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Evaluation of various cottonseed products on the growth and digestibility performance in Florida pompano *Trachinotus carolinus*

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A B S T R A C T

Two 10-week growth trials and digestibility trials were conducted to assess the efficacy of various cottonseed products in practical diets for the Florida pompano *Trachinotus carolinus*. In trial 1, a basal diet containing 150 g/kg of fishmeal (FM150) was modified by the iso-nitrogenous replacement of fishmeal (FM) with cold pressed cottonseed flour (CSF) resulting in diets containing 100 g/kg (FM100), 50 g/kg (FM50) and 0 g/kg (FM0) of fishmeal. To determine if lysine was limiting in the FM0 diet, two additional diets were developed using a high lysine corn protein concentrate (FM0-LCPC) or a crystalline source of lysine (FM0-CL). In trial 2, the basal diet was formulated to contain 457.0 g/kg of soybean meal (SBM) and 150 g/kg of poultry by-product meal (PBM), the other three diets were made by replacing SBM on an iso-nitrogenous and iso-lipidic basis with varying cottonseed flours (CSF) which included cottonseed flour (CSF), gossypol-extracted cottonseed flour (ECSF), and glandless cottonseed flour (GCSF). In trial 1, results showed that the use of up to 196 g/kg of CSF with a lysine supplement does not inhibit growth performance of the fish. In trial 2, no significant differences were observed in growth performance of the fish maintained on the various diets. Although gossypol was detected in the livers of fish offered diets with gossypol, no differences were observed in the cells of the liver samples or organization of the liver cells amongst any of the treatments. After each growth trial, apparent digestibility coefficients for dry matter (ADMD), energy (AED), and protein (APD) were determined for primary ingredients. In the first digestibility trial, no significant differences were observed with regard to digestibility coefficients for FM, CSF, and corn protein concentrate (CPC). In the second digestibility trial, no significant differences were observed on ADMD and AED for the ingredients GCSF, CSF, and ECSF. However, the APD values for ECSF were found to be significantly lower than those values of GCSF and CSF. Results of this study provide support that cottonseed products are acceptable for use in practical diets for Florida pompano.

Statement of relevance: In this study, we assessed the efficacy of various cottonseed products in practical diets for the Florida pompano. These findings will be useful because the feed producers need the growth and digestibility data of the cottonseed products as an alternative ingredient to use in the diet formulation. The conclusions in this manuscript provide valuable information which researchers and feed producers need to use the cottonseed products in diets of this species.

1. Introduction

Florida pompano, *Trachinotus carolinus*, is an important species with excellent potential for mariculture in the United States (Gonzalez-Felix et al., 2010; Rossi and Davis, 2012). This is due to desirable characteristics including high market prices, tolerance of a wide range of salinities, captive breeding, and acceptance of pelleted feeds (Pfeiffer and Riche, 2011; Weirich et al., 2009). To make this a profitable and enticing enterprise, a cost-efficient feed for this species must be developed.

Cost savings can be found by using plant based protein sources such as those obtained from soybeans, corn, and cottonseed. Cottonseed meal (CSM) has been utilized in ruminant animal feeds for years (Mbahinzireki et al., 2001). Many studies have assessed CSM as a protein source in numerous fish species (Cai et al., 2011; Dabrowski et al., 2000; Dorsa et al., 1982; El-Sayed, 1990; Jackson et al., 1982; Lim and Lee, 2008, 2009; Mbahinzireki et al., 2001; Rinchard et al., 2003; Robinson and Brent, 1989; Robinson and Li, 1994, 2008; Yue and Zhou, 2008). The disadvantages of CSM include the presence of gossypol and the low level of lysine which restrains its use in monogastric animals, including fish (Li and Robinson, 2006; NRC, 2011). The toxicity of gossypol has been reported in several species of fish (Barros et al., 2000; Barros et al., 2002; Dorsa et al., 1982; Herman, 1970; Mbahinzireki et al., 2001). Dorsa et al. (1982) have noted that free gossypol was primarily accumulated in the liver and kidney tissue in channel catfish *Ictalurus punctatus*. Gossypol can also be found bound to peptides, particularly on lysine residues. Bound gossypol is not quickly absorbed and may

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inhibit lysine absorption contributing to a potential lysine deficiency (NRC, 2011). With lysine supplementation, up to about 50% CSM can partially replace fishmeal (FM) or soybean meal (SBM) without negative impacts on the growth performance of tilapia *Oreochromis* sp. (Mbahinzireki et al., 2001) and channel catfish (Robinson and Li, 2008).

