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Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*

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ABSTRACT

Achieving true sustainability in fish farming requires the replacement of most of the fish meal and fish oil utilized as feedstuffs. The present experiment reports 2 feeding trials that resulted in the total replacement of fish meal and fish oil in juvenile cobia (*Rachycentron canadum*). The first trial was conceived as a 2×3 factorial design with three levels of fish meal replacement (FMR; 50, 75 and 100% of dietary protein) by soy protein concentrate (SPC), and two levels of mannan oligosaccharide (MOS) supplementation (0 or 0.3% of the diet). Since MOS has been reported to promote gut health and integrity, it was included in order to verify whether it would ease high levels of FMR. Lipids were supplied by menhaden oil. In the second feeding trial, fish meal was replaced by various combinations of SPC and soybean meal, again with or without MOS supplementation. In addition, some diets were supplemented with purified amino acids. Lipids were supplied by fish oil. A final diet (NOFM) was formulated using SPC, a marine worm meal, a nucleotide-rich yeast extract protein source, and MOS. In this last diet, lipids were supplied with a mix of soy oil and a DHA-rich algal meal, thereby completely eliminating both fish meal and fish oil. Over both feeding trials, juvenile cobia consistently exhibited excellent performance at 75% FMR and less. MOS did not have a significant effect, although a beneficial trend was observed in the first trial at 100% FMR. In the second trial, the fish fed the NOFM diet exhibited one of the best weight gains and feed efficiencies, with no mortality and no impact on muscle and liver composition. This result illustrates the crucial importance of the selection of feedstuffs for FMR and fish oil, since the NOFM diet did not receive amino acid supplementation. While this represents the first successful elimination of fish meal and fish oil in aquafeeds for cobia, the consistent, successful replacement of 94% of the fish meal in the other diets is actually more promising to the future as they solely utilized commodities traded (soy products) as replacement sources, which is the only road to true environmental and economical sustainability for the aquaculture industry.

1. Introduction

Over the past several years, intense focus has been trained upon the reduction and/or elimination of fish meal protein in aquafeeds, especially those designed for high-level marine carnivores. This goal has also been driven by the desire and need for the aquaculture industry to achieve true sustainability, all the while attempting to fill the massive seafood deficit that can only be overcome through aquaculture production (Lunger et al., 2006; FAO, 2007). Sustainable replacements for fish meal protein are most often those of plant origin, especially the grains, pulses and oilseeds (Lunger et al., 2006; Gaylord et al., 2006; Gatlin et al., 2007). Soybean meal (SBM) has been one of the most studied alternatives to fish meal, but has several limitations, including anti-nutritional factors, low levels of methionine and adverse effects on the intestinal integrity of some carnivorous species (Gatlin et al., 2007). Additionally, SBM is relatively low in crude protein levels, especially when compared to fish meal. Hence, complete replacement of fish meal in aquafeeds designed for carnivorous species requiring higher levels of dietary protein is problematic due to these lower crude protein levels. With the inevitable increase in the price of fish meal, as well as the realization for the need of alternate proteins to drive the industry forward, more emphasis has been placed upon technologies that can concentrate protein content from traditionally lower-protein sources, resulting in products such as corn gluten meal and soy protein concentrate (SPC; Barrows et al., 2007). These technologies have provided new, alternative sources of dietary protein which, in many cases, have crude protein levels similar to fish meal. As production capacities of these plant-based protein concentrates continue to increase, price and availability will make these products more cost-

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effective. However, their use and optimal inclusion rates in aquafeeds designed for high-level marine carnivores must be ascertained, and many of these recently developed feedstuffs have similar problems in terms of inadequate amino acid profiles.

In our facility, recent research has concentrated upon total fish meal replacement in aquafeeds designed for cobia (*Rachycentron canadum*, L.) utilizing a wide variety of alternate protein sources with varying levels of success (Craig and McLean, 2005; Lunger et al., 2006, 2007a; McLean and Craig unpublished data). Over the course of these studies, diets containing 100% replacement of fish meal have been investigated using a yeast-based protein source (Lunger et al., 2006, 2007a), with the finding that the addition of taurine in diets with high levels of fish meal replacement (FMR) significantly improved production characteristics (Lunger et al., 2007b). Positive impacts of taurine supplementation upon weight gain also have been observed in rainbow trout (Gaylord et al., 2006) and olive flounder (Kim et al., 2005, 2007). Unpublished results from our laboratory from feeding trials conducted with cobia indicated that supplementation of other amino acids such as methionine and lysine in addition to taurine is imperative if complete replacement of fish meal is to be achieved without detrimental impacts on production characteristics. Due to the outstanding nutritional qualities of fish meal, which include a well-balanced amino acid profile, high digestibility and palatability, and the presence of potential growth factors, it is highly improbable that complete replacement will be possible with a single alternative protein source (Craig and McLean, 2005). Drawing upon our previous findings, recent studies have investigated a blend of alternate protein sources, including yeast-based feedstuffs, *Neried* sp. worm meals, SBM, and other organic alternative protein sources (Lunger et al., 2007a), in combination with and without specific amino acid supplementation. This study sought to build upon these previous findings by utilizing various combinations of soy protein products and other alternative protein sources in aquafeeds that can be considered commercially feasible in terms of cost-effectiveness. Additionally, the use of mannan oligosaccharides (MOS), which have been shown to benefit larval cobia intestinal development (Salze et al., 2008), was evaluated in feeds for juvenile cobia. The addition of MOS was investigated to determine whether this feed additive could enhance the digestion and assimilation of high levels of plant protein incorporated as SBM and SPC in diets for juvenile cobia, thereby resulting in better feed efficiency ratio values. Based on over four years of cobia nutritional research, the present paper synthesizes two separate feeding trials: the first trial emphasizing fish meal replacement using SPC, the second trial furthering the exclusion, and elimination, of reduction fisheries byproducts — including fish oil — with a blend of alternative feedstuffs in diets utilizing more commercially applicable formulations.

