Analysis of Florida Pompano *Trachinotus carolinus* performance when fed practical diets of increasing levels of cotton seed flour processed by varying methods.

by

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A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 3, 2014

Keywords: Trachinotus carolinus, Florida pompano,
Fishmeal Replacement, Cottonseed Meal, Gossypol

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Abstract

Florida pompano is a propitious species for marine aquaculture, and high demand has made the development of practical feed formulations essential. The cost of feed can be reduced by alternative plant protein sources such as cottonseed meal or flour. Albeit this is a cost effective protein source, the presence of gossypol in the meal can cause adverse health affects and limits the level of inclusion. Selective breeding and/or processing methods designed to reduce the total gossypol content could be utilized to improve the quality of the product and allow high inclusion levels. In order to assess the effects of cottonseed meal and gossypol content on Florida pompano two growth studies were performed. The first trial compared the effects of increasing inclusion of traditional cottonseed meal and need for lysine supplementation, while the second compared the effects of three cottonseed products and their resulting gossypol levels. The first growth trial showed that use of up to 19.6% cottonseed flour with a lysine supplement does not inhibit growth performance of the fish and is an acceptable protein source. The second growth trial showed that diets containing genetically low gossypol cottonseed flour resulted in significantly higher final mean weights and percent weight gains of than fish diets formulated using traditional cottonseed flour or gossypol-extracted flour. All diets are comparable to the soybean meal reference diet with respect to mean final weight and percent weight gain of fish, FCR and survival. Following each trial, The digestibility of the primary protein sources were evaluated to provide basic data on digestibility coefficients. In the first trial, fishmeal, cottonseed meal, and corn gluten meal showed no significant difference as primary ingredients in regard to mean apparent dry matter digestibility (ADMD), mean apparent protein digestibility (APD), or mean apparent energy digestibility (AED). No significant difference between the mean apparent digestibility coefficients (ADC) values for cottonseed meal, extracted cottonseed meal, and glandless cottonseed meal diets were found with respect to ADMD for ingredients (ADMDI) and AEDI. However; ethanol (EtOH) extracted cottonseed flour was found to be significantly lower in apparent protein digestibility of the ingredient APD. These results provide support that cottonseed flour, especially that of a genetically low gossypol variety, is acceptable to use in feed for Florida Pompano. Diets including any of these primary ingredients show comparable performance and digestibility to fishmeal-based diets.

Acknowledgments

I would first like to extend my gratitude and love to my family for their constant support. My mom especially has helped me beyond belief with not only school but in life. I would also like to thank my major professor Dr. D. Allen Davis, his support and guidance have been indispensable throughout this process. I would like to thank my committee members Dr. Daniels and Dr. Chiba for taking time to help guide me. I wish to express my gratitude to my lab mates, Bochao Hu, Denghang Yu, Guillaume Salze, Guilherme Sassi, Katia Rodrigues, Samuel Addo, Sirirat Chatvijtkul, Xiaoyun Fang, Yan-gen Zhou, and Xuan Qiu, who helped tremendously and made the experience a pleasure. I would also like to give a big thank you to everyone at the Claude Peteet Mariculture Center, especially Melanie Rhodes, and the School of Fisheries, Aquaculture, and Aquatic Sciences, and the professors at Auburn University for giving me the opportunity to study and do research, gaining invaluable experience and knowledge. I would like to thank all of my friends, new and old, for their support and friendship over the years allowing me to work toward my dreams and stay floating through the craziness of life. Last, but not least, I would like to extend my deepest thanks to the employees and interns at Mote Marine Laboratories who I am grateful to be able to call my friends, without whom I would never have gotten to this point. I am forever thankful.

Table of Contents

List of Tables

Introduction

The Florida Pompano, *Trachinotus carolinus*, is a member of the jack family. The Florida pompano is a propitious species for marine aquaculture; as such, there is considerable interest in advancing the development of practical feed formulations. These fish are fast growing with high tolerance for a range of salinities (Main *et al* 2007). They have a considerably high market value and are sought after by commercial and sport fisherman due to their flaky texture and mild flavor, which makes aquaculture a potentially profitable enterprise (Main *et al* 2007). To make this a profitable and enticing enterprise, the costs of feed and fish growout must become cost efficient.

