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Replacement of fish meal by a novel non-GM variety of soybean meal in cobia, *Rachycentron canadum*: Ingredient nutrient digestibility and growth performance

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ABSTRACT

A constraint for the expansion of cobia aquaculture is the availability of high quality formulated diets which reduce or eliminate fish meal (FM) protein. Therefore, the nutritive value of a novel soybean cultivar, Navita™ (Navita, non-genetically modified and selectively bred soy), and regular, commodity soybean meal (SBM, de-hulled, defatted, roasted and solvent-extracted) was evaluated for cobia, *Rachycentron canadum* via separate digestibility and growth trials. In the first experiment Navita's apparent digestibility coefficients (ADC) were higher than those of SBM for nearly every nutrient evaluated. Crude protein ADCs were 82 and 69% for Navita and SBM, respectively. Apparent DC for amino acids ranged from 68 to 109% for Navita whereas, amino acid ADCs for SBM varied from 42 to 98%. The feeding trial utilized fish of a size that more closely resembles commercial cobia stocking (1.8 kg), and was conducted over a 91-day period. Experimental diets (iso-nitrogenous and iso-energetic) were formulated such that 67% of the FM protein in the reference diet was replaced by either a combination of SBM + soy protein concentrate (SPC, Solae Profine®) labeled MXSB-diet, or by a combination of SPC + Navita; Navita-diet, hereafter. A fourth experimental diet had 80% of the FM protein replaced by a combination of Navita + SPC and was identified as Navita-high. No significant differences ($P>0.05$) were observed in fish fed the experimental diets for feed conversion ratio, protein efficiency ratio, feed efficiency, mean daily intake, gross protein intake, gross energy intake, visceral somatic index, muscle ratio, and hepatosomatic index. Fish fed the Navita-high diet had the lowest fish in:fish out ratio (FIFO) at 0.9 ± 0.16. These results indicate that Navita meal can be incorporated at very high levels in the diet of marine carnivorous fish such as cobia with no detriment to performance, making it a prime candidate for FM replacement in aquafeeds.

1. Introduction

Aquaculture is a rapidly growing food-producing industry; however, there is still considerable potential for increased efficiency and efficacy of aquaculture through development of nutritious and cost-effective alternatives to traditional marine protein feedstuffs such as fish meal (FM). For cobia aquaculture, this is particularly relevant as suitable diets which reduce or eliminate FM protein are not yet commercially available. This species is the only member of the family Rachycentridae which is distributed worldwide in tropical and subtropical waters (Benetti et al., 2008; Ditty and Shaw, 1992; Shaffer and Nakamura, 1989). Cobia is well recognized for its fast growth and excellent meat quality and has been intensively farmed since the 1990s (Liao et al., 2004). The technology for reliable broodstock spawning and mass production of cobia fingerlings has been long established at the University of Miami Experimental Hatchery (UMEH). However, while the fundamental technology for cobia production is in place, more research is needed to resolve bottle-neck issues of cobia culture, particularly at the grow-out stage. One such limitation is the formulation of commercial diets with reduced FM levels that are both economical and environmentally friendly, while also maintaining optimal growth performance and disease resistance. The global FM supply remains relatively static but demand and price continue to increase (Naylor et al., 2009). Therefore, FM is a raw material that cannot be relied upon for aquaculture expansion. In contrast, soybean meal (SBM) has long been recognized as an excellent source of protein for animals and humans (Baker, 2000). However, SBM inclusion levels as replacement of FM in aquafeeds are limited by species-specific digestive physiology and by the presence of both heat-resistant and thermo-labile anti-nutritional factors (ANFs). While solvent-extracting and cooking may significantly reduce the biological activity of temperature-sensitive protease inhibitors, this processing also renders protein less available for absorption at the gastrointestinal level. Therefore, the present study was conducted to evaluate the apparent digestibility coefficients (ADCs) of protein and amino acids of a novel

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variety of non-genetically modified (GM) soybean product (Navita™) and contrast these coefficients against those obtained from fish fed conventional SBM. In addition, a study oriented at maximizing FM replacement in cobia diets, using fish close to marketable size was performed. The use of protein sources such as standard SBM, soy protein concentrates (SPC) and non-GM soy from selective breeding programs could greatly improve the profitability and ecologic sustainability of the industry. The overarching objective was to improve both the ecological and economic efficiencies of formulated feeds for cobia, *Rachycentron canadum*.