Different strategies have been developed to reduce gossypol content of CSMs. This includes gene manipulation of cultivated cotton strains as well as extraction of gossypol during processing. When using a solvent-extracted cotton seed meal, up to 20% inclusion in the feed did not significantly influence weigh gains, FCR, and survival of channel catfish when compared to fish maintained on feeds without the meal (Robinson and Brent, 1989). Additionally, glandless CSM had limited effects on growth and can replace as much as 50% of the typical SBM. When these feeds were supplemented with lysine, 100% of the SBM could be replaced without adverse effects on performance (Robinson, 1991).

Not only does the amount of gossypol included in the diets affect the growth and feasibility of including CSM in practical diets, the ability for these species to digest and utilize the protein and energy in the ingredients does as well. Anti-nutrients in ingredients such as gossypol in CSM limit the digestibility. The digestibility of certain CSM in tilapia decreases as the gossypol levels increase (Mbahinzireki et al., 2001). There are other factors including the level of inclusion in the diets, the processing methods used to produce the diets such as temperature, and the culture conditions of the fish (NRC, 2011). In species such as red drum *Sciaenops ocellatus* and rainbow trout *Oncorhynchus mykiss*, there is a positive correlation between the protein digestibility and the protein content for wide varieties of ingredients, including the animal or plant protein sources (McGoogan and Reigh, 1996; Serrano et al., 1992; Smith et al., 1995).

A number of studies have reported the apparent digestibility coefficient values for CSM in fish. For example, the apparent protein digestibility values were: 81.6–87.9% in rainbow trout *O. mykiss* (Cheng and Hardy, 2002), 76.35%–84.5% in red drum *S. ocellatus* (Gaylord and Gatlin, 1996; McGoogan and Reigh, 1996), 78.6–81.8% in Nile tilapia *Oreochromis niloticus* (Guimarães et al., 2008), 78.0–81.0% in juvenile and grower rockfish *Sebastes schlegeli* (Lee, 2002), 83.7–87.6% in Siberian sturgeon *Acipenser baerii* (Liu et al., 2009), 83.8% in hybrid striped bass *Morone saxatilis* ♀ × *Morone chrysops* ♂ (Sullivan and Reigh, 1995), 75.4%–80.0% in gilthead sea bream *Sparus auratus* L. (Nengas et al., 1995). The previously mentioned studies indicate that the CSM has a suitable range of apparent protein digestibility values in numerous fish species.

Presently there is limited information on cotton seed processed under more modern conditions as well as the newer varieties such as low gossypol cotton seed. In general the use of specialize seed preparation, mechanical extraction to remove oils and fine grinding, a higher protein and lipid cottonseed flour (CSF) product can be produced. In this study, two trials were conducted to 1) evaluate the response of Florida pompano to graded levels of cold pressed CSF, 2) determine if CSF, that differs in the methods in which gossypol is extracted, or not extracted, influences performance of the fish when included in diets, and 3) to determine digestibility coefficients for primary protein sources used in the growth trials.

2. Materials and methods

2.1. Growth trials — diets, culture systems, and feeding

The mechanically extracted CSF used in this research were produced by Cotton Incorporated (Cary, North Carolina, USA) using a proprietary steam de-hulling process. The fuzzy cottonseed hulls were separated from the kernels using an oscillating screener (Rotex, Runcorn, Halton, UK) fitted with a #6 mesh screen. The kernels were processed through a mechanical press (Ag Oil Press, Eau Claire, Wisconsin, USA) for oil extraction. The cottonseed cake produced by the press was ground in a rotary plate grinder (La Milpa, Tiffin Ohio, USA) and screened on an oscillating screener (Rotex, Runcorn, Halton, UK) fitted with a #60 mesh screen. The meal that passed through the screen was used to produce the experimental diets. For trial 1, the meal contained 49.2% protein, 12.7% fat and 3.05% fiber. In trial 2, the protein and lipid contents of the flours were 49.2% and 16.2% for CSF; 55.1% and 7.0% for Extracted CSF (ECSF) and 60.76% and 9.25% for the glandless CSF (GCSF), respectively.