As the cost of marine feed ingredients continues to increase, it is a top priority to replace expensive meals with less costly ingredients, such as plant-based proteins like soybean meal. However, further cost savings can be found by using other plant proteins, such as cottonseed meals. There have been a number of studies that have successfully implemented cottonseed meal in replacement of fishmeal at certain levels in the feed for catfish, carp and tilapia (Mbahinzireki *et al.*, 2001, Robinson and Li, 2008, Cai *et al.*, 2010).

As summarized by the NRC (2011), of main concern with utilizing cottonseed meal is the risk of gossypol toxicity. Cottonseed meal has been utilized in ruminant animal feeds for years; however, free gossypol can be toxic to single stomached animals, including fish. Gossypol is found in discrete pigment glands located in various parts of the plant. On a cellular level, gossypol binds to microsomal membranes and phospholipids inhibiting DNA synthesis and the depletion of available molecules such as lysine and iron, needed in normal function (NRC 2011). Specifically, when gossypol binds to lysine, the total lysine within the ingredient is no longer accessible for utilization, which typically results in lysine deficiency (Robinson and Li, 2008).

Within aquaculture, the more prevalent effects of gossypol include reduced feed consumption, a reduction in growth,   and reduced reproductive success, amongst others; however, death is rare within fish species (NRC 2011). The intensity of the toxicity varies based on many conditions, for example, species, environmental conditions, age, and diet formulation (NRC, 2011; Mbahinzireki *et al.*, 2001). Due to the unknown levels of toxicity of gossypol among different species, cottonseed meal and flour have been the center of many experiments attempting to obtain levels that are acceptable to specific fish in order to formulate diets including cottonseed meal. This also concerns consumers who question the effect on humans of the fish fed cottonseed meal; however, when gossypol levels in trout fillets were examined, the gossypol levels were below the detectable limits of the test illustrating little chance of adverse effects from consumption of fish fed cottonseed meal (Cheng and Hardy, 2002).

During studies that examined the effects of cottonseed meal, the liver was one of the most severely affected tissues within the body. Recent studies have noted that the liver exhibits the most severe effects and highest concentrations of gossypol (Dorsa *et al.* 1982). Two main symptoms are exhibited when faced with an increase in gossypol. One of these is diffuse coagulative necrosis typically diagnosed by shrunken cells, cell-to-cell contact reduction, intensely eosinophilic cytoplasm, and pyknotic and haryorrhetic nuclei. The second main histological symptom is haptocellular vacuolization, a loss of cell structure (Evans *et al.* 2010, Francis *et al*. 2001). As shown by Evans (2010), symptoms were moderately severe in livers of channel catfish fingerlings (initial average weight of 6.22 g), by the end of a twelve-week trial consisting of gossypol-rich feeds. In fact, concentrations of as low as 0.1% gossypol in feed for rainbow trout fry resulted in affected liver tissues (Francis *et al*. 2001). In these previous studies, seemingly healthy liver tissue showed scattered areas of affected tissue (Evans *et al*. 2010). One concerning finding in Francis *et al.* (2001) was that the gossypol that bound and damaged the kidneys and livers of the fingerling carp remained bound even after a 10-week feeding regimen with a gossypol free diet. If cottonseed meal is to be successfully utilized, the effects of gossypol on the tissues of fish and the amount of cottonseed that can be consumed per species without buildup or damage to fish kidneys or livers need to be researched.

