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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*

Guillaume Salze [a], Ewen McLean [b], P. Rush Battle [b], Michael H. Schwarz [c], Steven R. Craig [a,*]

[a] Department of Large Animal Clinical Sciences, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Phase II Duck Pond Drive, Blacksburg, VA 24061, United States

[b] Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, 100 Cheatham Hall, Blacksburg, VA 24061, United States

[c] Virginia Seafood Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, 102 S. King Street, Hampton, VA 23669, United States

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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-

* Corresponding author. Virginia Cobia Farms, 108 Battleground Avenue, Saltville, VA 24370, United States. Tel.: +1 276 496 4372x4; fax: +1 276 496 0268.
E-mail address: scraig@virginiacobiafarms.biz (S.R. Craig).

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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).