2. Materials and methods

2.1. Experimental system and husbandry

Both studies were undertaken using a recirculating aquaculture life support system, equipped with 300 L fiberglass, circular tanks (see Lunger et al., 2006). The number of tanks used in each experiment varied based on the number of diets. The recirculating system was serviced with a KMT-based (Kaldnes Miljøteknologi, Tønsberg, Norway) fluidized bed biofilter that also acted as a sump, a bubble-bead filter (Aquaculture Technologies Inc., Metaire, LA) for solids removal, a protein skimmer (R&B Aquatics, Waring, TX), and a UV sterilizer (Aquatic Ecosystems, Apopka, FL). The biofilter was oxygenated using diffusion air lines connected to a 1 hp Sweetwater remote drive regenerative blower (Aquatic Ecosystems, Apopka, FL). Water temperature was maintained at 28 °C by a thermostatically controlled heater placed in the sump. All fish were subjected to a 12:12 light:dark cycle using a combination of fluorescent and incandescent lighting to simulate dawn and dusk. Water quality parameters were monitored (3 times a week) during the feeding trials. Water temperature (28 °C) and pH (8.2) were monitored using a Hanna Instrument 9024 pH meter (Aquatic Ecosystems, Apopka, FL). Salinity was maintained at 18 ppt using Crystal Sea synthetic sea salt (Marineland, Baltimore, MD) added to well water and monitored using a refractometer. Dissolved oxygen (7.0 ± 0.1 ppm) and total ammonia nitrogen (0.12 ± 0.01 ppm) were measured using a YSI 85 Series dissolved oxygen meter (YSI Inc., Yellow Springs, OH) and by spectrophotometric analysis (Hach Inc., Loveland, CO), respectively. Nitrite (0.074 ± 0.012 ppm) and nitrate (97.7 ± 2.2 ppm) levels were quantified once a week by spectrophotometric analysis.

Juvenile cobia (*R. canadum*) were supplied by the Virginia Seafood Agricultural Research and Extension Center (VSAREC, Hampton, VA). Fish were transported to the lab, where they were acclimated and maintained for approximately 60 days. Upon commencement of the feeding trials, seven (81.7 ± 0.3 g, initial mean weight ± SEM) and five (104.0 ± 0.8 g) juvenile cobia for feeding trials 1 and 2, respectively, were randomly placed into each tank. Fish were hand-fed the experimental diets (three tanks per diet) twice daily, at 0900 and 1600 h for 6 weeks, initially starting at 7% body weight (bw) per day, and gradually decreasing to 5% bw d^{-1}, equally divided between the two daily feedings. This maintained a level of apparent satiation without overfeeding. Fish were group-weighed weekly to adjust the feeding rates and to monitor growth performance.

2.1.1. Feeding trial 1

Experimental feeds for the first feeding trial were produced as summarized in Table 1. Seven diets were formulated and fed in triplicate, thus leading to the stocking of 21 tanks. All diets were formulated to provide 45% crude protein and 12% total lipid (dry-matter basis) and supply approximately 13.7–14.3 kJ available energy/g dry diet.

The first feeding trial was designed as a 3 × 2 factorial with fish meal replacement level by SPC as one factor (50, 75 and 100% of

Table 1
Composition of the experimental diets (45% crude protein, 12% lipid on a dry weight basis) utilized in feeding trial 1. Diets are designated in reference to the fish meal replacement ratio: 50%, 25%, and 0% of the protein were supplied via fish meal in the 50/50, 25/75, and 0/100 diets, respectively. The + indicates mannan oligosaccharide (MOS) supplementation. Values are in g/100 g of dry diet.

