), and 40% inclusion of organically certified soybean meal, soybean isolate, or hemp. Two additional diets were formulated to contain a mixture of all organic protein sources at 23% with 8% fish meal, or organic protein sources at 25% and no fish meal. Diets were fed to triplicate groups of juvenile cobia (initial weight 10g/fish) in 300 l circular tanks in recirculating aquaculture system. Weight gain ranged from 167 to 1138% increase from initial weight and was similar for all fish fed diets containing 40% of any given alternate protein source. Fish fed the blended diet with 8% fish meal exhibited significantly lower weight gain, SGR, and FE ratio values than all other fish. Cobia fed the diet without any fish meal did not survive to the end of the study. Biological indices such as muscle ratio (MR), visceral somatic index (VSI), and packed cell volume (PCV) were all similar between fish fed the control diet and those fed diets with up to 40% alternate protein. Fish fed the diet with only 8% fish meal had significantly lower MR, PCV, and plasma protein, and significantly higher VSI. All fish exhibited similar fillet proximate composition for protein, lipid, dry matter, and ash except for those fed the diet containing 8% fish meal. Alternate protein sources appear to affect the fillet texture of cobia. Generally speaking, plant protein sources returned higher values for all measured textural parameters than the fish meal control. At all time points and within all texture parameters, cobia fed the diet containing hemp returned the highest values except for distance to rupture in the final time point. Results indicated that up to 40% of 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. My 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 value-added products.

Key words: cobia, *Rachycentron canadum,* yeast, soybean, hemp, fish meal, texture analysis, fillet quality

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1. Introduction

Aquaculture has been one of the fastest growing food-producing sectors in the world for more than two decades. Due to its rapid growth (approximately 10% per year since 1984), aquaculture production rather than the traditional fisheries industry is seen as the major source of future supplies of food fish (Watanabe, 2002). The consumption of fish globally has risen from approximately 93 million tonnes in 1998 to 103 million tonnes in 2003 (FAO, 2004) and is expected to continue rising. Such an increase in fish consumption certainly will stimulate an increase in aquaculture production and an increase in demand for fish meal supplies. Since the production of fish meal has remained relatively stable over the past 15 years, a situation that is unlikely to improve, fish meal can no longer be considered a sustainable resource for aquafeeds (FAO, 2004). As such, this finite resource will only become more expensive over time. Feed costs already account for half of the total cost of aquaculture production, with protein being the most expensive feed component (Bassompierre et al., 1997). The recent interest in culture of carnivorous species intensifies this problem, since carnivorous fish require greater amounts of protein, and fish meal represents the primary source for aquafeed formulations. Formulating cost-efficient diets for carnivorous species becomes problematic, and it is no surprise that less expensive, alternate protein sources are being examined.

Numerous alternate protein sources are available, but to prove useful in aquaculture they should be economically competitive, capable of being produced in large quantities (Hardy, 2004), contain balanced amino acid profiles and proper crude protein levels, and not compromise the growth or health of the fish (Hardy and Tacon, 2002). It is also helpful if they are easily handled and stored and do not lead to environmental contamination from release of phosphorous and nitrogen (Lunger et al., 2006). Few protein sources meet all of these criteria, but one of the most promising is soybean meal. Soybean meal (SBM) represents a sustainably produced feedstuff and one that supports cost-effective formulation of diets (Chou et al., 2004). However, SBM contains some antinutritional factors (ANF) such as protease (trypsin) inhibitors, phytohaemagglutinin (lectins), anti-vitamins, phytic acid, saponins, and phytoestrogens (Francis et al., 2001). Yeast-based protein sources also have been used as a dietary replacement for fish meal. Yeast proteins have relatively high crude protein levels and are a source of dietary nucleotides, which have been shown to promote increases in growth (Ramadan and Atef, 1991; Adamek et al., 1996; Burrells et al., 2001b). Another promising alternate protein source is hemp

meal. Hemp meal has high crude protein levels, a good amino acid profile, serves as a dietary source of omega-6 and omega-3 fatty acids and may have potential health benefits (Brousseau and Schaefer, 2000; Callaway, 2004). Unpublished results from an animal feeding trial conducted in Finland have also indicated that hemp meal is just as good as soybean meal when used in diets for farmed fish (Callaway, 2004). An additional benefit of all of these alternate protein sources is that they can be produced and certified organically. While organically certified proteins are presently more expensive than their conventionally produced counterparts, and hemp and yeast-based products are in some cases more costly than fish meal, increasing usage of fish meal combined with higher fuel costs may act to reduce present price differentials.

The organic sector of agriculture is a 28 billion dollar industry, with more than 31 million hectares of farmland under organic management worldwide (IFOAM, 2006). There are currently no approved standards for organic aquaculture production in the United States, but a preliminary report has been published (USDA-NOP, 2006). Due to emerging diseases, such as bovine spongiform encephalopathy (BSE) and avian flu, consumers are becoming more health conscious and expect higher levels of food quality and safety. Organic agriculture is rapidly expanding for these reasons. It is important for the future success of aquaculture to access this lucrative market. Although species such as trout, tilapia and shrimp are already farmed with organic certification in other parts of the world (Craig and McLean, 2005), production of organically certified marine carnivores will be technically and biologically difficult. Due to the high price of fish meal, limited supplies, and it's highly restricted use in diets for organically certified fish, organically certified alternate protein sources will need to be incorporated into aqua diets. This could be problematic due to palatability issues, low crude protein content and inappropriate amino acid profiles that plague many alternate protein sources. An additional concern is that organic diet certifications may not allow addition of synthetic amino acids (Bart Reid, member of the USDA organic aquaculture task force, Personal communication). One approach to surmount this barrier is to incorporate multiple alternate protein sources that have complementary amino acid profiles in diets for organically certified fish (Craig and McLean, 2005).

While the predominant concern with fish meal replacement usually relates to growth performance, another issue of concern is how these alternate protein sources will affect final product quality (de Francesco et al., 2004). Processors and consumers judge fish quality based

on perceived freshness, fat content, color, and texture (Ronsholdt and McLean 2004; Torrissen et al., 2001; Rasmmussen et al., 2000), all of which could potentially be affected by alternate protein incorporation into aquafeeds. The final aspect of the suitability of a protein source for fish meal replacement must take into account the impacts on flesh quality traits. Although a substantial amount of work has been conducted on alternate protein sources, relatively few studies have investigated this issue. Accordingly, the present study was designed to evaluate the use of both yeast and plant-based organically certified proteins as replacements for fish meal and to evaluate their impacts on final product quality in juvenile cobia (*Rachycentron canadum*).

2. Materials and Methods

2.1 System and husbandry

The experiment was carried out at the Virginia Tech Aquaculture Center (VTAC) in Blacksburg, Virginia in a custom-designed recirculating life support system (RLS) as described previously (Lunger et al., 2006). Briefly, the RLS consisted of twenty-four, 300-L circular fiberglass tanks incorporating a bubble bead filter (BBF-2 Aquaculture Technologies Inc., Metaire, LA, USA) to remove suspended solids, KMT fluidized bed with media (Kaldnes Inc., Providence, RI, USA) for biological filtration, a UV light sterilizer (Emperor Aquatics, Pottstown, PA, USA), side-looped protein skimmer (R&B Aquatic Distribution, Waring, TX, USA) to filter out smaller solids and decrease turbidity, and a thermostatically-controlled heater that was placed in the sump to maintain water temperature at 27 degrees Celsius. Salinity was maintained at 20 ± 1 ppt with the addition of synthetic sea salt (Marine Enterprises International, Baltimore, MD, USA) and was measured daily with a refractometer (Aquatic Ecosystems, Apopka, FL). All fish were subjected to a 12:12 light:dark cycle with the use of fluorescent lighting positioned 8 meters above the tanks.

Cobia juveniles were brought to the VTAC and were acclimated to culture conditions in eight, 1000-L tanks for two months. At the start of the study, ten individuals were randomly allocated to each tank (initial weight 10g/fish). Tanks were arbitrarily assigned to one of eight diets (n=3 tanks/diet), and the fish were hand-fed twice daily at 09.00 and 16.00. Feeding rates were determined based on tank weights, and rations were divided equally between the two feedings. The fish were initially fed 10% body weight, which was gradually decreased to 6% by

the end of the study to maintain a level of satiation without over-feeding. Fish in each tank were weighed weekly to monitor growth and adjust feeding rates.

2.2 Diets

Seven diets were formulated with the use of four organic protein sources. These included: 1. NuPro®, a yeast-based protein source (Alltech Inc., Nicholasville, KY, USA), which contained a mixture of nucleotides, peptides, and the contents of the cytoplasm, 2. soybean meal (SBM) from Professional Proteins (Washington, IA, USA), 3. soybean isolate from Nutriant (Cedar Falls, IA, USA), and 4. hemp from Manitoba Harvest Hemp Foods and Oil (Winnipeg, Manitoba, Canada). An additional diet (Diet 1) formulated as the control diet used Special Select® menhaden fish meal from Omega Proteins (Hammond, LA, USA) as the sole protein source. All diets were formulated to be isonitrogenous and isocaloric (except for Diet 7; see Table 3.1) providing 45% crude protein, 10% lipid and 320 kcal of available energy /100g dry diet. NuPro® replaced 25 and 40% of the fish meal in diets 2 and 3, respectively, while soybean meal (Diet 4), soybean isolate (Diet 5), and hemp (Diet 6) all replaced 40% of the fish meal in each respective diet. NuPro®, the organically certifiable yeast-based protein source, was chosen to replace 25% fish meal protein to reevaluate results obtained from Lunger et al. (2006), and the 40% replacement level was chosen because it appears that 40% fish meal replacement is the maximum level for cobia without adversely affecting growth or biological indices (Chou et al., 2004; Zhou et al., 2005; Lunger et al., 2006). Two additional diets were formulated with a combination of all organic protein sources. Each organic protein source in one diet (Diet 7) replaced 23% fish meal, (i.e., 8% protein from fish meal). Diet 8 replaced all the fish meal protein using 25% of each of the organic sources. Menhaden fish oil (Omega Oils, Reedville, VA, USA) was used as the lipid source and dextrin was used as the carbohydrate source in all diets. Calcium phosphate was added to the blended diets with higher levels of fish meal replacement to balance dietary phosphorus levels.

All diets were manufactured at the VTAC. Each ingredient was weighed and placed into a V-mixer (Patterson Kelly, East Stroudsburg, PA) for 15 minutes. Ingredients then were placed in a bowl for further mixing and the addition of fish oil and distilled water. Feed was placed into a 30-liter mixer (Hobart, Troy, OH) and pelleted by hand to obtain the desired size. Diets were air-dried to approximately 75% dry matter and stored in a freezer (-20°C) prior to feeding.

