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Replacement of fish meal in cobia (Rachycentron canadum) diets using an organically certified protein

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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. 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 two times 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 conversion ratio values, ranging from 1.9 to 5.8, 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 25% 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 25% 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 similar values as 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.

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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 improve (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 >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 (Catscartan 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. Due to the aforementioned restrictions, there exists, at present at least, 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 (Storebakken 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 zoonoses (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’s position as the leading food production growth industry (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 quantity 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 Prud, 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 in mind. The carnivorous cobia was used as an experimental animal whereas organically certified yeast-based proteins were employed as the alternative protein source.

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. 1). The RAS was comprised of twenty-four 300 l circular fiberglass tanks, a bubble-bead filter (BBF-2 Aquaculture Technologies Inc., Metairie, LA, USA) to remove suspended solids, 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 °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 ± 0.24 mg/l; total ammonia nitrogen, 0.40 ± 0.07 mg/l; nitrite, 0.32 ± 0.06 mg/l, nitrate, 8.78 ± 3.33 mg/l; and pH, 7.57 ± 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 two times 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, decreased to 7% during the final week of the feeding trial which maintained a level of apparent satiation without overfeeding. Tanks were group weighed weekly to adjust the feeding rates and monitor growth performance.

2.2. Diets

Solvent-extracted 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 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 due to the constraint to maintain the diets as isonitrogenous (Table 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 phosphorus levels. Diets were analyzed for proximate composition to verify formulation accuracy.

Table 1
Composition of the experimental diets (g/100g dry weight)

| Ingredient                  | Diet 1 (100/0) | Diet 2 (75/25) | Diet 3 (50/50) | Diet 4 (25/75) | Diet 5 (0/100) |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
| Extracted fish meal a       | 54.4           | 40.8           | 27.2           | 13.6           | 0              |
| NuPro™ b                    | 0              | 19.5           | 39.0           | 58.5           | 78             |
| Dextrin c                   | 9.5            | 9.5            | 9.5            | 9.5            | 3.6            |
| Menhaden fish oil d         | 9.6            | 9.6            | 9.5            | 9.5            | 9.4            |
| 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 g                       | 1.0            | 1.0            | 1.0            | 1.0            | 1.0            |
| CaPO₄ h                     | 0              | 0              | 0              | 1.0            | 1.0            |
| Cellufil i                  | 18.5           | 12.6           | 6.8            | 0              | 0              |
| Crude protein h             | 40             | 40             | 40             | 40             | 40             |
| Crude lipid h               | 11             | 11             | 11             | 11             | 11             |
| Available energy (kJ/100 g diet) h | 1243       | 1243           | 1243           | 1243           | 1142           |

a: Omega Proteins, Hammond, LA.
b: Alltech Incorporated, Nicholasville, VA.
c: US Biochemical Corporation, Aurora, IL.
d: Omega Oils, Reedville, VA.
e: ICN Corporation, Costa Mesa CA.
f: See Moon and Gatlin (1991).
g: Sigma-Aldrich, St. Louis, MO.
h: Calculated (convert to kilojoules).
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 conversion ratio (FCR) values, 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). Liver samples were only analyzed for lipid due to sample size.

2.4. Statistical analyses

All data were subjected to analysis of variance procedure utilizing SAS 9.1 (SAS, Cary, NC, USA). Where appropriate, data were also 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) 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 conversion ratio values ranged from 5.8 (Diet 5) to 1.9 (Diets 1 and 2) with the FCR decreasing \((P<0.0001)\) as inclusion rate of the yeast-based protein source increased (Table 2). Once again, there was no difference between Diet 1 and Diet 2 and these two diets produced the highest FCR values during the feeding trial. Survival also was significantly affected by dietary treatment as the fish fed Diet 5 had lower survival (63%) compared to an overall survival rate of 99% in cobia fed the remaining diets (Table 2).