Ingredients	Control	50/50	50/50+	25/75	25/75+	0/100	0/100+
Herring meal[a]	63.8	31.9	31.9	16.0	16.0	0.0	0.0
SPC[b]	0.0	32.9	32.9	49.4	49.4	65.9	65.9
Dextrin[c]	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Fish oil[d]	4.7	7.2	7.2	8.4	8.4	9.6	9.6
Mineral mix[e]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[f]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
CMC[c]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Amino acid mix[g]	0.0	1.0	1.0	1.0	1.0	1.0	1.0
BioMos®[h]	0.0	0.0	0.3	0.0	0.3	0.0	0.3
Cellufil[c]	9.5	6.0	4.7	4.2	2.9	2.5	1.2
Crude protein[i]	44.3	45.4	46.0	46.2	46.0	46.2	42.5
Crude lipid[i]	11.6	11.2	11.9	10.6	10.8	12.1	12.4
Available energy (kJ/g diet)[j]	13.9	14.0	14.3	13.9	13.9	14.5	13.9

[a] International Proteins, Minneapolis, MN.
[b] Soy Protein Concentrate, Profine VF, The Solae Company, St. Louis, MO.
[c] US Biochemical Corporation, Aurora, IL.
[d] OmegaPure menhaden oil, Omega Oils, Reedville, VA.
[e] ICN Corporation, Costa Mesa CA.
[f] See Moon and Gatlin (1991).
[g] 30% methionine, 20% lysine, 50% taurine.
[h] Alltech Incorporated, Nicholasville, KY.
[i] Analyzed.
[j] Calculated from physiological fuel values (16.7 J/g protein and carbohydrate; 37.7 J/g lipid).

dietary protein) and MOS inclusion as the other main factor (with and without). The diets were maintained isolipidic by increasing the levels of fish (menhaden) oil as fish meal levels decreased, and all diets except the control were supplemented with an amino acid mix (methionine, lysine, and taurine; 20/30/50 w/w/w, respectively). The remaining diets containing MOS were formulated by adding BioMos® (Alltech Inc., Nicholasville, KY), a commercial MOS product, at a level of 0.3% of the diet at the expense of cellulose.

2.1.2. Feeding trial 2

In the second feeding trial, a total of six diets were prepared (Table 2, 18 tanks stocked) based on the results of the first feeding trial, to further investigate MOS and amino acid supplementation in high plant-protein inclusion level diets for juvenile cobia, as well as to attempt to completely replace fish meal concomitantly with fish oil in a cobia diet. Experimental diets in this trial were formulated to more similar to commercial formulations utilized for juvenile cobia under production conditions. Dietary lipid levels were again held at 12% on a dry-matter basis. Total crude protein levels (45% of dry diet) were achieved with a combination of fish meal (25.3% of dry diet), SPC (12.6% of dry diet) and SBM (32.4% of dry diet). This formulation served as the control diet, as the fish meal content in commercial formulations is never 100% of the dietary protein and the ratios of these protein components more closely mimic commercial cobia formulations. Neither the amino acid mix nor MOS was included in this diet. The next two diets (SB and SB+) were formulated to contain lower levels of fish meal (12.6% of dry diet) and higher levels of SPC (25.3% of dry diet) while maintaining similar levels of SBM. Neither of these diets received the additional amino acid supplement utilized in the first feeding trial, but the SB+ diet was supplemented with MOS.

Table 2
Dietary composition of the experimental diets utilized in feeding trial 2. The + sign indicates MOS supplementation; SB = soybean-based diet; MXSB = soybean-based diet in which the incorporation of soybean meal has been maximized; NOFM = fish meal-free and fish oil-free diet. Values are in g/100 g of dry diet.

Ingredients	Control	SB	SB+	MXSB	MXSB+	NOFM
Herring meal[a]	25.3	12.6	12.6	8.5	8.5	0.0
SPC[b]	12.6	25.3	25.3	23.8	23.8	25.3
Soybean meal[c]	32.4	32.4	32.4	39.9	39.9	0.0
Worm meal[d]	0.0	0.0	0.0	0.0	0.0	30.0
Dextrin[e]	10.0	10.0	10.0	8.6	8.3	9.5
Soy oil	0.0	0.0	0.0	0.0	0.0	7.3
Fish oil[f]	8.7	10.0	10.0	10.2	10.2	0.0
DHA Gold[g]	0.0	0.0	0.0	0.0	0.0	1.5
Mineral mix[h]	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mix[i]	3.0	3.0	3.0	3.0	3.0	3.0
CMC[e]	1.0	1.0	1.0	1.0	1.0	1.0
Amino acid mix[j]	0.0	0.0	0.0	1.0	1.0	0.0
BioMos®[k]	0.0	0.0	0.3	0.0	0.3	0.3
NuPro®[k]	0.0	0.0	0.0	0.0	0.0	17.5
Cellufil[e]	3.0	1.7	1.4	0.0	0.0	0.6
Crude protein[l]	43.3	43.8	44.2	43.5	43.9	43.2
Crude lipid[l]	10.5	10.9	10.2	10.8	11.0	10.6
Available energy (kJ/g diet)[m]	12.9	13.1	12.9	12.8	12.9	12.8