2.3 Analysis

Ten fish were initially sampled at the beginning of the study to measure whole body values of crude protein, total lipid, dry matter and ash (AOAC, 1994). At the end of the study, all fish were weighed to obtain a final weight. Three fish were sampled from each tank (n=9 treatment^{-1}) and euthanized by an overdose of clove oil (3 mg/l; Sigma-Aldrich, St. Louis, MO, USA). Length was measured and each fish was weighed individually to calculate weight gain (% increase from initial weight), specific growth rate (SGR = ln final weight – ln initial weight / # of days), and feed efficiency ratio value (FE = g of weight gained / g food fed). These fish also were bled via caudal venipuncture for measurement of packed cell volume (PCV) and plasma protein values. Fillet, viscera, and liver were weighed to calculate the muscle ratio (MR = fillet weight *100 / total body weight), visceral somatic index (VSI = visceral mass weight *100 / total body weight), and hepatosomatic index (HSI = liver weight *100 / total body weight). Muscle and liver samples were kept for further analysis of crude protein, total lipid, dry matter and ash contents (AOAC, 1994). The remaining fish were euthanized with clove oil and filleted for texture analysis. Initial texture analysis was performed on muscle immediately following the end of the study. All other fillets were vacuum-packed and stored at -20°C for future sampling. Three fillets from each diet were sampled on February 1, 2006, March 15^{th,} 2006, and April 26th, 2006. A texture analyzer, (model TA-XT Plus, Stable Micro Systems, Surrey, England) was used to measure breaking force (g), distance to rupture (mm), gel strength (g*mm) and total energy [force (g)* time (sec)] on the dorsal side of the muscle. A spherical probe with a diameter of 5 mm was used for all tests with a 5 kg load cell, test speed of 1.10 mm/sec and 50% strain. The trigger force was set to 5.0 g. The probe moved downward a fixed distance of 15mm until it touched the fillet and continued downward until 50% compression was reached. One whole fish from each tank was frozen at the end of the study for future whole-body proximate analysis and the calculation of protein efficiency ratio (PER = total g gain / g protein fed) and protein retention (PR = whole body protein final – whole body protein initial / g protein fed).

2.4 Statistical Analysis

All data were analyzed by analysis of variance test using the statistical program SAS 9.1 (SAS, Cary, NC, USA). When appropriate, data also were subjected to a Duncan's multiple range test for means separation. Significant differences among the data were observed when $\alpha < 0.05$.

3. Results

Weight gain (increase from initial weight) ranged from 167 to 1138% (Table 3.2) and was significantly affected by amount and type of dietary protein source. There were no significant differences for weight gain ($P < 0.0001$) between diets containing 40% of NuPro®, soybean meal, soybean isolate or hemp. Fish fed diet 2 (25% NuPro®), however, exhibited higher weight gain than all other diets ($P < 0.0001$). The fish fed the 100% fish meal control diet showed significantly lower weight gain than the previously mentioned diets. The fish fed the diet comprising a mixture of alternate protein sources with 8% fish meal (Diet 7) expressed lower weight gain than all other diets ($P < 0.001$). Feed efficiency (FE) ratio values ranged from 0.19 for the 8% fish meal diet to 0.61 for the 25% yeast protein diet (Table 3.2). FE values followed the same trend as weight gain, with fish fed the 25% yeast-based diet having a significantly ($P < 0.0001$) higher value than all other diets, and fish fed the 8% fish meal diet having a significantly lower value than fish fed all other diets. The FE value for those fed the 40% yeast protein diet was significantly lower than those fed the 25% yeast protein diet. The fish consuming the control diet had a FE value that was lower than fish fed all other diets except those fed the 8% fish meal diet. Survival also was significantly affected by dietary treatment, with fish fed the 8% fish meal diet illustrating lower survival rates (53%) when compared to fish fed all other diets (Table 3.2). Cobia juveniles fed the diet containing no fish meal all died prior to the end of the study.

Fish fed the control diet (Diet 1) expressed higher muscle protein than fish fed the diets containing 40% yeast, 40% soybean meal, or 8% fish meal ($P = 0.0103$). Muscle protein values for fish fed diets containing 25% yeast, 40% soybean isolate and 40% hemp were all similar as were the muscle protein values for fish fed diets containing 40% yeast, 40% soybean meal and 8 % fish meal (Table 3.3). Muscle lipid ranged from 0.86 to 1.88% (wet weight basis : Table 3.3). Fish fed with the control diet and the 8% fish meal diet had lower values than fish provided with diets containing 40% yeast, soybean meal, soybean isolate, or hemp ($P = 0.0086$). Liver lipid ranged from 1.80 to 16.16% (wet weight basis : Table 3.3). Cobia juveniles fed the control diet and the 8% fish meal diet had the lowest liver lipid values, which differed from those of animals fed all other diets ($P = 0.0002$). Dry matter ranged from 21.7 to 24.6% (Table 3.3). Dry matter values for fish fed the 8% fish meal diet were significantly different from fish fed all other diets except for those fed the control diet ($P = 0.0012$). Dry matter levels in cobia fed the control diet,

the 25% yeast diet and the 40% soybean isolate and hemp diets were all similar. Cobia fed diets containing 40% yeast protein and 40% soybean meal had the highest values, but were not significantly different from those values recorded for cobia fed diets containing either 25% yeast, 40% soybean isolate or 40% hemp. Ash ranged from 5.9 to 8.9% (Table 3.3). Animals fed the diet containing only 8% fish meal had higher ash values than all other treatment groups ($P < 0.0001$).

Muscle ratios ranged from 13.9 to 30.5% ($P < 0.0001$). There were no significant differences between cobia fed the control diet or any of the diets with up to 40% fish meal replacement (Table 3.4). Muscle ratio for cobia fed the 8% fish meal diet was lower ($P < 0.01$) than for cobia fed all other diets. VSI ranged from 8.3 to 14.0% (Table 3.4). Fish fed the 8% fish meal diet had the highest VSI and differed from all other groups ($P < 0.0001$). HSI ranged from 1.1 to 2.6% (Table 3.4), with control diet (Diet 1) fed cobia having the lowest ($P < 0.0001$) and fish fed the 8% fish meal diet exhibiting the highest HSI values. Packed cell volume (PCV) ranged from 27.4 to 37.8% (Table 3.4), with cobia fed the 8% fish meal diet having the lowest and fish fed 40% soybean isolate diet reporting the highest PCV. Plasma protein values ranged from 1.8 to 3.8% (Table 3.4), with animals presented with the 8% fish meal diet expressing the lowest value relative to those fed all other diets ($P < 0.0001$).

Whole-body protein was significantly higher for fish fed the control diet, whereas there were no significant differences among other groups (Table 3.5). Whole-body lipid values were similar for all fish fed diets with up to 40% plant protein, but the control fish exhibited significantly lower values (Table 3.5). All whole-body dry matter values were within the same range, although some significant differences in means were observed (Table 3.5). All cobia had similar whole-body ash content except, that those fed the 8% fish meal diet were higher (Table 3.5). Fish fed diets containing up to 40% plant protein had similar protein efficiency ratios. The control fish had a significantly lower PER than fish fed diets containing up to 40% plant protein, but the fish fed the 8% fish meal diet demonstrated a PER significantly lower than controls (Table 3.5). All cobia had similar protein retention values, except that those fed the diet containing only 8% fish meal were lower (Table 3.5).

3.1 Texture Analysis

All texture parameters examined were impacted by protein source. Initial breaking force (Fig. 3.1) ranged from 295g (SBM) to 395g (Hemp); distance to rupture (Fig. 3.2) ranged from

3.0mm (Control) to 3.9mm (Hemp); gel strength (Fig. 3.3) ranged from 941g*mm (Control) to 1526g*mm (Hemp); and total energy (Fig. 3.4) ranged from 233 (SBM) to 390 (Hemp). For the final time-point, April 26, 2006, breaking force (Fig. 3.1) ranged from 234g (Control) to 312g (Hemp); distance to rupture (Fig. 3.2) ranged from 3.6mm (25% NuPro®) to 4.3mm (40% NuPro®); gel strength (Fig. 3.3) ranged from 882 g*mm (Control) to 1250 g*mm (Hemp); and total energy (Fig. 3.4) ranged from 271 (Control) to 374 (Hemp). All values for time points two (Feb. 1, 2006) and three (March 15, 2006) registered values intermediate to initial and final values and rankings for the respective treatments followed the same trends (Fig. 3.1-3.4).

4. Discussion

Even though there are no established standards for organic aquaculture in the United States, it is probable that inclusion of fish meal in aquafeeds will be highly restricted. Almost certainly, fish meals that are organically certifiable will have to originate from fully sustainable stocks and/or scraps from fish destined for human consumption. These sources will prove to be costly and most likely will not be traded on a commodity level. Additionally, specific synthetic amino acids will be allowed only if they reside on the National List of Approved Substances. As residence on this list must be approved every five years, there is no long-term guarantee that these ingredients will always be available for organically certified rations. This makes formulation of organic diets for carnivorous fishes very difficult. Most plant protein sources are limiting in terms of one or more essential amino acids. Therefore, it may be necessary to use a variety of plant protein sources with complementary amino acid profiles if fish meal is to be eliminated from organic aquafeed formulations (Craig and McLean, 2005). I attempted to achieve this in the current study with diets 7 and 8, which utilized a blend of all protein sources while also examining the impacts of individual organic protein sources on fish performance and quality parameters at a 40% inclusion level.

In the present study, all of the fish fed diets containing a particular organic protein source substituted up to 40% performed equally well. This result is in agreement with the results produced by Chou et al. (2004) and Zhou et al. (2005), which demonstrated that SBM could replace up to 40% of the fish meal in diets for juvenile cobia without adversely affecting fish performance or health. All fish exhibited high increases in weight gain and acceptable specific growth rate values compared to others obtained from our laboratory. Cobia that were fed a

mixture of organic protein sources and fish meal (Diet 7) performed poorly, expressing very low increases in weight gain, these fish had lower survival rates (53%) than fish fed the remaining diets. The fish that were fed the diet containing the mixture of organic plant protein sources with no fish meal (Diet 8) did not survive to the end of the study because they would not eat the feed. This small inclusion of fish meal in the former diet (8%) must have provided some essential nutrients or possibly served as a feed attractant that induced consumption of the feed and helped keep the fish alive. However, these results also indicate that simply utilizing blends of different alternate proteins will not always be sufficient as a fish meal replacement, especially in aquafeeds designed for high-level carnivores. The present study strongly supports the need for establishing quantitative amino acids requirements for cobia, many of which have not presently been determined.