2. Materials and methods

Care and handling of the fish as well as procedures used in this study were reviewed and approved by the University of Miami Animal Care and Use Committee. Navita™ meal was donated by Navita Premium Feed Ingredients (NPFI), West Des Moines, IA. This is a genetically unique, patented non-GM soy cultivar, which contains increased levels of protein and amino acids for animal feed and reduced ANF levels. Beans are made into a defatted meal by conventional methods but this particular variety is selectively bred to have up to 20% higher protein density than the commodity SBM and ultra-low levels of oligosaccharides (0.6%, raffinose + stachyose). In contrast, conventional SBM has 6% of oligosaccharides.

2.1. Digestibility trial — experimental diets

Digestibility diets (Table 1) were manufactured at the Oceanic Institute, Waimanalo, HI and were comprised of a 70:30 mixture of the reference diet and the Navita™ meal. By including the reference diet at 70%, and with the crude protein contents inherent to the test ingredient, the resulting experimental diet had an analyzed crude protein and lipid values of 45 and 12%, respectively, which satisfies the requirements defined for cobia (Fraser and Davies, 2009). Yttrium oxide (Y_2O_3) was included at 0.5% of the reference diet as an inert, indigestible marker. The dietary ingredients were first ground to a uniform particle size (<1 mm) in a hammer mill, then batch-mixed in a commercial ribbon blender (Ross 42-A-10, Charles Ross and Son Co., Hauppauge, NY), preconditioned at 94–99 °C and extruded (Wenger X-20, Wenger Manufacturing, Sabetha, KS) at 99–138 °C through a 5-mm die to produce buoyant pellets.

Table 1
Reference and test diets for determination of apparent digestibility coefficients in cobia.

Ingredient	Reference diet	Navita[a]	Regular soybean meal[b]
	g/kg of diet		
Pollock fish meal[c]	612.6		
Dextrin	120.0		
Menhaden oil	69.4		
Mineral premix[d]	50.0		
Vitamin premix[e]	40.0	700.0	700.0
Carboxylmethyl cellulose[f]	30.0		
Cellufil[f]	73.0		
Yttrium oxide[g]	5.0		
Test ingredient	–	300.0	300.0
Total	1000	1000	1000

[a] Navita Premium Feed Ingredients, West Des Moines, IA, (g/kg) 558.8 crude protein, 893.3 dry matter, 5.8 crude lipid, 31.0 crude fiber, 58.1 ash, non-genetically modified soy.
[b] Archer Daniels Midland, Decatur, IL, 480.0 crude protein, 933.8 dry matter, 18.4 crude lipid, 38.0 crude fiber, 61.9 ash, commodity roasted/cooked and hexane extracted, genetically modified soy.
[c] Alaska Pollock (*Theragra clalcogramma*) meal Genuine Alaska Pollock Producers, Seattle, WA, (g/kg) 698.8 crude protein, 734.5 dry matter, 7.9 crude lipid.
[d] Composition (g/kg): $Ca(H_2PO_4)_2{\cdot}H_2O$, 136.00; $Ca(C_6H_{10}O_6){\cdot}5H_2O$, 348.553; $FeSO_4{\cdot}7H_2O$, 5.00; $MgSO_4{\cdot}7H_2O$, 132.00; K_2HPO_4, 240.00; $NaH_2PO_4{\cdot}H_2O$, 88.00; NaCl, 45.00; $AlCl_3{\cdot}6H_2O$, 0.084; KI, 0.15; $CuSO_4{\cdot}5H_2O$, 0.50; $MnSO_4{\cdot}H_2O$, 0.70; $CoCl_2{\cdot}6H_2O$, 1.00; $ZnSO_4{\cdot}7H_2O$, 3.00; $NaSeO_3$, 0.0127.
[e] Composition (g/kg): ascorbic acid, 50; DL-calcium pantothenate, 5.0; choline chloride, 36.2; inositol, 5.0; menadione sodium bisulfite, 2.0; niacin, 5.0; pyridoxine HCl, 1.0; riboflavin, 3.0; thiamine mononitrate, 0.5; DL-α-tocopherol acetate (250 IU/g), 8.0; vitamin A palmitate (500,000 IU/g), 0.2; micro-mix, 10.0; cellulose, 874.1. Micro-mix composition (g/100 g): biotin, 0.50; folic acid, 1.8; vitamin B12, 0.02; cholecalciferol (40 IU/μg), 0.02; cellulose, 97.66.
[f] USB-Affymetrix, Cleveland, OH.
[g] Sigma-Aldrich Company, St. Louis, MO.