[a] International Proteins, Minneapolis, MN.
[b] Soy Protein Concentrate, Profine VF, The Solae Company, St. Louis, MO.
[c] Professional Proteins Ltd., Hi Protein (48%) soybean meal; Washington, IA.
[d] SeaBait®, Shoreline Polychaeate Farms, UK.
[e] US Biochemical Corporation, Aurora, IL.
[f] OmegaPure menhaden oil, Omega Oils, Reedville, VA.
[g] Advanced BioNutrition, Columbia, MD.
[h] ICN Corporation, Costa Mesa CA.
[i] See Moon and Gatlin (1991).
[j] 30% methionine, 20% lysine, 50% taurine.
[k] Alltech Incorporated, Nicholasville, KY.
[l] Analyzed.
[m] Calculated from physiological fuel values (16.7 J/g for protein and carbohydrate; 37.7 J/g for lipid).

Two additional diets were formulated to contain the maximum inclusion levels of soy products, in order to minimize overall protein costs. In these diets (MXSB and MXSB+), protein levels from fish meal were minimized and the soybean meal were maximized, resulting in 8.5% of dry diet supplied by the fish meal, 32.8% by the soy concentrate, and 39.9% by soybean meal. Both MXSB and MXSB+ diets were supplemented with the amino acid mix, and MXSB+ also included MOS, to further investigate the impacts of MOS supplementation.

A final experimental diet was designed to eliminate fish meal and fish oil entirely from the formulation. This diet formulation was based not only upon published results from our laboratory (Craig and McLean, 2005; Craig et al., 2006; Lunger et al., 2006, 2007a,b), but also incorporating the results of several unpublished feeding trials involving novel alternate protein sources in aquafeeds for juvenile cobia at our laboratory. In this diet (NOFM), the dietary protein component was composed of 30% of dry diet of a *Nereid* worm meal (SeaBait®, Shoreline Polychaete Farms, UK), 25.3% of dry diet of SPC, and 17.5% of dry diet of NuPro® (Alltech Inc., Nicholasville, KY), a yeast-based protein source high in nucleotides. Lipids were supplied by soy oil, endogenous lipids from the worm meal, and from DHA-enriched algae meal (AquaGrow Gold, Advanced BioNutrition, Columbia, MD). Importantly, this diet was not supplemented with amino acids, but incorporated MOS.

2.2. Diet manufacture

All diets were manufactured onsite, where the dry components of the diet were first thoroughly mixed in a Patterson–Kelley twin shell® Batch V-mixer (Patterson–Kelley Co. Inc., East Stroudsburg, PA) prior to being transferred into a Hobart D300 Floor Mixer (Hobart Co., Troy, OH), where oil was added and further mixed. The amount of distilled water required for pelleting (20–40% of feed weight) then was added to the mixture and mixed until a pebble-like consistency was achieved. The mixture then was pressure pelleted using an appropriate diet to provide pellets of suitable size for the fish. After air-drying, feed moisture content was approximately 15% and accurate dry matter determinations (AOAC, 1994) made so that feed quantity was based upon a dry-matter basis. Bulk diets were frozen at −20 °C and smaller portions were thawed and refrigerated as needed.

2.3. Data acquisition

At the end of the trials, all fish were weighed to obtain a final weight and three fish from each tank (feeding trial 1) and two fish per tank (feeding trial 2) were sampled for biological indices and proximate composition. The fish were euthanized with an overdose of anesthetic (buffered MS-222, 250 mg L^{-1}; Sigma-Aldrich, St. Louis, MO) prior to being weighed. Overall weight gain, specific growth rate (SGR; 100*Ln (final weight/initial weight)/(trial duration)), and feed efficiency ratio values were then calculated. The fish were subsequently dissected and the visceral mass, liver, and filets were weighed to establish the viscerasomatic index (VSI; visceral weight/total body weight*100), hepatosomatic index (HSI; liver weight/total body weight*100), and muscle ratio (MR; total filet weight/total body weight*100), respectively. In addition to the above production parameters and biological indices, proximate analyses were performed (AOAC, 1994) on muscle (dry matter, crude protein, lipid, and ash) and liver (lipid) in the second feeding trial.

2.4. Statistical analyses

In the first feeding trial, all data were subjected to factorial analysis of variance (ANOVA) procedures utilizing JMP 7.0.1 (SAS, Cary, NC, USA). In the second trial, standard ANOVA procedures were utilized and, when appropriate, Tukey–Kramer HSD was used for multiple comparisons of the means (α<0.05).