As for the 100% fish meal control diet, weight gain and SGR values were within the normal range for cobia previously obtained in our laboratories, but were significantly lower than fish fed diets with up to 40% fish meal replacement. We examined the in vitro protein digestibility (Bassompierre et al., 1997) of the various protein sources, and found that for the menhaden fish meal was significantly lower than that of the other protein sources. This may explain the superior performance of fish fed diets containing the organically certifiable protein sources. Even though weight gain and SGRs were significantly lower for the control diet, these fish still fared well in comparison to the others in terms of biological indices. In fact, all biological indices for all cobia were within an acceptable range when compared to previous reports for cobia (Chou et al., 2004; Zhou et al., 2005) except for fish fed diet 7 (23% organic protein/8% fish meal).

Muscle lipid and liver lipid values were all low and within a similar range for cobia fed all diets except for diet 7. These values were comparable to those previously reported for cobia (Chou et al., 2004; Zhou et al., 2005; Lunger et al., 2006). Fish fed the control diet had significantly lower muscle and liver lipid levels than fish fed diets with up to 40% replacement of fish meal, but all were in the acceptable range for cobia. This illustrates that the use of organic proteins in diets for juvenile cobia will not cause the deposition of unfavorable amounts of lipid in their muscle or livers. Packed cell volume and plasma protein percentages were all similar for diets 1 through 6. This indicates that all cobia were in good health and could also be a consequence of the high metabolic activity, and thus rapid growth, observed in this species

(Lunger et al., 2006; Sun et al. 2006a; 2006b). The significantly lower levels for both packed cell volume and plasma protein for diet 7 indicate that these fish were in poor health, and that their optimal nutritional requirements were not being met. While there were no significant differences for VSI values between fish fed the control diet or the organic diets with up to 40% fish meal replacement, there was a significant increase in VSI for the fish that were fed a diet containing only 8% fish meal. This also was reported by Lunger et al. (2006), in which the data showed that when 75 and 100% of the fish meal was replaced by an organic protein source, the VSI values significantly increased. This may indicate that cobia alter their digestive physiology in order to increase absorptive capabilities in response to these potentially nutrient-deficient diets. These observations and results from previous studies argue strongly for intensive future research upon the digestive physiology of cobia.

With regards to whole-body analysis of the cobia, fish fed the control diet had significantly higher levels of protein and lower levels of lipid compared to fish fed diets containing up to 40% inclusion of an organic protein source. Nevertheless, these data correlated well with the individual proximate analyses for muscle protein, lipid, and hepatic lipid levels. Zhou et al. (2005) reported similar whole body lipid, muscle, and liver lipid levels in similar-sized cobia fed diets containing up to 60% soybean meal.

Cobia fed the various organic protein sources exhibited equal protein efficiency ratios, which again indicated no detrimental impact of alternate organic protein incorporation. Compared to other published studies with cobia (Chou et al., 2004; Zhou et al., 2005), the values obtained in the present study are lower. While Zhou et al. (2005) observed higher PERs ranging from 1.3 to 1.8, the overall weight gain was one quarter of that observed in the present study.

The texture of fish can be defined by its dryness, hardness, and juiciness, and typically is tested in the industry by the 'finger method' (Lie, 2001). We utilized the texture profile analysis method since it correlates well with sensory evaluation (Torrissen et al., 2001; Schubring, 2005). This represents the first time texture analysis has been applied to cobia fillets. At all time points and for all texture parameters, cobia fed the diet containing hemp returned the highest values, except for distance to rupture in the final time point. Another interesting point, based upon the two diets containing NuPro®, indicate that texture may not be a function of the quantity of alternate protein, but rather one of type. My results suggest that alternate proteins have differential effects upon final product quality. Generally speaking, plant protein sources returned

higher values for all measured texture parameters than the fish meal control, indicating firmer flesh. This may have implications in terms of cobia processing and development of industrial products. For example, texture influences extrusion parameters during the production of preformed products such as fish sticks, surimi, fish nuggets, and other value-added products (Thiébaud et al., 1996). These products have traditionally relied upon groundfish such as cod, hake, haddock, and sole. These fisheries are currently either in serious decline or are highly managed and regulated. A sustainable aquacultured product that can replace these fishes in the institutional food market would go along way towards relieving pressure on these depleted fish stocks. Due to the rapid growth rates of cobia combined with its high quality white flesh, this species has the capability to serve as a replacement to the more traditional white fish market.

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Table 3.1 Composition of the experimental diets (g/100g dry weight).

Ingredient Fish meal:Plant protein	Diet 1 100/0	Diet 2 75/25	Diet 3 60/40	Diet 4 60/40	Diet 5 60/40	Diet 6 60/40	Diet 7 8/23	Diet 8 0/25
Fish meal[1]	65.9	48.2	38.6	38.6	38.6	38.6	5.14	0
NuPro®[2]	0	21.9	35.1	0	0	0	20.2	21.9
Soybean meal[3]	0	0	0	38.8	0	0	22.3	24.2
Soybean isolate[4]	0	0	0	0	22.5	0	13	14.1
Hemp[5]	0	0	0	0	0	37.5	21.8	23.7
Dextrin[6]	7.5	7.5	7.5	7.5	7.5	7.5	1.9	0.2
Lipid[7]	2.6	4.8	5.4	4.1	5.3	4.7	6.65	6.9
Mineral mix[8]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[9]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
CMC[6]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$CaPO_4$[10]	0	0	0	0	0	0	1.0	1.0
Cellufil[6]	16.0	9.6	5.4	3.0	18.1	3.3	0	0
Crude protein[11]	45	45	45	45	45	45	45	45
Crude lipid[11]	10	10	10	10	10	10	10	10
Available energy (kcals/100gdiet)[11]	300	300	300	300	300	300	277.6	270.8

[1] Omega Proteins, Hammond, LA.
[2] Alltech Incorporated, Nicholasville, KY.
[3] Professional Proteins Ltd., Washington, IA.
[4] Nutriant, Cedar Falls, IA.
[5] Manitoba Harvest, Manitoba, Canada
[6] US Biochemical Corporation, Aurora, IL.
[7] Omega Oils, Reedville, VA.
[8] ICN Corporation, Costa Mesa, CA.
[9] See Moon and Gatlin (1991).
[10] Sigma-Aldrich, St. Louis, MO.
[11] Calculated.

Table 3.2 Weight gain, feed efficiency ratio values (FE), specific growth rates (SGR) and survival percentages in juvenile cobia fed experimental diets in which fish meal (FM) was replaced with different organic protein sources[1].

Diet (FM : Organic protein)	Weight gain[2] % increase	SGR[3]	FE[4]	Survival (%)
100/0 Control	753.33^{c}	4.37^{c}	0.51^{c}	100^{a}
75/25 Yeast protein	1138.63^{a}	5.13^{a}	0.61^{a}	96^{a}
60/40 Yeast protein	1010.43^{b}	4.91^{ab}	0.56^{b}	100^{a}
60/40 Soybean meal	1014.03^{b}	4.92^{ab}	0.57^{ab}	100^{a}
60/40 Soybean isolate	964.17^{b}	4.82^{b}	0.60^{ab}	100^{a}
60/40 Hemp	927.53^{b}	4.75^{b}	0.57^{ab}	100^{a}
8/23 Yeast protein, SBM, SBI, Hemp	167.67^{d}	2.00^{d}	0.19^{d}	53^{b}
Pooled SE	36.07	0.08	0.01	4.71
$P < F$	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[1] Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] Weight gain = (final tank weight – initial weight)/initial tank weight.
[3] SGR = (ln final wt. – ln initial wt.)/49 days
[4] FE = g gained/g fed.

Table 3.3 Muscle protein, muscle lipid, muscle dry matter and ash and liver lipid in juvenile cobia fed experimental diets in which fish meal (FM) was replaced with multiple organic protein sources.

Diet (FM : Organic protein)	Muscle Protein[1]	Muscle Lipid[2]	Muscle dry matter[1]	Muscle ash[1]	Liver Lipid[2]
100/0 Control	89.02^{a}	0.91^{b}	22.68^{bc}	6.67^{b}	3.04^{b}
75/25 Yeast protein	85.85^{ab}	1.51^{ab}	23.73^{ab}	5.89^{c}	11.59^{a}
60/40 Yeast protein	82.91^{b}	1.66^{a}	24.50^{a}	6.29^{bc}	16.16^{a}
60/40 Soybean meal	82.58^{b}	1.84^{a}	24.63^{a}	6.28^{bc}	12.26^{a}
60/40 Soybean isolate	86.16^{ab}	1.88^{a}	23.64^{ab}	6.37^{bc}	13.12^{a}
60/40 Hemp	85.26^{ab}	1.85^{a}	23.61^{ab}	6.26^{bc}	13.77^{a}
8/23 Yeast protein, SBM, SBI, Hemp	84.83^{b}	0.86^{b}	21.72^{c}	8.93^{a}	1.80^{b}
Pooled SE	2.13	0.30	0.66	0.24	1.55
$P < F$	0.0103	0.0086	0.0012	< 0.0001	0.0002

[1] Means of 3 fish per tank ($N = 9$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$). Values are presented on a dry matter basis.
[2] Means of 2 fish per tank ($N = 6$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$). Values are presented on a wet weight basis.

Table 3.4 Biological indices, including muscle ratio (MR), visceral somatic index (VSI), hepatosomatic index (HSI), packed cell volume (hematocrit) and plasma protein concentrations, in juvenile cobia fed experimental diets with different organic protein sources[1].

Diet (FM : Organic protein)	MR[2]	VSI[3]	HSI[4]	Packed cell volume (%)	Plasma protein (%)
100/0 Control	28.31^{a}	8.29^{b}	1.12^{d}	34.39^{ab}	3.26^{c}
75/25 Yeast protein	30.53^{a}	9.55^{b}	2.17^{bc}	35.38^{ab}	3.75^{a}
60/40 Yeast protein	27.83^{a}	9.48^{b}	2.41^{ab}	34.94^{ab}	3.76^{a}
60/40 Soybean meal	29.78^{a}	9.08^{b}	1.73^{c}	34.83^{ab}	3.71^{a}
60/40 Soybean isolate	27.64^{a}	8.59^{b}	1.78^{c}	37.75^{a}	3.69^{ab}
60/40 Hemp	26.58^{a}	9.64^{b}	1.97^{c}	33.50^{b}	3.39^{bc}
8/23 Yeast protein, SBM, SBI, Hemp	13.89^{b}	14.02^{a}	2.64^{a}	27.40^{c}	1.77^{d}
Pooled SE	2.23	1.07	0.26	1.78	0.16
$P < F$	< 0.0001	< 0.0001	< 0.0001	0.0002	< 0.0001

[1] Means of 3 fish per tank ($N = 9$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] MR = muscle weight*100/body weight
[3] VSI = VSI weight*100/body weight
[4] HSI= HSI weight*100/body weight

Table 3.5 Whole-body proximate analysis, protein efficiency ration (PER), and protein retention (PR) for juvenile cobia fed experimental diets in which fish meal was replaced with multiple organic protein sources[1].