2.2. Digestibility trial — fish and experimental conditions

Juvenile cobia (initially weighing 286 ± 10 g/fish), raised in tanks at UMEH (Miami, FL) were seined and graded. These fish were stocked into four, 4500-L fiberglass round tanks at a density of 80 fish/tank. Sand-filtered well water entered each tank at a rate of 50 L/min (1600% exchange per day) and exited via an internal standpipe. Supplemental aeration was provided to each tank to maintain dissolved oxygen levels near saturation. Tanks were covered by a shading mesh with natural illumination. Water temperature, salinity and dissolved oxygen concentration were monitored daily (YSI-57) and averaged 24.4 °C, 33.5 ppt and 6.3 ppm, respectively. Water quality parameters were monitored weekly for pH, ammonia, nitrite and nitrate using a Hach™ water quality test kit and remained within recommended levels for cobia (Benetti et al., 2007) throughout the experiment. After a 7-day conditioning period during which all fish were fed the reference diet once per day (900 h) to apparent satiation, feeding of the test diet was initiated. Fish in each tank received the experimental diet for 7 days prior to the collection of fecal samples. Feces were collected 3–4 h post feeding at each collection period by the fecal stripping technique described by Austreng (1978) and Hajen et al. (1993), with modifications. Fish were stripped one day per week, for three consecutive weeks. Briefly, at the appointed collection period fish were dip-netted from the tank and a solution of MS-222 (500 mg/L; Finiquel, Argent Chemical, Redmond WA) was sprayed onto the gill cavity and the opercula held closed until complete relaxation of abdominal muscles was attained. Immediately afterwards, the gills were completely rinsed with a gentle stream of seawater for 30 s, flushing any residual anesthetic. Gentle pressure was then applied to the lower abdomen of the fish, thus expressing feces from the distal intestine onto clean stainless steel bowls. Care was taken to exclude urine, mucus, and other contaminants from the collection vessels. After fecal collection, fish were introduced into a plastic tub with oxygenated seawater until complete recovery. Fecal samples were oven-dried (60 °C) overnight, stored frozen (−80 °C) and sent on dry ice to the Fish Nutrition Laboratory of Texas A&M University for analyses. Yttrium oxide was analyzed by inductivity coupled plasma mass spectrometry (ICP-AES analysis, Perkin Elmer Optia 3000DV; Perkin Elmer, Wellesley, MA, USA) at 371 nm. All yttrium analyses were conducted in duplicate. Proximate composition of diets and fecal samples was analyzed using established methodologies for dry matter, crude protein (AOAC, 2005), lipids (Folch et al., 1957) and ash (AOAC, 2005). Crude protein was estimated by measuring total nitrogen by the Dumas method (Ebeling, 1968) and multiplied by 6.25. Dry matter was determined by heating the samples at 125 °C for 3 h, and ash was quantified after heating at, 650 °C for 3 h (AOAC, 1990). Crude lipid was determined by chloroform and methanol extraction (Folch et al., 1957). Gross energy content was measured by combustion via bomb calorimeter (Parr Instrument Company, Moline, IL, USA) using benzoic acid as a standard. Amino acid content was quantified after acid hydrolysis with 6 N HCl according to procedures described by Pohlenz et al. (2012). Calcium and Phosphorus were quantified by Inductively Coupled Plasma Spectroscopy. Plasma amino acids were assayed via HPLC following a fluorometric technique (Buentello and Gatlin, 2000) using pre-column derivatization with o-phthaldialdehyde (Sigma, St. Louis, MO). Navita ADCs for crude protein and amino acids were calculated from a comparison of the apparent nutrient digestibility of the reference diet and experimental diets as described by Forster (1999).

2.3. Feeding trial — systems, experimental diets and feeding

The feeding trial was conducted in 12 cylindrical, fiberglass tanks (4000-L each) operated as a flow-through system with filtered water