3. Results

3.1. Feeding trial 1

3.1.1. Weight gain, FE and survival

Weight gain, specific growth rate (SGR), feed efficiency (FE) and survival were all significantly affected by the fish meal replacement (FMR) level in the experimental diets (Table 3). At the end of the first feeding trial, juvenile cobia fed diets with either 50 or 75% FMR achieved equal weight gain, ranging from 199 to 205% over the six week period. Similarly, fish fed these same diets had significantly higher FE ratio values when compared to fish fed the diets containing 100% FMR. Survival was also significantly higher in fish fed the 50 and 75% FMR replacement diets, averaging 90% compared to an average survival of 50% in fish fed the 100% FMR diets. Addition of MOS had no significant effects on weight gain, FE ratio values or survival in the first feeding trial. Fish fed the control diet had intermediate performances in terms of cumulative growth, FE, and survival (167%, 30.7%, and 90.5%, respectively). However, these values were not statistically different from any other treatments.

3.1.2. Biological indices

Fish meal replacement also had significant impacts on hepatosomatic index (HSI) and muscle ratio (MR), but not on visceral somatic index (VSI; Table 4). Fish fed the 50% FMR diets had significantly larger HSI (1.25%) compared with fish fed the 100% FMR diets (1.05%). Cobia fed the diet containing 75% FMR had intermediate HSI values of 1.2%. In terms of MR, a similar trend was observed to that just described, with fish fed the 50% FMR diets having significantly higher MR values (32.6%) compared to fish fed the 100% FMR diets (29.2%), with those fed the 75% FMR diets having intermediate values (31.4%). Visceral somatic indices were not impacted by FMR, with means ranging from 8.3% in fish fed the 75% FMR diet to 9.0% in fish fed the 100% FMR diet, and with intermediate values recorded for fish fed the 50% FMR diet (8.6%). Inclusion of MOS had no significant effects on VSI, HSI, or MR.

3.2. Feeding trial 2

3.2.1. Weight gain, FE and survival

In the second feeding trial, diet impacted weight gain significantly: fish fed the SB and SB+ diets, without amino acid supplementation, displaying the lowest overall weight gain (99 and 121% respectively, Table 5). Fish fed the MXSB, MXSB+, and NOFM diets achieved significantly greater weight gains. Juvenile cobia fed all the latter diets had similar weight gains, ranging from 218% in fish fed the NOFM diet to 242% in fish fed the MXSB diet. Fish fed the control diet exhibited an intermediate weight gain of 187%.

Specific growth rates were significantly impacted by diets, logically mirroring the results observed with respect to weight gain. Observed SGR ranged from 1.64 to 1.88% in fish fed the SB and SB+ diets, respectively, to 2.75 to 2.91% in fish fed the NOFM and MXSB diets, respectively. Fish fed the control diets presented an intermediate SGR of 2.50%.

Feed efficiency ratio values were impacted significantly by diet, following a trend similar to that observed with regards to weight gain (Table 5). Fish fed the MXSB (59%), MXSB+ (59%), NOFM (56%) and control diets (51%) had statistically higher FE ratio values than fish fed the SB and SB+ diets (34% and 40%, respectively).

3.2.2. Biological indices and tissues analyses

Biological indices were all significantly impacted by dietary treatments (Table 6). With respect to VSI, fish fed the SB and SB+ (10.62 and 10.78%, respectively) diets had significantly higher VSI ratios than fish fed the MXSB diet (9.07%). Fish fed the remaining diets had intermediate responses ranging from 9.33 (MXSB+) to 9.67% (control). Hepatosomatic indices were also significantly affected by dietary treatments as fish fed the MXSB and MXSB+ diets returned the lowest HSI levels (1.68 and 1.75%, respectively), compared to those observed in fish fed the SB+ diet (2.55%). Fish fed the remaining diets had intermediate responses ranging from 2.02 (control) to 2.30% (SB). Finally, muscle ratio was significantly decreased in fish fed the SB and SB+ diets (30.06 and 31.63%, respectively). Fish fed the

Table 3
Weight gain, feed efficiency ratio values (FE), specific growth rates (SGR) and survival percentages of juvenile cobia in trial 1 fed diets in which fish meal was replaced by soy protein concentrate with and without MOS supplementation.

Dietary treatment		Wt gain[1]	FE[2]	SGR[3]	Survival
FMR	MOS				
50	−	199	37.0	2.55	81.0
50	+	205	43.0	2.58	95.2
75	−	199	41.9	2.54	100.0
75	+	199	37.5	2.54	90.5
100	−	116	17.4	1.76	38.1
100	+	153	28.3	2.15	61.9
ANOVA P<F					
FMR		0.0007	0.0015	0.0007	0.0042
		50, 75>100	50, 75>100	50, 75>100	50, 75>100
MOS		0.7785	0.3163	0.8361	0.3965
FMR*MOS		0.3926	0.2015	0.2924	0.3597
Pooled SE		0.0352	0.0028	0.0292	0.0229

FMR: fish meal replacement; MOS: mannan oligosaccharide.
[1]Weight gain, as percent increase from initial weight.
[2]Feed efficiency ratio (g gained/g fed).
[3]Specific growth rate (% d^{-1}).

Table 4
Biological indices of juvenile cobia in the first feeding trial.