Diet (FM: Organic protein)	Protein %DM	Lipid %wet	DM	Ash	PER[2]	PR[3]
100/0 Control	69.6^{a}	1.69^{c}	23.3^{b}	16.0^{bc}	0.92^{c}	63.90^{bc}
75/25 Yeast protein	61.7^{bc}	3.44^{ab}	27.6^{a}	14.0^{c}	1.15^{a}	70.97^{a}
60/40 Yeast protein	57.6^{c}	4.55^{a}	28.1^{a}	14.5^{bc}	1.06^{b}	61.03^{c}
60/40 Soybean meal	62.2^{b}	3.34^{ab}	26.7^{a}	16.2^{b}	1.11^{ab}	68.77^{ab}
60/40 Soybean isolate	63.2^{b}	3.72^{ab}	26.6^{a}	15.4^{bc}	1.16^{a}	72.93^{a}
60/40 Hemp	62.8^{b}	3.21^{b}	26.5^{a}	15.6^{bc}	1.10^{ab}	69.13^{ab}
8/23 Yeast protein, SBM, SBI, Hemp	62.3^{b}	1.34^{c}	22.8^{b}	26.4^{a}	0.38^{d}	23.55^{d}
Pooled SE	1.31	0.39	0.56	0.62	0.02	2.03
$P < F$	0.0013	0.0012	0.0001	< 0.0001	< 0.0001	< 0.0001

[1] Means of 3 fish per treatment. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] PER = total g gain/ g protein fed.
[3] PR = (whole body protein final – whole body protein initial)/g protein fed.

Figure 3.1 Breaking force (g) of cobia fillets at four different time points.

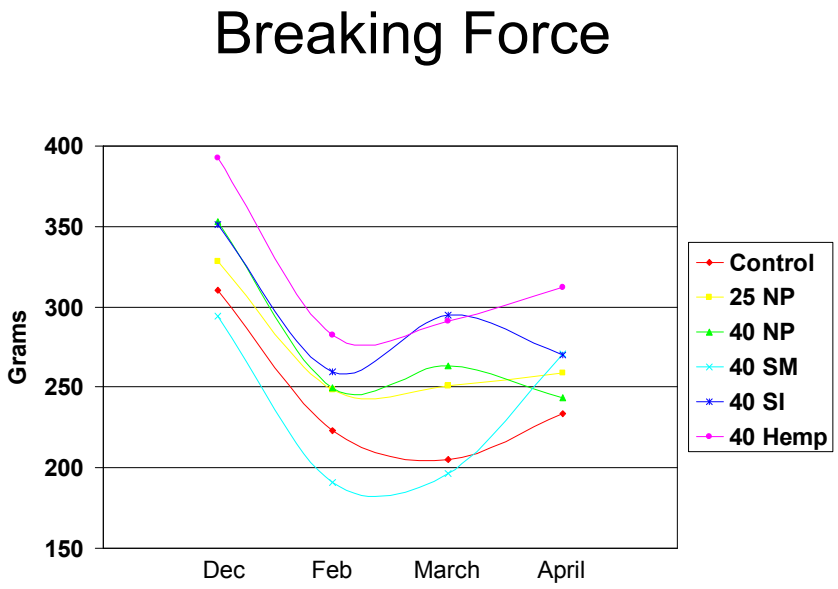


Figure 3.2 Distance to rupture (mm) of cobia fillets at four different time points.

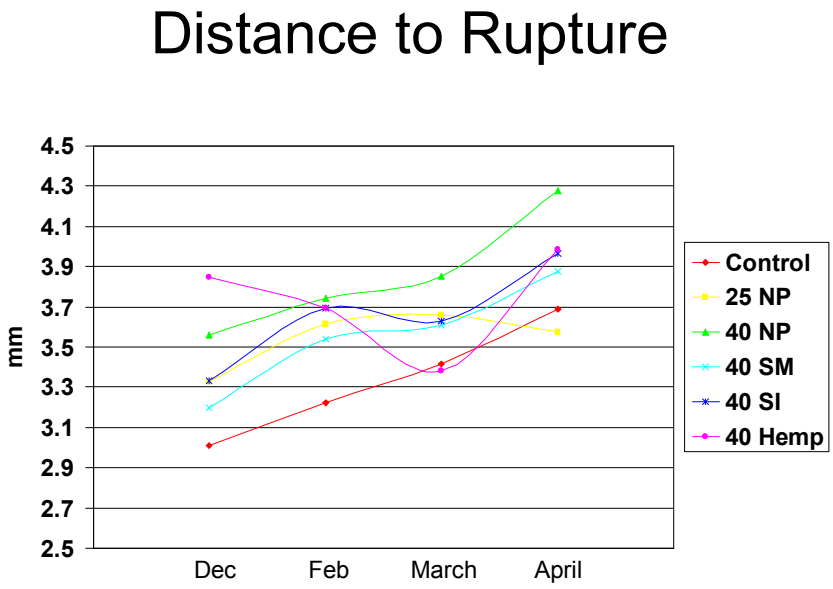


Figure 3.3 Gel strength (g*mm) of cobia fillets at four different time points.

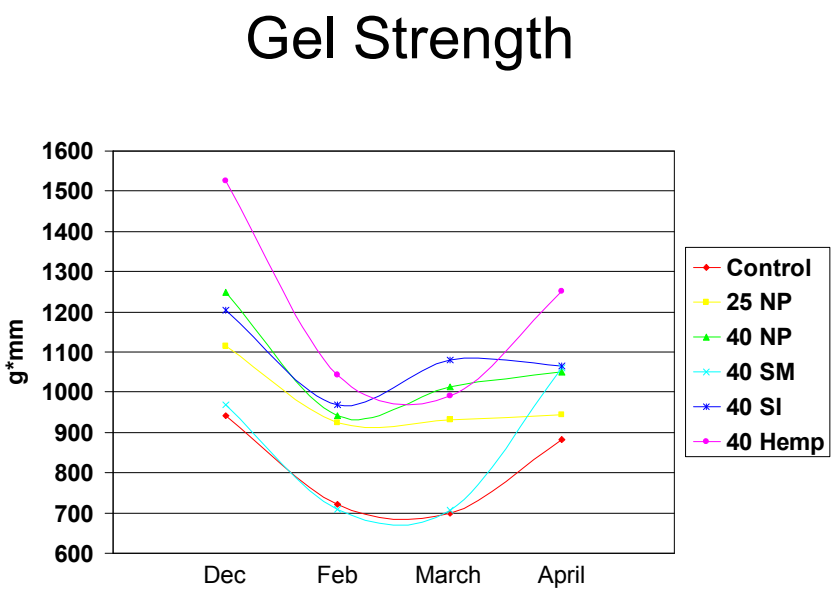
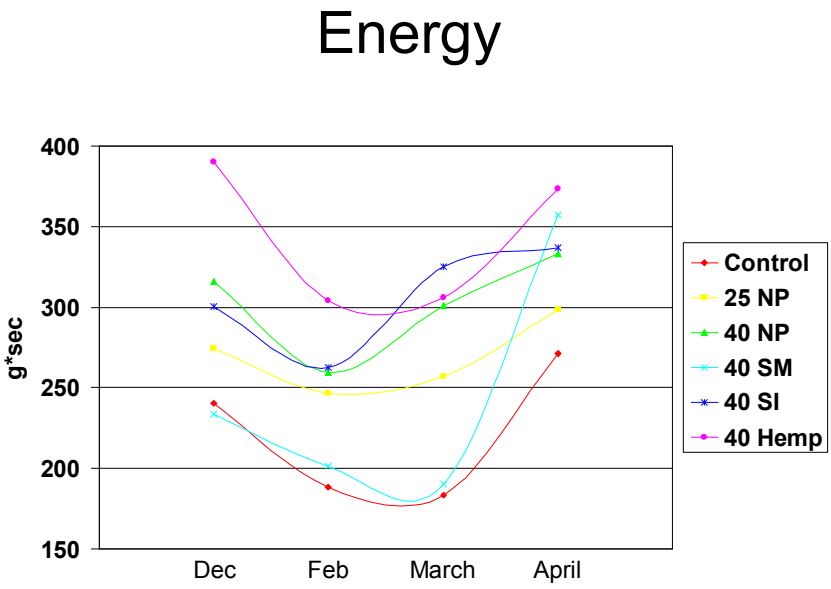


Figure 3.4 Total energy (g*sec) of cobia fillets at four different time points.



CHAPTER 4

Taurine addition to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia, Rachycentron canadum

Abstract

Two separate 8-week feeding trials were conducted to examine the impacts of fish meal replacement with an organically certifiable yeast-based protein source with and without supplementation of methionine, tryptophan, and taurine to diets for juvenile cobia. In the first trial, diets were formulated to contain 41% crude protein and 13% lipid, and a yeast-based protein replaced fish meal at 50 or 75% with or without 0.5g/100g dry diet taurine supplementation. The control diet contained 100% fish meal. Methionine and tryptophan were added to all diets except the control to resemble the amino acid profile of fish meal. Results from this study indicated that fish fed diets supplemented with taurine exhibited significantly higher weight gains and better feed efficiencies than all other fish. Diet affected biological indices such as muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI). The 75% yeast-based protein diet without taurine returned the lowest MR values and the highest VSI and HSI values. Muscle proximate analysis were also significantly affected by diet, with fish fed the taurine-supplemented diets producing the best results and the 75% yeast-based diet without taurine returning poor results. In the second trial, diets were formulated to contain 43% crude protein and 11% lipid, with the control diet containing 100% fish meal and the same yeast-based protein replacing fish meal at 50, 75, or 100%. All diets except the control were supplemented with taurine. Results from this study indicated that increasing amount of yeast led to decreased weight gains and feed efficiencies regardless of taurine supplementation. MR values tended to decrease while VSI and HSI values tended to increase with increasing fish meal replacement. No between-diet differences were recorded for muscle protein, lipid, dry matter and ash levels. Results of both studies showed that taurine supplementation has a significant effect on growth and feed efficiency of juvenile cobia. Additionally, alternate proteins, of yeast-based origin can be utilized at very high levels in diets for cobia with proper amino acid supplementation.