In trial 1, the basal diet (400 g/kg protein, 80 g/kg lipid) was formulated using SBM, poultry by-product meal (PBM), and FM as the primary protein sources. The FM in the basal diet was replaced on an iso-nitrogenous basis with CSF and to produce diets with 100 g/kg, 50 g/kg and 0 g/kg FM, while maintaining the diets and iso-lipidic with fish oil (Table 1). Two additional FM-free diets with lysine supplemented by a high lysine corn protein concentrate (LCPC) or a crystalline source of lysine were evaluated. Proximate analysis and amino acid profiles for these diets were presented in Tables 2 and 3 (analyzed at Midwest Laboratories, Inc., Omaha, Nebraska, USA).

In trial 2, the basal diet (400 g/kg protein, 100 g/kg lipid) was formulated to contain 457.0 g/kg of SBM and 150 g/kg of poultry by-product meal (PBM). Test diets were made by replacing SBM on an iso-nitrogenous basis with CSF, ECSF, or GCSF. (Table 1). Dietary lipids were maintained by adjusting cotton seed oil levels while keeping fish oil levels constant. Lysine and taurine were supplemented to maintain similar dietary levels of these amino acids across diets. Diet proximate and amino acid compositions are presented in Tables 2 and 3, respectively (analyzed at University of Missouri, Agricultural Experiment Station Chemical Laboratories, Columbia, Missouri, USA). Gossypol levels were also analyzed in test ingredients and test diets (Table 4).

Experimental diets in both growth trials were produced in Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, Alabama, USA) using standard protocol. All dry ingredients and oil were weighed, mixed for appropriately 15 min, and then boiling water was added to obtain a mash appropriate for extrusion. The diets were formed using a food mixer (Hobart Corporation, Troy, Ohio, USA) with 3 mm of die, and the pellets were dried to less than 10% moisture. Subsequently, the diets were crumbled, packed in the plastic bags and stored in the freezer (−20 °C) until needed.

Juvenile pompano (~1 g mean weight) were obtained from a commercial hatchery Trout Lodge Marine Farms/Proaquatix (Vero Beach, Florida, USA). Fish were loaded into a hauling tank equipped with a supplemental oxygen supply system and transported to Claude Peteet Mariculture Center (CPMC) in Gulf Shores, Alabama, USA. The fish were acclimated by slowly replacing the hauling water (25.5 °C temperature, 24.7 mg/L dissolved oxygen, 28.7 g/L salinity and 7.2 pH) with local salt water (25.7 °C temperature, 5.6 mg/L dissolved oxygen, 30.6 g/L salinity, and 7.8 pH), before being transferred into a 5-m^3 fiberglass tank with an independent biological filter, air lift pumps and supplemental aeration provided by a 2.0-hp. regenerative air blower (Pentair Aquatic Eco-Systems, Inc., Apopka, Florida, USA) and air diffusers. Fish remained in this tank for approximately 30 days until adequate size for the growth trial was achieved. During this period, fish were fed to apparent satiation with a 1.5 mm commercial feed with 500 g/kg of crude protein and 150 g/kg of crude fat (FF Starter, Zeigler Bros. Inc., Gardners, Pennsylvania, USA).

Trial 1 used a semi-closed recirculating system consisting of a central reservoir (800-L) with 24 circular polyethylene tanks (0.85 m height × 1.22 m upper diameter, 1.04 m lower diameter), a 0.33-hp. sump pump and a bead filter, and supplemental aeration using a regenerative blower and air diffusers. Four replicate groups of 20 fish per tank (initial mean weight 12.5 g) were offered diets over 10 weeks. In trial 2, the semi-closed recirculating system was consisted of 12 circular fiberglass culture tanks (1.0 m^3), a biological filter, a reservoir tank (1.0 m^3), a water pump, and supplemental aeration provided by a regenerative