Dietary Treatment		VSI	HSI	MR
FMR	MOS			
50	−	8.4	1.2	33.5
50	+	8.8	1.3	30.6
75	−	8.7	1.3	31.4
75	+	8.1	1.1	31.4
100	−	9.0	1.0	28.8
100	+	9.0	1.1	29.6
ANOVA P<F				
FMR		0.2753	0.0050	0.0440
			(50>100)=75	(50>100)=75
MOS		0.2211	0.4590	0.0898
FMR*MOS		0.1337	0.1024	0.4317
Pooled SE		0.2731	0.0471	1.1735

FMR: fish meal replacement; VSI: viscera-somatic index; HSI: hepatosomatic index; MR: muscle ratio.

Table 5
Weight gain, feed efficiency ratio values (FE), specific growth rates (SGR) and survival percentages of juvenile cobia in the second feeding trial.

Dietary treatment	Wt gain[1]	FE[2]	SGR[3]	Survival
Control	187[ab]	51[a]	2.50[ab]	93
SB	99[c]	34[b]	1.64[c]	100
SB+	121[bc]	40[b]	1.88[bc]	100
MXSB	242[a]	59[a]	2.91[a]	100
MXSB+	232[a]	59[a]	2.86[a]	100
NOFM	218[a]	56[a]	2.75[a]	100
P<F	0.0002	<0.0001	<0.0001	0.4582
Pooled SE	16.41	2.3	0.142	2.72

Values with different superscripts within the same column were significantly different ($P<0.05$).
[1]Weight gain, as percent increase from initial weight.
[2]Feed efficiency ratio (g gained/g fed).
[3]Specific growth rate (% d^{-1}).

Table 6
Biological indices for juvenile cobia in the second feeding trial.

	VSI	HSI	MR
Control	9.7^{ab}	2.02^{ab}	37.0^{a}
SB	10.6^{a}	2.30^{ab}	30.1^{b}
SB+	10.8^{a}	2.55^{a}	31.6^{b}
MXSB	9.1^{b}	1.68^{b}	37.0^{a}
MXSB+	9.3^{ab}	1.75^{b}	36.9^{a}
NOFM	9.4^{ab}	2.17^{ab}	36.9^{a}
P<*F*	0.0052	0.0022	<0.0001
Pooled SE	0.339	0.162	1.065

Values with different superscripts within the same column were significantly different ($P<0.05$); VSI: viscera-somatic index; HSI: hepatosomatic index; MR: muscle ratio.

remaining diets exhibited significantly higher MR values, ranging from 36.87 (MXSB+) to 37.02% (control).

Tissue composition in cobia from the second feeding trial was significantly impacted by dietary treatment (Table 7). Filets from fish fed the MXSB+ diet had higher protein content (19.7%), while filets from fish fed the SB+ diet had the lowest (18.9%). Finally, the ash content of fish fed the NOFM was about 50% higher than that of fish fed other diets (1.53%, vs. 1.03%). Muscle dry matter averaged 24.3 ± 0.19% across all treatments, and lipid content ranged from 3.29% (control) to 4.13% (SB). Liver lipid contents were not statistically influenced by dietary treatment, although a trend may be considered (Table 7): levels ranged from 16.58% (SB) to 29.13% (control).

4. Discussion

This represents the fourth time that high FMR levels of 75% of dietary protein or higher have been incorporated into diets for juvenile cobia without negative impact on production characteristics in our lab (Lunger et al., 2007b; others Craig and McLean, unpublished data). Such fish meal replacement levels have been achieved by including supplemental amino acids, particularly taurine, methionine and lysine. In addition to underlying amino acid requirements, combining various alternate protein sources (e.g. yeast-based protein, SBM, SPC) reinforces previous hypotheses that a single alternate protein source cannot effectively replace fish meal in diets designed for high-level marine carnivores (Craig and McLean, 2005).

In the first feeding trial, juvenile cobia responded well to FMR levels of 50 and 75% of dietary protein, showing equal weight gain and greater than 91% survival. Fish fed an internal control diet consisting of 100% herring meal as the protein source performed statistically equal, but numerically lower, than fish fed the FMR 50 and 75% diets (167% increase from initial weight, data not presented). Conversely, when 100% of the fish meal was replaced with a single ingredient (SPC), even with amino acid supplementation, production performances declined significantly in all growth parameters (Table 3).

Table 7
Proximate analysis of muscle and liver of juvenile cobia in experiment. Values for protein and lipid presented as g/100 g wet weight.