Key words: amino acid, alternate protein, yeast, quality, protein efficiency ratio

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1. Introduction

In aquaculture production, fish meal typically is regarded as the main protein source in diets for carnivorous fishes due to its high level of protein, excellent amino acid profile which provides adequate levels of all essential amino acids, low carbohydrate level, high digestibility, and few antinutritional factors (Zhou et al., 2004). As aquaculture production continues to increase, so too has the industry's demand for fish meal. However, due to the stagnant supply of fish meal, prices inevitably will increase with demand (FAO, 2004). This has amplified the need to investigate alternative protein sources. The use of plant proteins in diets for carnivorous species creates a challenge since they typically require higher levels of protein in their diet and plant proteins are less palatable. Nevertheless, several studies have shown promising results using plant-based protein sources in aquafeed formulations (Gomes et al., 1995; McGoogan and Gatlin, 1997; Tidwell and Allan, 2001; Fagbenro and Davies, 2001; Forster, 2002; Pereira and Oliva-Teles, 2003; Chou et al., 2004). Plant protein sources that have received the most interest are soybean meal and corn gluten meal due to their good amino acid profiles, except for methionine which is limiting in soybean meal (El-Sayed, 1999), and lysine which is limiting in corn gluten mean (Pereira and Oliva-Teles, 2003).

Total replacement of fish meal and soybean meal with an organically certifiable yeast-based protein has been reported in diets for tilapia without impacting weight gain (Craig and McLean, 2005). In a previous study using cobia (*Rachycentron canadum*), a carnivorous species, the same yeast-based protein could replace 25% of the fish meal protein without impacting growth parameters (Lunger et al., 2006). Further dietary inclusion rates for this protein might be problematic, however, due to amino acid imbalances (Craig and McLean, 2006).

Methionine is an essential amino acid required by fish for normal growth and metabolic functions (NRC, 1993). Since many plant proteins are deficient in methionine, it is typically the first limiting amino acid in diets which replace fish meal with large amounts of plant protein. Methionine deficiencies result in reduced growth rates, feed efficiency and survival (Goff and Gatlin, 2004), such that in diets high in plant protein methionine supplementation must be used (Jackson and Capper, 1982, Murai et al., 1982, Takagi et al., 2001).

Taurine is not considered to be an essential amino acid because it can be synthesized by fish. As in mammals, Yokoyama et al. (1997) demonstrated that rainbow trout synthesized taurine from cysteine. In the mammalian system, taurine is synthesized through many enzymatic

reactions; but the enzyme L-cysteinesulphinate decarboxylase seems to be rate-limiting (Jacobsen and Smith, 1968). Activity of this enzyme varies among fishes depending upon species and size. For example, in the yellowtail, as well as in bluefin and skipjack tunas, L-cysteinesulphinate decarboxylase activity is not present, whereas in Japanese flounder there is only low activity (Yokoyama et al., 2001).

Taurine typically is found in large quantities in fish meal and animal by-products, but is almost non-existent in plant meals. Even when all essential amino acid requirements are met in plant-based diets, growth still seems to be low when compared to fish meal-based diets (Gaylord et al., in press). Therefore, taurine supplementation may be required for plant-based diets. Indeed, dietary taurine additions improved weight gain and feed efficiency in olive flounder (Park et al., 2002; Kim et al., 2005) and rainbow trout (Gaylord et al., in press). Based on this information, the current studies were undertaken to examine whether higher levels of fish meal could be replaced in cobia diets utilizing yeast-based proteins and specific amino acid supplementation including methionine, tryptophan and taurine.

2. Materials and Methods

2.1 Experiment 1

2.1.1 Experimental system and husbandry

All studies were undertaken using a recirculating aquaculture system (Figure 4.1). The 3400-L recirculation configuration (flow rate = 4 L/min-aquaria) comprised 24, 110-L glass aquaria (15 aquaria dedicated to this study) serviced with a 750-L (200 gal) KMT-based (Kaldnes Miljøteknologi, Tønsberg, Norway) fluidized bed biofilter, a bubble-bead filter (Aquaculture Technologies Inc., Metaire, LA, USA) for solids removal, a protein skimmer (R&B Aquatics, Waring, Texas), and a 40-watt UV sterilizer (Aquatic Ecosystems, Apopka, FL, USA). The fluidized bed was oxygenated using diffusion air lines connected to a 1 hp Sweetwater remote drive regenerative blower (Aquatic Ecosystems, Apopka, FL, USA). During the feeding trial, water temperature (mean ± S.D.: 26.5 ± 1.5 ^{o}C) and pH (7.82 ± 0.27) were monitored 3 times a week using a Hanna Instrument 9024 pH meter (Aquatic Ecosystems, Apopka, FL, USA). Dissolved oxygen (5.33 ± 0.98 mg/L) and total ammonia nitrogen (0.49 ± 0.24 mg/L) also were measured three times a week using an YSI 85 Series dissolved oxygen meter (YSI Inc., Yellow Springs, OH) and by spectrophotometric analysis (Hach Inc., Loveland, CO, USA),

respectively. Nitrite (mean = 0.59 ± 0.49 mg/L) and nitrate (mean = 61 ± 10 mg/L) levels were quantified once a week by spectrophotometric analysis. Salinity was monitored and maintained near 17 ppt (17.5 ± 1.6 ppt) using Crystal Sea synthetic sea salt (Marineland, Baltimore, MD, USA). Photoperiod was manipulated using phosphorescent tubes positioned 1.8 m above the system with a 12h photophase-scotophase cycle using an automated timer with a half-hour dusk/dawn period.

Juvenile cobia were supplied by the Virginia Seafood Agricultural Research and Extension Center (Hampton, VA, USA). Fish were transported to the Virginia Tech Aquaculture Center (VTAC, Blacksburg, VA) and were acclimated and maintained in eight 500-L tanks for approximately 2 months. Upon commencement of the experiment, seven juvenile cobia, (mean individual weight with one standard deviation, 9.8 ± 0.3 g), were randomly placed into each of 15 experimental tanks. Fish were hand-fed the experimental diets (Table 4.1) twice daily, at 09.00 and 16.00 h. The ration was divided equally between the two feedings. Fish were fed 10, 10, 10, 8, 7, 6, 5, and 4 % body weight per day, during weeks 1, 2, 3, 4, 5, 6, 7, and 8, respectively. This feeding regime maintained a level of apparent satiation without overfeeding. Fish in tanks were group-weighed weekly to adjust the feeding rates and monitor growth performance.

2.1.2 Diets

Experimental feeds were produced as summarized in Table 4.1. The control diet was composed of 100% herring fish meal, with the remaining diets produced by replacing fish meal with NuPro® (Alltech Inc., Nicholasville, KY) at 50 and 75% on a nitrogenous basis. Methionine (0.3%) and tryptophan (0.2%) were added to these diets based upon amino acid analysis of previous diets utilizing these replacement levels. Two additional diets were manufactured by adding taurine (0.5%) to complement each fish meal replacement level. Each feed treatment was performed in triplicate. The dry dietary components of the diet were first mixed in a Patterson-Kelley twin shell® Batch V-mixer (Patterson-Kelley Co. Inc., East Stroudsburg, PA, USA) for 20 minutes and then homogenized into a paste by adding menhaden fish oil. The amount of distilled water required for pelleting (20-40% of feed weight) then was added, and the mixture was further homogenized. The paste was extruded through a Hobart D300 Floor Mixer (Hobart Co., Troy, OH, USA) without steam, using an appropriate die to provide pellets of suitable size for the fish. After air-drying, feed was frozen at -10°C until

needed. To determine dry matter, duplicate samples from each feed were heated at 135°C for 2 hrs in a gravity oven (Blue M Electric, Blue Island, IL, USA). Prior to use as feed, small quantities were thawed and refrigerated.

2.1.3 Data acquisition

At the end of the feeding trial, three fish from each tank (N=9 treatment^{-1}) were euthanized by an overdose of clove oil (3mg/l Sigma-Aldrich, St. Louis, MO, USA) and bled via caudal venipuncture for measurement of packed cell volume (PCV) and plasma protein levels. Fish were measured for length, weight, weight gain, and feed efficiency ratio. Survival, visceral somatic index (VSI), hepatosomatic index (HSI), and muscle ratio (MR) were calculated. Muscle and liver samples also were collected for proximate analysis, including crude protein, total lipid, dry matter and ash (AOAC, 1994). Plasma taurine, methionine and tryptophan were quantified according to a modified method of Petritis et al. (1999) using an Agilent 1100 series LCMSD Trap (Agilent Technologies, Palo Alto, CA). Injection volume was 15 µl. Separation was carried out on a gradient at a flow rate of 0.6 ml/min. Mobile phase A was 0.1% heptafluorobutyric acid (Sigma-Aldrich Co., St. Louis, MO) and mobile phase B was 100% acetonitrile. Samples were injected on a 5 µm, 125 x 4 mm Purospher RP-18E column with 4 x 4 mm precolumn (Agilent Technologies, Palo Alto, CA) using an automated injection sequence. Mass spectrometry was performed in positive ion mode with nebulizer pressure at 45 psi, drying gas flow at 9.5 l/min, drying gas temperature at 350°C, and capillary voltage at 3500 V. Prior to injection, 60 µl of plasma from each of three fish per tank was pooled, for a total of 180 µl of plasma per tank. The pooled plasmas were then divided into three 50 µl aliquots. Each aliquot of pooled plasma was diluted with 50 µl of mobile phase A. Plasma proteins were then precipitated out with 300 µl of 100% methanol, followed by incubation for 10 minutes at room temperature and centrifugation at 17500 x g at 4°C for five minutes (Piraud et al. 2005). Next, the resulting supernatants were transferred directly to autosampler vials with inserts and injected. Amino acid concentrations were calculated using QuantAnalysis software (Version 1.6, Build 121, Bruker Daltonik, Billerica, MA).

2.1.4 Statistical analyses

All data were subjected to analysis of variance procedure utilizing SAS 9.1 (SAS, Cary, NC, USA). When appropriate, a post-hoc test (Duncan's multiple range test) was used to check for significant differences ($\alpha < 0.05$) between the means.

