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Evaluation of genetically-improved (glandless) and genetically-modified low-gossypol cottonseed meal as alternative protein sources in the diet of juvenile southern flounder *Paralichthys lethostigma* reared in a recirculating aquaculture system

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ABSTRACT

Cottonseed meal (CSM) proteins from genetically-improved (glandless) low-gossypol seed (GI-CSM, 52.1% crude protein, CP), genetically-modified low-gossypol seed (GMO-CSM, 56.0% CP) and from an untreated regular (glanded) seed (R-CSM 49.9% CP) were evaluated to replace fish meal (FM) protein (59.5% CP) in juvenile southern flounder *Paralichthys lethostigma* diets. Eight isonitrogenous (45% CP) and isolipidic (16% crude lipid, CL) diets were formulated. A control diet (0% CSM) contained 40% fishmeal (FM) and other practical protein sources. Six diets replaced 50, 75 and 100% FM protein with GI-CSM or GMO-CSM protein. One diet replaced 100% FM protein by R-CSM protein. Fifteen fish (mean = 1.81 g) were stocked in each of twenty-four 75-L tanks (N = 3 per treatment) and were fed the treatment diets for eight weeks. Final percent weight gain was not significantly ($P > 0.05$) different in fish fed the GI- and GMO- and R-CSM protein diets (898–1405%) compared with the control diet (0% CSM) (1242%), but percent weight gain was greater ($P < 0.05$) in fish fed the 50% GI-CSM diet (1405%) versus the 100% GI-CSM diet (898%). Feed conversion ratio was excellent for all treatments (FCR = 0.70–1.00), with no treatment differences ($P < 0.05$). Protein efficiency ratios (PER) were also not significantly different among the treatments (2.26–3.24), although the lowest value was for 100% R-CSM diets. After 8 weeks of feeding, survival of fish ranged from 80 to 91%, with no treatment differences. Apparent protein digestibility of diets was significantly higher for the fish fed 75% and 100% GI-CSM and 100% GMO-CSM protein diets (83.5, 83.5 and 86.5%, respectively) compared with the control diet (79.4%). After 8 weeks, no significant ($P > 0.05$) interactive effects between CSM sources and FM replacement levels on final weight, FCR and PER were observed. Arginine levels in the diets increased as CSM was increased, consistent with the high arginine concentrations found in CSM. Liver gossypol was only detectable in fish fed the 100% R-CSM diet (37 μg/g). Replacing up to 75% FM protein by GI- or GMO-CSM protein did not affect on whole body omega-3 PUFAs, or liver gossypol. The results suggest that up to 75% of fish meal protein may be replaced by GI- or GMO-CSM protein in the diet of juvenile flounder without adverse effects on growth performance and body composition.

Statement of relevance: Cottonseed meal (CSM) is a potentially cost-effective alternative plant protein source for use in aquafeeds. The results suggest that a 75% of fishmeal protein could be replaced by genetically-improved and genetically modified (GMO) low gossypol based cottonseed flour protein in the diet of southern flounder. These ultra-low gossypol cottonseed flour proteins could be inexpensive protein sources for the commercial culture of southern flounder and other finfish species.

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1. Introduction

To reduce the amount and cost of wild-caught fish used as a source of protein in aquafeeds and the nutrient levels in effluent waste, the identification of effective alternative protein sources to FM is imperative (Trushenski et al., 2006). However, there are species-specific limits to how much FM can be replaced by alternative plant protein sources without negatively affecting fish growth, feed efficiency and body composition (Gatlin III et al., 2007).

1.1. Cottonseed meal

Cottonseed meal (CSM) is a potentially cost-effective alternative plant protein source for use in aquafeeds (Anderson et al., 2016; Cook et al., 2016). CSM is produced by a several step process that removes the hulls and separates the oil. Seed is first passed through a series of knives and shakers to separate the hull from the kernels. The kernels are flaked and cooked and then the oil is expressed either by pressing or solvent extraction with hexane. The recovered oil is the most valuable by-product of the seed and it is primarily used in cooking and food production (Lin et al., 2015). After removing the oil from the kernels, the remaining defatted kernel tissue is known as CSM. CSM is much cheaper per unit of protein than FM and other FM replacement protein sources, due to the large global production of cotton and cottonseed by-products. However, high levels of the antinutrient gossypol in regular CSM (R-CSM) limit the incorporation of this ingredient in aquafeeds. Gossypol is a terpene-based secondary metabolite that has a role in the cotton plant's defense against pests and possibly diseases (Romano and Scheffler, 2008). Gossypol is produced and deposited in “glands” in the stems, leaves and green bolls of the plant, and in the seed (Lusas and Jividen, 1987). Anti-nutritional and anti-fertility effects have been reported in warm-blooded animals and fish fed cottonseed products containing high levels of gossypol (Blom et al., 2001; Henry et al., 2001; Romano and Scheffler, 2008). Furthermore, gossypol is known to bind with lysine, rendering this essential amino acid less available in fish feed (Wilson et al., 1981).