	Muscle				Liver
	Dry matter	Protein	Lipid	Ash	Lipid
Control	23.67	19.0^{ab}	3.29	1.12^{b}	29.1
SB	24.36	18.9^{ab}	4.13	0.95^{b}	16.6
SB+	23.82	18.9^{b}	3.59	1.06^{b}	18.6
MXSB	24.67	19.5^{ab}	3.87	1.06^{b}	16.7
MXSB+	24.69	19.7^{a}	3.70	0.96^{b}	17.7
NOFM	24.69	19.5^{ab}	3.45	1.53^{a}	24.1
P<*F*	0.1028	0.0167	0.3276	0.0007	0.0765
Pooled SE	0.3210	0.1903	0.2702	0.0887	3.4037

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

The use of dietary MOS has been investigated in fish with ambiguous results: improvements in growth and health status in rainbow trout, *Oncorhynchus mykiss* (Yilmaz et al., 2007), and European sea bass, *Dicentrarchus labrax*, (Torrecillas et al., 2007) were observed, while no significant effects were discerned in trials with Nile tilapia *Oreochromis niloticus* (Craig and McLean, 2003), Gulf of Mexico sturgeon *Acipenser oxyrinchus desotoi* (Pryor et al., 2003) and European sea bass (Sweetman and Davies, 2006). No significant benefits were observed from dietary MOS supplementation in either of the present experiments with juvenile cobia. Nonetheless, when replacing 100% of the fish meal, a beneficial trend was observed in the first trial, as fish fed the 100+ diet experienced a 24% increase in weight gain and a 38% increase in survival on average, when compared to the non-MOS supplemented 100% FMR diet. While these numbers were not significantly different, they may still be relevant from a commercial production stand-point when using diets with high FMR levels. Based on these data, as well as those observed by Salze et al. (2008) where beneficial effects were noted in larval cobia, producers should be practical in their choices regarding dietary MOS supplementation.

In the second feeding trial, fish meal provided approximately 9% of the dietary protein in the SB/SB+ diets, while only 6% in the MXSB/MXSB+ diets. Despite the higher fish meal inclusion, fish fed the former diet only achieved half the growth of that of fish fed the MXSB/MXSB+ diets (Table 5). Since the SB/SB+ diets were not supplemented with the amino acid mix, this emphasizes the critical importance of such supplementation when high replacement levels (>75% of dietary protein) of dietary fish meal are employed. The amino acid mix was developed in our lab based upon novel findings with respect to taurine supplementation in feeds for juvenile cobia (Lunger et al., 2007b). During the course of many other unpublished cobia studies, experimental diets were analyzed for amino acid composition which aided in the refinement of the present amino acid supplement regime and these data support the efficacy of supplemental amino acid incorporation.

One of the most important and far-reaching aspects of this experiment was the successful, total elimination of fish meal concomitantly with fish oil in a cobia aquafeed. In the second feeding trial, this was achieved with a unique combination of alternate protein and lipid sources, as well as dietary additives (MOS). The dietary protein components of the NOFM were comprised of a *Nereid* sp. worm meal, SPC, and NuPro®, each supplying complementary amino acid and nutrient profiles. *Nereid* worms are marine in origin (Dall et al., 1991) and thus their lipid component contains high levels of long chain highly unsaturated fatty acids (HUFA) of the n−3 family required by marine fishes. The SPC, containing 71% crude protein, provided the majority of the dietary protein. Finally, NuPro®, a yeast-based protein source, complemented the protein supply while also adding valuable nutrients such as nucleotides, di- and tri-peptides, and other potential nutrients from the yeast cytoplasm. However, the present data are insufficient to infer whether cobia benefited from these nutrients. Noteworthy are the overall performances of fish fed this diet, given that it was not supplemented with the amino acid mix. These findings highlight the importance of selecting alternate protein sources based upon appropriate dietary amino acid profiles for the considered species.

In addition, NOFM was also devoid of any fish oil, but utilized two alternate lipid sources, namely soy oil, which supplied over 60% of the dietary lipid component, indicating that juvenile cobia can effectively utilize alternate, non-marine lipid sources. The other component of the dietary lipid in the NOFM diet was a concentrated marine algal meal. Importantly, this meal contained 45% lipid, of which 20% was docosahexaenoic acid (DHA). The other significant source of dietary n−3 HUFA in this formulation were the lipids derived from the worm meal: its lipid component accounts for 14% g of its mass, a majority of which (13.5%) are n−3 HUFA. Therefore, the combination of n−3

HUFA from the algal and worm meals added up to approximately 0.9% of the diet. The outstanding growth performance of fish fed the NOFM diet suggests that this dietary n−3 HUFA level was sufficient to satisfy cobia juvenile requirements for these n−3 HUFA. Although the precise requirement of juvenile cobia for n−3 HUFA has not yet been determined, 0.9% dry diet is consistent with previously determined requirements in other marine species. Red drum (*Sciaenops ocellatus*), which is often compared with cobia, requires 0.5% dry diet of n−3 dietary HUFA (Lochmann and Gatlin, 1993). Requirements for n−3 HUFA in other marine species such as red seabream (*Pagrus major*), yellowtail (*Seriola lalandi*) and turbot (*Psetta maxima*) range from 0.5 to 2.0% of dry diet (National Research Council, 1993; Lee et al., 2003).