2.2 Experiment 2

2.2.1 Experimental system and husbandry

The same experimental system was used for this study (Fig. 4.1). However, only 12 tanks were used. Water quality parameters were identical as those recorded during the first study. The 12 tanks initially were stocked with seven fish each (mean individual weight ~28 g per fish) and were hand-fed as previously described. Cobia were fed between 8 and 4% body weight to maintain apparent satiation. Fish in tanks were group weighed weekly to adjust the feeding rates and monitor growth performance.

2.2.2 Diets

Diets were manufactured in a manner identical to those employed in the first experiment. A 100% fish meal diet was used as the control and three other experimental diets were formulated. The yeast-based, organically certified product NuPro® (Alltech Inc., Nicholasville, KY, USA) was used to replace 50, 75, or 100% fish meal in diets 2, 3, and 4, respectively. Taurine also was added to each diet at 0.5% (Table 4.2).

2.2.3 Analysis

Data acquisition and statistical analyses were undertaken as described above.

3. Results

3.1 Experiment 1

Weight gain (% increase from initial weight) ranged from 337 to 1350% and was significantly affected by diet (Table 4.3). Cobia that were fed the 50 and 75% yeast-based diets supplemented with taurine (Diets 3 and 5, respectively) had significantly higher ($P < 0.0001$) weight gains than those fed the control diet (Diet 1) and the 50 and 75% yeast-based diets without taurine supplementation (Diets 2 and 4, respectively). Fish fed diet 4 (75% yeast-based protein without taurine) had significantly poorer growth than all other fish. Specific growth rates (SGR) ranged from 2.46 to 4.61% body weight per day and also were significantly affected by diet (Table 4.3). SGRs for cobia fed the control diet (Diet 1) and the 50 and 75% yeast-based

diets with taurine were similar, while fish fed the 50% and 75% yeast-based diets without supplemental taurine exhibited lower SGR values, with those fed the 75% yeast-based protein diet exhibiting the lowest ($P < 0.0001$). Feed efficiency (FE) ratio values ranged from 0.25 to 0.55 (Table 4.3) and followed a similar trend to weight gain. Cobia fed diets supplemented with taurine had significantly higher FE values than the control. FE values for the control diet and the 50% yeast protein diet without taurine were the same, but cobia fed the 75% yeast-based diet without taurine had significantly lower FE values than all others ($P < 0.0001$). Survival was variable, with most mortalities resulting from an air line failure in week 6 of the trial. I noticed that fish fed the diets containing supplemental taurine more readily survived this mechanical failure.

Muscle ratios ranged from 17.6 to 30.0% and also followed a similar trend as weight gain. Fish fed control, 50% and 75% yeast-based diets with taurine expressed similar muscle ratios (Table 4.4). Cobia fed 50% replacement protein without taurine had similar muscle ratio values to control and 75% replacement plus taurine diets, which were lower than that recorded for fish fed 50% yeast-based protein plus taurine. Fish fed 75% replacement protein without taurine had lower muscle ratio values than those in all other treatments ($P < 0.0001$; Table 4.4). Visceral somatic index (VSI) ranged from 9.0 to 14.1% (Table 4.4) with significant impact of diet. Fish fed the control diet had lower VSI values than all other treatments, while the highest VSI was observed in fish fed 75% protein replacement without taurine ($P < 0.0001$). Hepatosomatic index ranged from 1.59% for the control diet to 5.07% for the 75% protein replacement diet without taurine (Table 4.4). HSI values followed the same trend as seen for VSI (Table 4.4).

Muscle protein ranged from 78.4-83.1% (dry weight basis) and was only slightly affected by diet (Table 4.5). Fish fed the alternate protein with taurine expressed lowest muscle protein content, which was the same as cobia provided with 50% yeast protein without taurine. Muscle lipid ranged from 1.42 to 3.02% (wet weight basis : Table 4.5) with cobia receiving the 75% yeast-based protein diet without taurine expressing lowest levels ($P < 0.02$). Liver lipid ranged from 15.9 to 30.8% (wet weight basis) and was significantly affected by diet (Table 4.5). Muscle dry matter ranged from 22.0 to 25.4% and differed across all groups, while muscle ash ranged from 5.5 to 7.5% (Table 4.5). Fish fed the diet containing 75% yeast protein without taurine

supplementation had significantly higher muscle ash content, while fish fed both diets that were supplemented with taurine had significantly lower ash contents than all other fish.

Plasma amino acid values for taurine (nmol/ml) ranged from 5 to 555 (Table 4.6). Fish fed the control diet had significantly higher plasma taurine levels. Fish fed the diets supplemented with taurine had intermediate plasma taurine levels (222 and 223 nmol/ml) while fish fed the unsupplemented diets had the lowest plasma taurine levels (26 and 5 nmol/ml). Plasma methionine and tryptophan levels ranged from 33 to 48 nmol/ml and 8.7 to 12.0 nmol/ml, respectively (Table 4.6), and did not differ significantly with respect to diet.

3.2 Experiment 2

All diets were supplemented with taurine, except for the 100% fish meal control. Weight gain ranged from 632 to 1205% and was significantly affected by diet (Table 4.7). Fish fed the control and 50/50 diets expressed identical and highest growth increases ($P > 0.05$). As the percentage of replacement protein increased, weight gain decreased; fish fed 100% replacement exhibited the lowest overall weight increase ($P = 0.0010$). Specific growth rates (SGR) and feed efficiency (FE) ratio values followed the same trend as weight gain, decreasing with increasing level of fish meal replacement (Table 4.7). Fish fed the control diet however, had significantly higher FE values than all other diets ($P = 0.0008$).

Muscle ratio (MR) ranged from 21.9 to 27.9% with the highest value being expressed by control diet-fed fish (Table 4.8). The visceral somatic index (VSI) ranged from 8.2 to 10.8% and was significantly affected by diet (Table 4.8). VSI tended to increase with increasing percentage of fish meal replacement, with fish fed 100% replacement expressing the highest VSI ($P < 0.0001$). Hepatosomatic index (HSI) ranged from 1.64 to 3.31% and also was significantly affected by diet. As with VSI, animals maintained on the 100% fish meal replacement diet had the highest HSI values (Table 4.8).

No between diet differences were recorded for muscle protein (85-87% dry weight), muscle lipid (1.1-1.8% wet weight), liver lipid (4.1-10.2% wet weight), dry matter (22.7-23.2%) and ash levels (5.7-6.0%) (Table 4.9).

Plasma amino acid values for taurine and tryptophan (nmol/ml) ranged from 249 to 382 and 13.2 to 16.0, respectively (Table 4.10), and did not differ significantly with respect to diet. Plasma methionine levels were significantly impacted by fish meal replacement with levels

ranging from 39 to 87 nmol/ml (Table 4.10). Fish fed the control and the 50/50 diets had similar plasma methionine levels (87 and 76 nmol/ml, respectively) which then decreased significantly with increasing yeast inclusion rates (64 nmol/ml for the 25/75 diet and 39 nmol/ml for 100% yeast diet).

4. Discussion

The production of fish meal has remained relatively stable over the past 15 years, and this situation is unlikely to improve (FAO, 2004). Indeed, it has been suggested that the availability of fish meal will decline in the future, such that fish meal no longer can be considered a sustainable protein source for aquafeeds (Craig and McLean, 2006). Accordingly, alternate proteins are needed to replace fish meal, especially for diets of carnivorous species. Plant proteins are probably the most widely used alternative to fish meal, but they pose problems, including lower crude protein levels, palatability issues, amino acid deficiencies and the occurrence of antinutritional factors such as trypsin inhibitors (Francis et al., 2001). Taurine is not considered to be an essential amino acid for fish. It is a free amino acid that is present in large quantities in various tissues of marine fishes (Park et al., 2002). In mammals, taurine plays important roles in osmoregulation, bile acid conjugation, membrane stabilization, hormone release, modulation of neurotransmitters, and antioxidation (Sturman, 1988; Huxtable, 1992). It is also a required amino acid in diets for cats due to their low cysteinesulfinate decarboxylase activity (Knopf et al., 1978). Carnivorous fishes in the wild consume large quantities of taurine since it is highly abundant in animal tissues, but this does not apply when aqua diets contain large amounts of plant protein sources, which are naturally low in taurine. Therefore, it may be necessary to supplement these diets with taurine and other amino acids to reap benefits.

In feeding trial 1, supplementing taurine at 0.5g/100g dry weight to diets that contained large amounts of a yeast-based protein source resulted in significantly more weight gain and generally exhibited better feed efficiency ratios than animals fed the control diet. Growth rates and feed efficiency ratios also have been improved with taurine supplementation in species such as Japanese flounder (Park et al., 2002; Kim et al., 2003; Kim et al., 2005a; 2005b), sea bass (Martinez et al., 2004), yellowtail (Matsunari, et al., 2005), rainbow trout (Gaylord et al., in press), and black tiger shrimp (Shiau and Chou, 1994). In my second trial, weight gain, specific growth rate, and feed efficiency ratios decreased with increasing level of dietary fish meal

replacement even with taurine supplementation. The same trend was observed when the same yeast-based protein was substituted at identical levels without taurine (Lunger et al., 2006). However, the diet containing 100% of the yeast-based protein source in Lunger et. al (2006) exhibited only 86% increase from initial weight utilizing 11 g fish, whereas in the present study, the diet containing 100% of the yeast-based protein source returned over 380% increase from initial weight starting with much larger fish (28 g initial weight).

In the first trial described here, fillet proximate composition was comparable among fish fed all diets, except for those containing 75% protein replacement without taurine. These fish exhibited lower levels of muscle and liver lipid, but higher ash content. Similar results were reported by Lunger et al. (2006), who showed that fish fed diets containing 75% yeast protein deposited less muscle and liver lipid than fish fed at 25 or 50% replacement levels. In my second trial, all fish exhibited similar muscle proximate values, although Lunger et al. (2006) indicated that muscle protein, lipid, and liver lipid tended to decrease with increasing levels of dietary yeast-protein. The reason for this anomaly remains unclear, but could be explained by feed intake.

In my previous study, feed intake of fish fed diets containing high levels of yeast protein was low. The diet containing 100% of the yeast-based protein source in Lunger et al (2006) was repeatedly spit out by the cobia, whereas the same diet with taurine addition in the present trial was readily and eagerly consumed by the juvenile cobia, indicating no palatability issues. It has been reported that taurine can act as a feed attractant. Sea bass fry were observed to preferentially consume a diet supplemented with 0.2% taurine (Martinez et al., 2004). Taurine supplementation of juvenile cobia diets may have made the diets more palatable, thus increasing feed intake and subsequently weight gain, but due to the feeding strategy employed in the current set of experiments, consumption could not be absolutely quantified.