Muscle protein also tended to decrease as inclusion of the yeast-based protein source increased with a range of 17.8–19.7% (Table 3). Muscle protein in fish fed the control diet (Diet 1) and the Diet 2 did not differ. Muscle lipid ranged from 0.5 to 1.8 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 3). Dry matter and ash ranged from 20.3% to 24.6% wet weight, 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 to 25.3 and decreased \((P<0.001)\) with increasing levels of yeast-based protein inclusion (Table 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 4). Hepatosomatic index (HSI) ranged from 2.2 to 4.6 and was significantly

### Table 2

| Diet (Fish meal : yeast-based protein source) | Weight gain | FCR | Survival (%) |
|---------------------------------------------|-------------|-----|--------------|
| 100/0                                       | 512 \(^{a}\) | 1.9 \(^{a}\) | 100 \(^{a}\) |
| 75/25                                       | 503 \(^{a}\) | 1.9 \(^{a}\) | 100 \(^{a}\) |
| 50/50                                       | 432 \(^{a}\) | 2.4 \(^{a}\) | 97 \(^{a}\) |
| 25/75                                       | 238 \(^{a}\) | 3 \(^{b}\) | 100 \(^{a}\) |
| 0/100                                       | 86 \(^{b}\)  | 5.8 \(^{b}\) | 63 \(^{b}\) |
| Pooled SE                                   | 15.56       | 0.14 | 7.60         |

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

### Table 3

| 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 \(^{bc}\) | 22.0 \(^{ab}\) | 6.2 \(^{b}\) | 16.16 \(^{e}\) |
| 75/25                                     | 19.7 \(^{a}\)  | 1.80 \(^{a}\)  | 23.7 \(^{a}\)  | 5.97 \(^{b}\) | 24.64 \(^{e}\) |
| 50/50                                     | 19.0 \(^{b}\)  | 1.30 \(^{a}\)  | 22.40 \(^{a}\) | 6.40 \(^{b}\) | 20.87 \(^{b}\) |
| 25/75                                     | 18.3 \(^{b}\)  | 0.93 \(^{bc}\) | 21.70 \(^{ab}\) | 6.10 \(^{b}\) | 13.37 \(^{e}\) |
| 0/100                                     | 17.8 \(^{d}\)  | 0.50 \(^{a}\)  | 20.30 \(^{b}\) | 9.80 \(^{a}\) | 4.19 \(^{d}\)  |
| Pooled SE                                 | 1.10          | 0.25         | 0.50              | 0.44       | 1.90        |

\(^{a}\)Means of 3 fish per tank \((N=9\) treatment \(^{-1}\)). Means with different superscripts in the same column differed significantly \((P<0.05)\).
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 continues to surround the certifiability of by-catch from commercial fisheries, as well as 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 met 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 biological value due to essential amino acid deficiencies and/or imbalances and poor digestibility (Hardy, 1996; Francis et al., 2001). These issues may be amplified with 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 any 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 only began to decrease following 50% inclusion rate of the yeast-based protein source. These results are thereby similar to observations for other species of juvenile carnivorous fish in which fish meal was replaced using yeast-based products by 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 cobra 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, returning low weight gain and feed response, reduced FCR 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 assigned to the 100% yeast-based diet after feeding.

Table 4

| Diet fish meal: | MR2 | VSI3 | HSI4 | Packed cell volume (%) | Plasma protein (%) |
|----------------|-----|------|------|------------------------|-------------------|
| yeast-based protein source |       |      |      |                        |                   |
| 100/0          | 25.3a | 10.8c | 2.1c | 40b                    | 3.8b              |
| 75/25          | 25.9a | 12.0bc | 3.3c | 49a                    | 4.6a              |
| 50/50          | 15.4b | 12.9b | 3.9b | 51a                    | 4.2b              |
| 25/75          | 16.7b | 15.4a | 4.6a | 48a                    | 3.8b              |
| 0/100          | 10.1c | 16.4a | 2.7d | 32b                    | 2.4c              |
| Pooled SE      | 2.62  | 0.72  | 0.33 | 6.0                    | 0.44              |

1Means of 3 fish per tank (N=9 treatment−1). Means with different superscripts in the same column differed significantly (P<0.05).
2MR=muscle weight*100/body weight.
3VSI=VSI weight*100/body weight.
4HSI=HSI weight*100/body weight.
Although feed intake was not directly measured, this observation indicates that palatability was poor in this diet. Additionally, amino acid analysis of the diets did not indicate a severe nutrient deficiency with respect to essential amino acids such as lysine and methionine. Likewise, the significantly reduced biological indices (lower VSIS, 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 immune-depression and environmental stress (Lara-Flores et al., 2002; Olvera-Nova 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 obvious 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 demanding of more thorough investigation.

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