These data are especially germane to the ongoing concerns regarding sustainable production of marine carnivores — often species with higher market values. The complete elimination of both fish meal and fish oil has now been successfully achieved in aquafeeds for cobia juveniles. However, the ground-breaking NOFM formulation relied on novel, unique and sometimes expensive, alternative feedstuffs, and therefore may hinder the feasibility and sustainability of such diets from an economical perspective. Hence, the consistent, successful replacement of up to 94% of the fish meal protein achieved here is actually more promising to the future of the aquaculture industry and the surrounding issues of true sustainability. Indeed, the use of commodity traded plant-protein sources as alternatives to fish meal represents the only practical avenue to true environmental and economical sustainability in the global aquaculture industry.

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References

AOAC, 1994. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA.

Barrows, F.T., Gaylord, T.G., Stone, D.A.J., Smith, C.E., 2007. Effect of protein source and nutrient density on growth efficiency, histology and plasma amino acid concentration of rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research 38, 1747–1758.

Craig, S.R., McLean, E., 2003. Effect of Dietary Inclusion of Bio-Mos Upon Performance Characteristics of Nile Tilapia, Alltech 19th Annual Symposium on Biotechnology and the Food Industry, Lexington, Kentucky, USA.

Craig, S.R., McLean, E., 2005. The Organic Aquaculture Movement: A Role for NuPro™ as an Alternative Protein Source. In: Lyons, T.P., Jacques, K.A. (Eds.), Nutritional Biotechnology in the Feed and Food Industries — Proceedings of Alltech's 21st Annual Symposium. Alltech, Lexington, KY, pp. 285–293.

Craig, S.R., Schwarz, M.H., McLean, E., 2006. Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. Aquaculture 261, 384–391.

Dall, W., Smith, D.M., Moore, L.E., 1991. Biochemical composition of some prey species of *Penaeus esculentus* Haswell (Penaeidae: Decapoda). Aquaculture 96, 151–166.

FAO, 2007. The State of World Fisheries and Aquaculture — 2006 (SOFIA). Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, p. 180.

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

Gaylord, T.G., Teague, A.M., Barrows, F.T., 2006. Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Journal of the World Aquaculture Society 37, 509–517.

Kim, S.-K., Takeuchi, T., Akimoto, A., Furuita, H., Yamamoto, T., Yokoyama, M., Murata, Y., 2005. Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder *Paralichthys olivaceus*. Fisheries Science 71, 627–632.

Kim, S.-K., Matsunari, H., Takeuchi, T., Yokoyama, M., Murata, Y., Ishihara, K., 2007. Effect of different dietary taurine levels on the conjugated bile acid composition and growth performance of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*. Aquaculture 273, 595–601.

Lee, S.-M., Lee, J.H., Kim, K.-D., 2003. Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). Aquaculture 225, 269–281.

Lochmann, R.T., Gatlin, D.M., 1993. Essential fatty acid requirement of juvenile red drum (*Sciaenops ocellatus*). Fish Physiology and Biochemistry 12, 221–235.

Lunger, A.N., Craig, S.R., McLean, E., 2006. Replacement of fish meal in cobia (*Rachycentron canadum*) diets using an organically certified protein. Aquaculture 257, 393–399.

Lunger, A.N., McLean, E., Craig, S.R., 2007a. The effects of organic protein supplementation upon growth, feed conversion and texture quality parameters of juvenile cobia (*Rachycentron canadum*). Aquaculture 264, 342–352.

Lunger, A.N., McLean, E., Gaylord, T.G., Kuhn, D., Craig, S.R., 2007b. Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). Aquaculture 271, 401–410.

Moon, H.Y., Gatlin III, D.M., 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95, 97–106.

National Research Council, 1993. Nutrient Requirements of Fish. National Acad. Press, Washington, DC.

Pryor, G.S., Royes, J.B., Chapman, F.A., Miles, R.D., 2003. Mannanoligosaccharides in fish nutrition: effects of dietary supplementation on growth and gastrointestinal villi structure in gulf of Mexico sturgeon. North American Journal of Aquaculture 65, 106–111.

Salze, G., McLean, E., Schwarz, M.H., Craig, S.R., 2008. Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. Aquaculture 274, 148–152.

Sweetman, J.W., Davies, S., 2006. Improving Growth Performance and Health Status of Aquaculture Stocks in Europe through the Use of Bio-Mos®. In: Lyons, T.P., Jacques, K.A. (Eds.), Nutritional Biotechnology in the Feed and Food Industries, Proceedings of Alltech's 22nd Annual Symposium. Nottingham University Press, Nottingham, UK, pp. 445–452.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L., Izquierdo, M.S., 2007. Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish & Shellfish Immunology 23, 969–981.

Yilmaz, E., Genc, M.A., Genc, E., 2007. Effects of dietary mannan oligosaccharides on growth, body composition, and intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. Israeli Journal of Aquaculture-Bamidgeh 59, 182–188.