Plasma amino acid analysis revealed no significant differences in methionine and tryptophan levels when these amino acids were supplemented in the diets in the first experiment. However, significant differences were observed when taurine was supplemented. Although these levels did not rise to the levels observed in the control diet which contained 100% fish meal protein, they were significantly elevated over those unsupplemented with taurine. The elevated plasma taurine levels appear to correlate with the improved weight gain when taurine was supplemented to diets 3 and 5 in the first feeding trial. Whether this result was solely due to

metabolic actions of taurine (i.e. possible essentiality of taurine) or simply the impacts of increased feed intake is questionable. Plasma taurine levels in the second trial were similar to those observed in the supplemented diets in the first trial. Cobia fed the diets with 50% fish meal protein replacement and taurine supplementation showed equivalent growth to that of the control fish. However, diets containing greater than 50% of the yeast product led to reduced growth. In trial 2, methionine was not supplemented to any of the diets and plasma data revealed that when greater than 50% yeast protein was utilized, plasma methionine concentrations were reduced compared to control fish. This may indicate a methionine deficiency in the yeast protein, as suggested by Craig and McLean (2006). The methionine requirement for cobia has recently been determined to be 1.05% of dry diet (Zhou et al., 2006). Although dietary amino acid levels were not determined in the present study, the plasma data is indicative of a methionine deficiency. Plasma tryptophan data do not appear to indicate that the lack of tryptophan supplementation in trial 2 was responsible for the reduced weight gain in fish fed the higher levels of yeast protein inclusion.

Evidence from the present study indicates that cobia require taurine supplementation when fed diets containing high levels of plant-based protein sources. Martinez et al. (2004) reported that sea bass also may need dietary taurine supplementation under certain feeding practices. Rainbow trout that were fed a plant-based diet needed taurine supplemented at 5g/kg dry diet in order to match the growth of fish fed a fish meal-based control diet (Gaylord et al., in press). Kim et al. (2003) reported that juvenile olive flounder required taurine supplementation, whereas fingerling olive flounder did not. Optimal levels of taurine supplementation were suggested to be 15mg/g (Kim et al., 2005b) or 15-20mg/g (Park et al., 2001). Results thus indicate that taurine supplementation is necessary for some fish species. This could be particularly true for marine species, since taurine plays a critical role in osmoregulation and typically comprises more than 50% of the free amino acid pool (Lombardini et al., 1979). Fish raised in seawater may have a greater demand for dietary taurine than fish held in fresh water and a fish's ability to convert cysteine to taurine may be based on their environmental salinity adaptation (Gaylord et al., in press). Therefore, the osmotic stabilization provided by taurine may be related to its effects on growth and the fact that supplementation to diets improves growth in numerous species (Kim et al., 2003). Some species may be unable or poor at synthesizing taurine *de novo* from cysteine. This result may be due to the activity level of L-

cysteinesulphinate decarboxylase, which in turn might be influenced by the natural feeding habits of a particular species or from previous feeding history (Gaylord et al., in press). Carnivorous fishes therefore, may be less able to synthesize taurine due to their naturally high intake, while herbivorous/omnivorous fish may be more capable of such synthesis due to the paucity of taurine in their natural diets. The activity level of the enzyme L-cysteinesulphinate decarboxylase in cobia was not examined in this study, but it does represent another area for future research and would be beneficial for determining the essentiality of taurine in diets for cobia. Species with rapid growth rates, such as cobia, may also experience an increased demand on the *de novo* synthesis of taurine which cannot be met, especially when fish meal is replaced in diets by plant protein sources devoid of taurine. When a non-essential amino acid, such as taurine, is added to diets, it may be possible to conserve essential amino acids as well (Cowey, 1994), which could lead to improved growth rates. In trial 1, fish were fed diets containing 50 and 75% yeast-based protein supplemented with taurine, methionine and tryptophan, or methionine and tryptophan alone. Of these, the best performance was recorded for the 50 and 75% replacement levels supplemented with all three amino acids. It appeared in trial 2 that diets supplemented with taurine alone was ineffective in preventing growth suppression when concentrations of yeast protein exceeded 50% of the total dietary protein. Comparatively, in trial 1, fish fed diets supplemented with methionine and tryptophan alone returned inferior weight gains compared to fish fed the other diets. Taken together, these data appear to indicate that when high levels of yeast protein are included in cobia aquafeeds, taurine supplementation may be more critical than methionine and tryptophan. An explanation for these results could be that taurine alone was able to allow cobia to conserve the small amount of methionine and tryptophan that was present in the unsupplemented diets, thereby improving growth rates.

Due to the wide range of biological impacts associated with taurine, including anticonvulsant activity, muscle membrane stabilization, bile salt synthesis, cell proliferation and viability and antioxidant activities to name a few (Huxtable, 1992), reasons for improved growth rates observed in the present study with the addition of taurine are purely speculation. This particular area of research is very limited, and results such as these certainly warrant future investigations. It is obvious from the results of both of the present studies that taurine supplementation has a significant effect on growth and feed efficiency of juvenile cobia. These findings could dramatically change the amount and types of alternate proteins that can be

incorporated into diets for juvenile cobia and decrease the industry's reliance on fish meal supplies for the continued aquaculture of cobia. The results from this study also argue for the importance of determining quantitative amino acid requirements for cobia, many of which are presently undetermined.

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Table 4.1 Composition of diet (g/100g on a dry matter basis): 41% crude protein, 13% lipid, and 300 kcals/100g.

Ingredient Fish meal:Yeast protein	Diet 1 100:0	Diet 2 50:50	Diet 3 50:50 + Tau	Diet 4 25:75	Diet 5 25:75 + Tau
Herring meal[a]	58.6	29.3	29.3	14.6	14.6
Yeast protein[b]	0.0	40.0	40.0	59.9	59.9
Dextrin[c]	4.9	4.9	4.9	4.9	4.9
Menhaden Fish oil[d]	7.1	9.3	9.3	10.3	10.3
Mineral mix[e]	4.0	4.0	4.0	4.0	4.0
Vitamin mix[f]	3.0	3.0	3.0	3.0	3.0
CMC[c]	1.0	1.0	1.0	1.0	1.0
$CaPO_4$[g]	0.0	1.0	1.0	1.0	1.0
Methionine[g]	0.0	0.3	0.3	0.3	0.3
Tryptophan[g]	0.0	0.2	0.2	0.2	0.2
Taurine[g]	0.0	0.0	0.5	0.0	0.5
Cellufill[c]	21.4	6.7	6.2	0.5	0.0
Crude protein	41	41	41	41	41
Crude lipid	13	13	13	13	13
Available energy (kcals/100g diet)	300	300	300	300	300

[a] International Proteins Corporation, Minneapolis, MN, USA.
[b] Alltech Incorporated, Nicholasville, KY, USA.
[c] U.S. Biochemical Corporation, Cleveland, Ohio, USA.
[d] Omega oils, Reedville, VA, USA.
[e] ICN Corporation, Costa Mesa, CA, USA.
[f] See Moon and Gatlin (1991).
[g] Sigma-Aldrich, St. Louis, MO

Table 4.2 Composition of diet (g/100g on a dry matter basis): 43% crude protein and 11% lipid

Ingredient	Diet 1 100:0	Diet 2 50:50 + Tau	Diet 3 25:75 + Tau	Diet 4 0:100 + Tau
Herring meal[a]	57.2	28.6	14.3	0.0
Yeast protein[b]	0.0	41.9	62.9	83.8
Dextrin[c]	9.5	5.6	4.8	3.4
Menhaden Fish oil[d]	6.0	8.1	8.5	8.3
Mineral mix[e]	4.0	4.0	4.0	4.0
Vitamin mix[f]	3.0	3.0	3.0	3.0
CMC[c]	1.0	1.0	1.0	1.0
$CaPO_4$[g]	0.0	1.0	1.0	1.0
Taurine[g]	0.0	0.5	0.5	0.5
Cellufil[c]	19.3	6.3	0.0	0.0
Crude protein	43	43	43	43
Crude lipid	11	11	11	11
Available energy (kcals/100g diet)	309	293.4	290.2	284.6

[a] International Proteins Corporation, Minneapolis, MN, USA.
[b] Alltech Incorporation, Nicholasville, KY, USA.
[c] US Biochemical Corporation, Cleveland, Ohio, USA.
[d] Omega oils, Reedville, VA, USA.
[e] ICN Corporation, Costa Mesa, CA, USA.
[f] See Moon and Gatlin (1991).
[g] Sigma-Aldrich, St. Louis, MO

Table 4.3 Weight gain, specific growth rate (SGR), feed efficiency ratio value (FE), and survival percentage of juvenile cobia fed experimental diets in which fish meal was replaced by a yeast-based protein[1].

Diet (Fish meal:Yeast)	Weight gain[2] (% increase)	SGR[3]	FE[4]	Survival %
100:0	981[b]	4.17[a]	0.39[b]	95[a]
50:50	753[c]	3.49[b]	0.41[b]	62[b]
50:50 + Tau	1350[a]	4.47[a]	0.55[a]	66[ab]
25:75	337[d]	2.46[c]	0.25[c]	33[c]
25:75 + Tau	1249[a]	4.61[a]	0.53[a]	90[ab]
Pooled SE	59.5	0.16	0.02	8.84
$P < F$	< 0.0001	< 0.0001	< 0.0001	0.0038

[1] Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] Weight gain = (final tank weight – initial weight)/initial tank weight.
[3] SGR = (ln final wt. – ln initial wt.)/49 days
[4] FE = g gained/g fed.

Table 4.4 Biological indices, including muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI), of juvenile cobia fed experimental diets in which fish meal was replaced with a yeast-based protein[1].

Diet (Fish meal:Yeast)	MR[2]	VSI[3]	HSI[4]
100:0 Control	27.2[ab]	9.0[d]	1.59[d]
50:50	24.4[b]	11.5[b]	4.03[b]
50:50 + Tau	30.0[a]	10.0[c]	3.11[c]
25:75	17.6[c]	14.1[a]	5.07[a]
25:75 + Tau	27.0[ab]	11.6[b]	3.65[bc]
Pooled SE	1.72	0.59	0.32
$P < F$	< 0.0001	< 0.0001	< 0.0001

[1] Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] MR = muscle weight*100/body weight
[3] VSI = VSI weight*100/body weight
[4] HSI= HSI weight*100/body weight

Table 4.5 Muscle protein, lipid, dry matter and ash, and liver lipid of juvenile cobia fed experimental diets in which fish meal was replaced with a yeast-based protein[1].

