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Full replacement of menhaden fish meal protein by low-gossypol cottonseed flour protein in the diet of juvenile black sea bass *Centropristis striata*

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ABSTRACT

Eight isonitrogenous (46% crude protein) and isolipidic (14% crude lipid) diets were formulated for juvenile black sea bass *Centropristis striata* to replace menhaden fish meal (FM) protein (59.5% CP) by three cottonseed meal (CSM) proteins: a CSM prepared from glandless seed (GCSM, 50.4% CP), a CSM that had been solvent extracted with acidic ethanol to remove the gossypol (SCSM, 53.8% CP), and a CSM prepared from regular (glanded) cottonseed (RCSM, 45% CP). Three diets replaced 50, 75 and 100% of FM protein with GCSM, and three diets replaced 50, 75 and 100% of FM protein with SCSM. One diet replaced 100% FM protein with RCSM protein. A control diet (0% CSM) was formulated with high FM protein and other practical protein sources. L-methionine and L-lysine were supplemented to the diets to equal the control diet. Fifteen fish were stocked in each of twenty-four 75-L tanks, and each test diet was fed to triplicate groups of fish (mean initial weight = 7.7 ± 0.10 g) for 56 days at 22–24 °C, 32–35 salinity, and ambient photoperiod conditions. Fish were fed twice per day to apparent satiation. Final (day 56) mean body weight (BW, 24.16–31.62 g), body weight gain (BWG, 211.2–311.3%), specific growth rate (SGR, 1.70–2.18%/d), and feed intake (FI, 0.37–0.43 g/fish/d) were not significantly different ($P > 0.05$) among the control, GCSM or SCSM treatments, but were reduced ($P < 0.05$) for the 100% RCSM treatment (20.09 g, 159.5%, 1.45%/d, and 0.25 g/fish/d, respectively). Lower palatability of the RCSM diet was attributable to the anti-nutrient compound gossypol. No significant differences in survival (84.1–97.8%), feed conversion ratio (FCR, 1.05–1.33), protein efficiency ratio (PER, 1.62–2.08), or whole body protein or lipid composition were observed among the fish fed the low-gossypol diets. Gossypol (25.9 mg/kg) was only detectable in the livers of fish fed the high-gossypol RCSM diet. Fish whole body essential amino acid compositions did not differ significantly among treatments. Whole body n − 3 PUFAs decreased, while n − 6 PUFAs increased with increasing CSM protein in the diets. The apparent digestibility coefficient of protein was high (83.1–87.1%) for all treatments. For juvenile black sea bass, 75% FM in the diet can be replaced with low-gossypol CSM protein prepared by solvent-extraction, and 100% of FM can be replaced with low-gossypol CSM protein prepared from glandless seed with no adverse effects on survival, growth or feed utilization.

Statement of relevance: Cotton seed flour is an inexpensive agricultural by-product. The results demonstrate successful replacement of fish meal protein with genetically-improved cottonseed flour protein in the diet of black sea bass. These findings may substantially lower aquafeed costs (a key operational cost) for finfish growout operations to boost profitability and accelerate expansion of commercial production of black sea bass and other finfish species.

1. Introduction

Fish meal (FM) carries large quantities of energy per unit weight and is a source of high-quality protein and highly digestible essential amino and fatty acids (Zinn et al., 2009; Cho and Kim, 2011), making it a popular source of protein in aquaculture feeds. However, a decrease in the supply of FM and increasing costs, due to higher demand and a decline in the sustainability of the reduction fisheries, have led to many studies on alternative protein sources to replace FM protein in aquaculture feeds (Tacon et al., 2006, 2011; Trushenski et al., 2006; Alam et al., 2011, 2012).

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1.1. Cottonseed meal

Cottonseed meal (CSM) has received limited attention as a potential FM replacement in aqua feeds; however, CSM protein is an increasingly attractive alternative to FM protein due to improved plant production and processing methods. After harvesting, cottonseed is separated from the lint (i.e., ginning); the seeds are then cleaned and passed through a series of knives and shakers to separate the hull from the kernels. After separating most of the hulls, the kernels are flaked and cooked in preparation for oil extraction either by pressing or more typically by solvent extraction with hexane. The recovered oil is currently the most valuable by-product of the seed (National Cotton Council of America, cotton.org), and it is primarily used in cooking and food production (Lin et al., 2015). Once the oil is removed from the kernels, the remaining defatted seed tissue forms CSM.

1.2. Production and demand of cottonseed meal

The farm value of U.S. cotton and cottonseed production is approximately $5 billion, and the annual business revenue stimulated by cotton exceeds $120 billion, making cotton America's number one value-added crop (National Cotton Council of America, 2015, cotton.org). China and India are the two leading countries in cotton production and both produce 7 million mt, double the amount produced in the United States (Indexmundi, 2015b). Due to the large global production of cotton as well as the large quantities of cottonseed by-products, CSM is much cheaper per unit of protein than FM and other FM replacements. In 2014, the price of CSM was $383–420 per ton compared to $1512 per ton for FM (Indexmundi, 2015a). Since CSM is one-fourth the price of fish meal, it could reduce aquaculture production costs substantially if it can be efficiently used as a protein source in aqua feeds.