1.2. Low-gossypol CSM as a FM replacement

The utilization of CSM as an ingredient in the feeds of animals and fish has been improved by reducing or eliminating gossypol. In late 1950's McMichael discovered the existence of mutant cotton plants without the lysigenous glands that contain gossypol (McMichael, 1959; Siccardi et al., 2012), rendering the seed largely gossypol-free and less toxic to non-ruminant animals. Low-gossypol seed has also obtained by seed-specific, RNAi-mediated silencing of a delta-cadinene synthase gene (Sunilkumar et al., 2006). This genetically modified (GMO) genotype has ultra-low levels of gossypol in the seed but, unlike glandless plants, it maintains normal levels of gossypol in the foliage, roots and floral tissues to protect the plant against pests and diseases (Rathore et al., 2012; Palle et al., 2013).

Regular high-gossypol CSM (R-CSM) protein has been used to replace FM protein at maximum levels of 35% in grass carp *Ctenopharyngodon idellus* (Zheng et al., 2012) and 30% in parrotfish *Oplegnathus fasciatus* (Lim and Lee, 2009). In comparison, low-gossypol CSM protein has been used to replace FM protein at levels ranging from 20 to 100% for hybrid striped bass *Morone saxatilis* ♀ × *Morone chrysops* ♂ (Sullivan and Reigh, 1995), channel catfish *Ictalurus punctatus* (Robinson and Rawles, 1983; Li et al., 2008; Dorsa et al., 1982), rainbow trout *Oncorhynchus mykiss* (Lee et al., 2006), Florida pompano *Trachinotus carolinus* (Riche and Williams, 2010; Cook et al., 2016), white shrimp *Litopenaeus vannamei* (Siccardi et al., 2012; Richardson et al., 2016) and black sea bass *Centropristis striata* (Anderson et al., 2016).

1.3. Southern flounder aquaculture

The southern flounder *Paralichthys lethostigma* is a flatfish in the family Paralichthyidae. It can be found in coastal waters from Albemarle Sound, North Carolina, through the South Atlantic states with the exception of South Florida. Southern flounder landings have declined, leading to more stringent fishery regulations and interest in culturing native flatfishes for stock enhancement or food fish production. The development of intensive culture methods for southern flounder in the southeastern United States is of great interest because of its euryhaline character and its status as a highly desirable food and recreational species and potential for commercial culture. Hatchery methodology for spawning and larval rearing is well investigated (Daniels and Watanabe, 2003; Watanabe et al., 2006). Dietary protein and lipid requirements of juvenile southern flounder have been established (Alam et al., 2009, 2011), and a number of studies have determined the substitution limits of alternative plant (i.e., soybean meal) (Alam et al., 201) and animal (i.e., poultry by-product meal) (Dawson, 2012) for FM protein in the diet of juvenile southern flounder. To date, no studies have been conducted to determine the efficacy of low-gossypol CSM protein as a replacement for FM protein in the diet of southern flounder. The objectives of this study were to determine, under controlled laboratory conditions, the effects of different levels of substitution of FM protein with low-gossypol CSM protein from genetically-improved (glandless) and genetically-modified (GMO) plants on growth performance, feed utilization, dietary protein digestibility, and body composition of southern flounder.

2. Materials and methods

2.1. Experimental fish

Juvenile southern flounder (approximately 90 days post-hatching) were cultured from eggs produced by photothermally conditioned captive broodstock held at the University of North Carolina Wilmington Center for Marine Science (UNCW-CMS) Aquaculture Facility (Wrightsville Beach, NC). Broodstock was induced to spawn using luteinizing hormone-releasing hormone analogue (LHRHa) implants (Watanabe et al., 2001; Watanabe et al., 2006). Eggs were hatched and larvae reared through juvenile stages using methods established at UNCW (Watanabe et al., 2001). Juveniles were reared in 500-L tanks on a commercial diet (Skretting Vancouver, British Columbia, 50% crude protein and 15% lipid) until they were stocked in the experimental tanks.

2.2. Experimental system

The experimental system consisted of twenty-four 75-L rectangular tanks located in an indoor climate–controlled laboratory supported by a recirculating aquaculture system (RAS). The RAS included a Kaldness moving bed (Anox Kaldness, Inc., Providence, Rhode Island) biofilter, a bead filter (Aquaculture Systems Technologies, LLC, New Orleans, Louisiana) to remove solids, a protein skimmer to remove small particulate and dissolved materials, and an ultraviolet sterilizer for disinfection. Each tank was supplied with diffused air supplemented with pure oxygen, and the water temperature was controlled by a heat pump. Total ammonia and nitrate in water were measured weekly using a portable data-logging spectrometer (HACH DR/2010 SPEC). Water temperature, dissolved oxygen, salinity, and pH were measured using a multi-parameter probe (YSI 556 MPS, GEO Scientific, Ltd., Vancouver, British Columbia).

2.3. Cottonseed meals

Three CSMs were prepared at Cotton Inc. (Cary, NC, USA) (Anderson et al., 2016). A high-gossypol CSM was prepared from