Diet (Fish meal:Yeast)	Muscle protein[1]	Muscle lipid[2]	Muscle dry matter[1]	Muscle ash[1]	Liver Lipid[2]
100:0 Control	83.1[a]	2.58[a]	23.12[c]	6.47[b]	18.14[b]
50:50	80.3[ab]	2.48[a]	24.28[b]	6.51[b]	30.80[a]
50:50 + Tau	78.9[b]	2.63[a]	25.40[a]	5.83[bc]	22.68[ab]
25:75	83.1[a]	1.42[b]	21.99[d]	7.52[a]	15.89[b]
25:75 + Tau	78.4[b]	3.02[a]	25.29[a]	5.50[c]	20.64[b]
Pooled SE	1.97	0.38	0.58	0.42	3.41
$P < F$	0.0151	0.0177	<0.0001	<0.0001	0.0382

[1] Means of 3 fish per tank ($N = 9$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$). Values are presented on a dry matter basis.
[2] Means of 1 fish per tank ($N = 3$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$). Values are presented on a wet weight basis.

Table 4.6 Plasma amino acid values of juvenile cobia in trial 1 fed diets in which fish meal was replaced with a yeast-based protein[1].

Diet (Fish meal:Yeast)	Taurine	Methionine	Tryptophan
100:0 Control	555.5[a]	47.8[a]	10.4[a]
50:50	26.1[c]	42.5[a]	8.7[a]
50:50 + Tau	221.8[b]	47.1[a]	12.0[a]
25:75	5.4[c]	42.3[a]	9.6[a]
25:75 + Tau	223.3[b]	32.9[a]	9.3[a]
Pooled SE	7.51	3.18	1.14
$P < F$	<0.0001	0.0513	0.3358

[1] Means of 3 individual fish per diet. Units are expressed as nmol/ml plasma.

Table 4.7 Weight gain, specific growth rate (SGR), feed efficiency ratio value (FE), and survival percentage of juvenile cobia fed experimental diets in which fish meal was replaced with a yeast-based protein[1].

Diet (Fish meal:Yeast)	Weight gain[2] (% increase)	SGR[3]	FE[4]	Survival
100/0 Control	524[a]	3.27[a]	0.51[a]	100[a]
50/50	516[ab]	3.24[a]	0.45[b]	100[a]
25/75	430[b]	2.98[a]	0.46[b]	100[a]
0/100	280[c]	2.38[b]	0.35[c]	95[a]
Pooled SE	27.16	0.09	0.02	2.33
$P < F$	0.0007	0.0004	0.0008	0.4411

[1] Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] Weight gain = (final tank weight – initial weight)/initial tank weight.
[3] SGR = (ln final wt. – ln initial wt.)/days of study.
[4] FE = g gained/g fed.

Table 4.8 Biological indices including muscle ratio (MR), visceral somatic index (VSI), and hepatosomatic index (HSI) of juvenile cobia fed experimental diets in which fish meal was replaced with yeast-based protein[1].

Diet (Fish meal:Yeast)	MR[2]	VSI[3]	HSI[4]
100:0 Control	27.9[a]	8.2[c]	1.64[c]
50:50	24.0[ab]	9.1[bc]	2.24[b]
25:75	21.9[b]	9.8[b]	2.60[b]
0:100	22.4[b]	10.8[a]	3.31[a]
Pooled SE	2.40	0.54	0.28
$P < F$	0.0180	< 0.0001	< 0.0001

[1] Means of 3 tanks treatment^{-1}. Means with different superscripts in the same column differed significantly ($P < 0.05$).
[2] MR = muscle weight*100/body weight
[3] VSI = VSI weight*100/body weight
[4] HSI= HSI weight*100/body weight

Table 4.9 Muscle protein, lipid, dry matter and ash, and liver lipid of juvenile cobia fed experimental diets in which fish meal was replaced with a yeast-based protein.

Diet (Fish meal:Yeast)	Muscle protein[1]	Muscle lipid[2]	Muscle dry matter[1]	Muscle ash[1]	Liver Lipid[2]
100:0 Control	85.3[a]	1.48[a]	22.86[a]	6.02[a]	5.91[ab]
50:50	85.5[a]	1.13[a]	22.73[a]	5.97[a]	9.47[a]
25:75	84.7[a]	1.78[a]	23.20[a]	5.69[a]	10.22[a]
0:100	86.6[a]	1.31[a]	22.75[a]	5.77[a]	4.11[b]
Pooled SE	1.59	0.30	0.44	0.16	1.45
$P < F$	0.8560	0.5144	0.8599	0.4608	0.0510

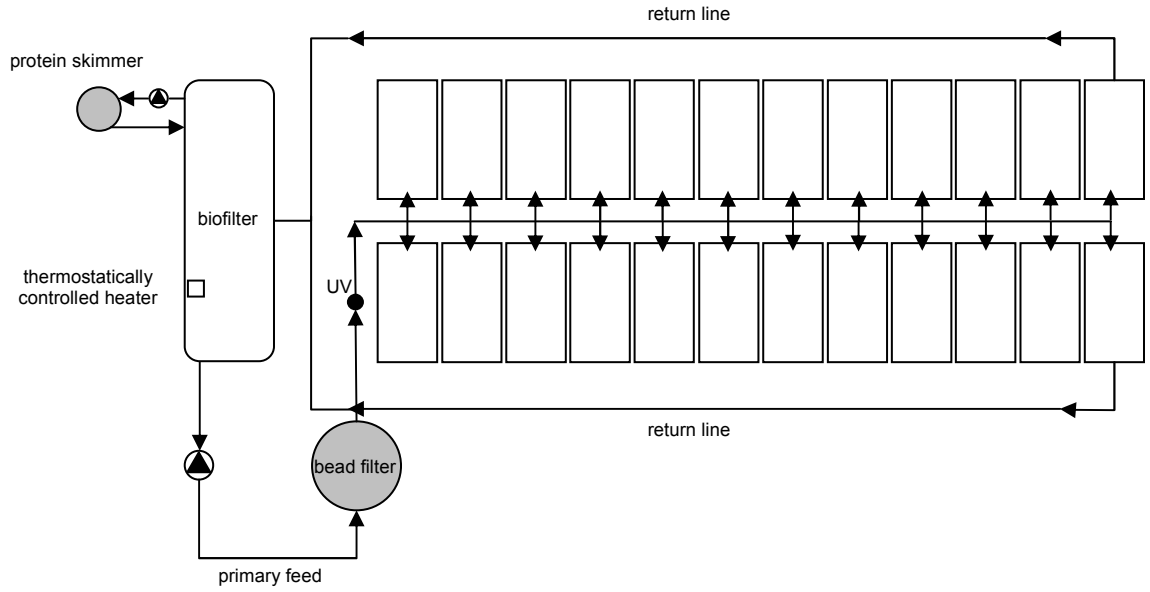
[1] Means of 1 fish per tank ($N = 3$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$). Values are presented on a dry matter basis.
[2] Means of 1 fish per tank ($N = 3$ treatment^{-1}). Means with different superscripts in the same column differed significantly ($P < 0.05$). Values are presented on a wet weight basis.

Table 4.10 Plasma amino acid values of juvenile cobia in trial 2 fed diets in which fish meal was replaced with a yeast-based protein[1].

Diet (Fish meal:Yeast)	Taurine	Methionine	Tryptophan
100:0 Control	306.9[a]	87.2[a]	15.5[a]
50:50	382.0[a]	76.4[a]	13.5[a]
25:75	336.8[a]	63.5[b]	16.0[a]
0:100	248.6[a]	38.9[c]	13.2[a]
Pooled SE	29.28	3.55	1.47
P < *F*	0.0635	<0.0001	0.4693

[1] Plasma was pooled from 5 fish per diet and run in triplicate. Units are expressed as nmol/ml plasma.

Figure 4.1 Schematic diagram of experimental recirculating aquaculture system used in the present study, highlighting major components. Each tank and the fluidized bed biofilter were aerated using filtered compressed air.



Chapter 5

Summary Conclusions

Results of studies presented in this thesis suggest that:

1.) The organically certifiable yeast-based protein, NuPro®, can replace up to 40% fish meal in diets for cobia, *Rachycentron canadum*, without amino acid supplementation. This inclusion level did not cause harmful effects on the health or biological indices of the fish and promoted excellent growth and feed efficiency.

2.) Other organically certifiable protein sources, such as soybean meal, soybean isolate, and hemp, also can replace up to 40% fish meal without detrimental effects. Further inclusion may be possible with amino acid supplementation.

3.) Simply blending alternate protein sources as a replacement for fish meal is not adequate unless specific amino acid requirements are satisfied. Therefore, quantitative essential amino acid requirements for cobia are mandatory if 100% replacement of fish meal is to be achieved.

4.) Alternate protein sources appear to have impacts on product texture, such as breaking force, distance to rupture, gel strength, and total energy. I present the first results of the effects of alternate proteins on final product quality in cobia.

5.) Taurine appears to be conditionally indispensible when cobia are fed diets containing high inclusion levels of yeast-based protein.

Future research:

This thesis provides a foundation for organic production of cobia. However, before this goal can be realized on a commercial scale, several areas of investigation must be advanced. Most important are the specific essential amino acid requirements which must be determined so that replacement of fish meal can occur on amino acid basis. Secondly, alternate lipid sources must be evaluated for their suitability as a replacement for fish oil, not only for economic viability of the industry, but also for future sustainability. Impacts of both of these issues on final product quality also must be addressed so that a high value, high demand product can be delivered to the consumer. Finally, these results need to be validated under commercial production scenarios. Since cobia grow so rapidly, impacts of nutritional modification might differ among life stages.

Vitae

Angela Nicole Lunger was born on March 9, 1982 in Youngstown, Ohio to Cheryl and Wayne Lunger. Angela is the eldest of two children. When she was four years old her family moved to Richmond, VA where she has lived until now. Growing up with animals present in her daily life led her to take particular interest in them. She decided she wanted to be a veterinarian early on and took a job as a kennel assistant at a local animal clinic at the age of 16. After high school, she attended North Carolina State University and graduated *magna cum laude* with a bachelor's degree in animal science. At NC State she was involved in the companion animal club, was a member and treasurer of Zeta Tau Alpha sorority and was a member of Gamma Beta Phi honor fraternity. While attending NC State, her goals however, had changed and she decided to become more involved in the research aspect of veterinary medicine. She applied to the Virginia Maryland Regional College of Veterinary Medicine and was granted an assistantship in the fall of 2004. Accordingly, she began to conduct research towards earning her master's degree in Biomedical and Veterinary Sciences.