An important constraint to the use of CSM in animal feeds is gossypol, a terpene-based secondary metabolite that has an important role in the cotton plant's defense mechanisms in that it protects against pests and possibly diseases (Romano and Scheffler, 2008). Gossypol is deposited in structures called "glands" in the stems, leaves and green bolls of the plant, and in the seed (Lusas and Jividen, 1987). Gossypol is known to have anti-nutritional and anti-fertility effects on warm-blooded animals and fish fed cottonseed products (Eisele, 1986; Blom et al., 2001; Henry et al., 2001; Romano and Scheffler, 2008) and is also known to bind with lysine, rendering this essential amino acid less available (Wilson et al., 1981). For these reasons, cottonseed and CSM have been overlooked as a potential fish meal replacement in aqua feeds.

1.3. Reduction of gossypol in cottonseed meal and it's use as fishmeal replacement

Much research has been conducted to reduce or eliminate gossypol in CSM to improve its utilization as an ingredient in the feeds of animals and fish. Although several approaches have been undertaken, two approaches have been most effective. The first approach was to prepare CSM from naturally mutant "glandless" seed that lack the lysigenous glands and most of the gossypol. Cotton plants that produced glandless seeds were first noted in the late 1950's when farmers visually noticed the existence of mutant plants without the dark-colored glands (Siccardi et al., 2012), rendering the seeds largely gossypol free. The second approach, which has been used sporadically, was to extract the gossypol from meal with an acidified polar solvent (Liadakis et al., 1993; Pelitire et al., 2014). Although requiring additional processing, this approach has been found to eliminate 90–95% of the gossypol present in regular high-gossypol CSM (Pelitire et al., 2014).

Research on the replacement of fish meal with CSM is more limited than with other alternative protein sources, such as soybeans, canola, linseed, corn gluten, lupine, pea, rapeseed, rice, and macroalgae. Regular, high-gossypol CSM protein has been successfully used to replace FM protein at moderate levels of 35% in grass carp *Ctenopharyngodon idellus* (Zheng et al., 2012) and 30% in parrotfish *Oplegnathus fasciatus* (Lim and Lee, 2009). Research on low-gossypol CSM conducted to date has shown variable results. Low-gossypol CSM from glandless seed has been incorporated in the diets of hybrid striped bass *Morone saxatilis* ♀ × *Morone chrysops* ♂ (Sullivan and Reigh, 1995), channel catfish *Ictalurus punctatus* (Robinson and Rawles, 1983; Li et al., 2008), rainbow trout *Oncorhynchus mykiss* (Lee et al., 2006), Florida pompano *Trachinotus carolinus* (Riche and Williams, 2010; Cook et al., 2016) and white shrimp *Litopenaeus vannamei* (Siccardi et al., 2012) at FM replacement levels ranging from 20 to 67% without a decrease in growth, feed conversion efficiency, or digestibility coefficients.

1.4. Black Sea bass

Black sea bass *Centropristis striata* is a commercially important marine finfish that occurs on hard bottom areas, such as shipwrecks and coral reefs from Cape Cod, Massachusetts, to Cape Canaveral, Florida, and feeds upon fish, decapods and amphipods (Musick and Mercer, 1977; Fischer, 1978). Total fishery landings in the South Atlantic Ocean from 1980 to 1990 averaged approximately 2.5 million lbs. with a peak harvest of 3.6 million lbs. in 1988, while landings from 2000 to 2010 averaged 1.15 million lbs. with a peak harvest of 1.75 million lbs. (SEDAR, 2013). This is attributable to increasingly stringent catch quotas and size limits that have been enforced since fishery management plans were enacted in 2006. These strict quotas and size requirements make black sea bass a good candidate for aquaculture to help meet growing demand.

1.5. Black Sea bass aquaculture

Due to high market price and demand and increasingly restrictive fishing regulations, development of culture techniques for black sea bass from the Atlantic Coast populations has been the focus of studies from Florida to New Hampshire (Watanabe, 2011). University of North Carolina Wilmington (UNCW) research results on black sea bass aquaculture have been promising and have shown that the species can be bred reliably in captivity and reared through fingerling stages (Berlinsky et al., 2000, 2005; Watanabe et al., 2003; Carrier et al., 2011; Watanabe et al., 2015), raised from fingerling to adult stages in recirculating aquaculture system with sustainable diets (Alam et al., 2008, 2012, 2015) and under high densities (Watanabe, 2011), and that the cultured product can garner lucrative niche markets (Wilde et al., 2008). Recent interest in the commercial production of black sea bass has increased, and private grow-out operations are beginning to emerge in North Carolina, Virginia, and Maine (Watanabe, 2011).

An important attribute of black sea bass for aquaculture is their ability to accept and grow well on a variety of alternative plant and animal protein sources to FM, including solvent-extracted soybean meal (Alam et al., 2012) and poultry by-product (Dawson, 2012; Watanabe, 2011). The objectives of this study were to determine the effects of different levels of substitution of FM protein with low-gossypol CSM proteins on growth performance, feed utilization, protein digestibility, and body composition of juvenile black sea bass *Centropristis striata* under controlled laboratory conditions.

2. Material and methods

2.1. Experimental animals and system

Juvenile black sea bass were cultured from eggs supplied by photothermally conditioned captive broodstock held at the UNCW-CMS Aquaculture Facility (Wrightsville Beach, NC). Adults were induced to spawn using luteinizing hormone-releasing hormone analogue (LHRHa) implants (Watanabe et al., 2003). Eggs were hatched and