Most previous studies have determined that cottonseed meal cannot be used as a protein replacement at high inclusion rates without reducing growth, feed conversion ratios (FCR), and survival. For vundu catfish (*Heterobranchus longifilis*) only a 30% inclusion of the total diet could be reached with no additional amino acid supplementation and, without negatively impacting the performance of the fish (Toko, *et al* 2008). For channel catfish (*Ictalurus punctatus*) and tilapia, no more than 50% of the total protein can be included even with supplemental lysine (Mbahinzireki *et al.*, 2001, Robinson and Li, 2008). For silver crucian carp (*Carassius carassius*), the inclusion level within the feed can be as high as 40% before negative effects are observed (Cai *et al.,* 2010). Seeing that cottonseed meal could only be included in small percentages, possibly due to gossypol or other anti-nutritional factors, could be attributed to the processing or genetics of the cottonseed, different methods have been developed to reduce plant production of gossypol, such as genetically manipulating the strains of cotton, or extracting gossypol. However, some methods in some species did not improve the inclusion rates. In an early experimental study, inclusion of up to 20% solvent extracted cottonseed meal (32% protein) in the feed did not significantly influence the mean weight gains, feed conversion ratio (FCR), or survival of channel catfish when compared to those of fish maintained on feeds without the meal (Robinson and Brent 1989). However, subsequent studies showed that fish fed diets including glandless cottonseed meal showed limited effects on growth with as much as 50%

of the typical soybean meal being replaced by glandless cottonseed meal. When these feeds were supplemented with lysine, 100% of the soybean meal could be replaced without adverse effects on performance (Robinson 1991). Depending on the species of fish, the effects of gossypol and the level of processed cottonseed meals that can be used in feeds will be different. The growth and health of alternate species are guides to formulation of experimental diets for pompano.

Not only does the amount of gossypol included in the diets affect the growth of the fish and the feasibility of including cottonseed meal in practical diets, the ability of the species discussed above to digest and utilize the protein and energy in the ingredients does as well. Anti-nutrients in ingredients, such as gossypol in cottonseed meal, limit the digestibility. The digestibility of certain cottonseed meals in tilapia decreases as the gossypol levels increase (Mbahinzireki *et al.*, 2001). There are other factors including the size of the meal used in the diets, the processing methods while producing the diets such as heat, and the culture conditions of the fish (NRC 2011). In species such as red drum (*Sciaenops ocellatus*) and sea trout (*Cynoscion nebulosus*), there is a positive correlation between the protein digestibility and the protein content in the ingredient (Serrano, 1992; Smith, 1995; Mcgoogan and Reigh, 1996; Allen *et al*., 2000).

A number of studies have reported that fish fed cottonseed meal diets have apparent protein digestibility values similar to those fed fishmeal. Rainbow trout (*Oncorhynchus mykiss*), hybrid striped bass (*Morone chrysops x Morone saxatilis*), silver perch (*Bidyanus bidyanus*), channel catfish, Nile tilapia (*Oreochromis niloticus*), and siberian sturgeon (*Acipenser baerii*) all had digestibility values ranging from 82%-88%, while red drum, gilthead sea bream (*Sparus aurata*), and rockfish had values ranging from 75-85%. The digestibility of menhaden fishmeal for these same species ranges from 77-96%, making cottonseed meal a good alternative to fishmeal (Cheng and Hardy, 2002; Sullivan and Reigh, 1995; Allen *et al*, 2000; Mcgoogan and Reigh, 1996; Wilson and Poe, 1985; Guimaraes *et al*, 2008; Liu *et al*, 2009; Gaylord and Gatlin, 1996; Nengas *et al*, 1995; Lee, 2002). However, in other studies on red drum, dry matter and energy digestibility fell well below that of fishmeal, calling into question whether this is a suitable substitute for some species (McGoogan, and Reigh, 1996).

Based on these findings, two growth trials were conducted, one testing the inclusion levels of cottonseed meal replacing 0%, 25%, 75%, and 100% of fishmeal in the diets for Florida pompano with and without lysine supplementation, and one to test cottonseed flours produced by varying processes. Typical cottonseed meal (CSM) contains at least 40% protein (Dorsa, *et al.*); however; as described by the provider of the products, cottonseed meal, which refers to products having between 40 and 50% protein, was used in the first growth and digestibility trials, while in the second set of trials, cottonseed flours, which refer to products containing a protein content of 50 to 65%, were used. These trials will provide information needed for the industry to begin formulating a practical diet for pompano that includes a reasonable percentage of cottonseed meal and determine which type of processed cottonseed flour provides the best growth for this species while reducing gossypol levels. Not only will this information be relevant to Florida pompano, but the improvement of cottonseed flours and the reduction of gossypol may have many dietary implications across aquaculture species and into other agriculture-based dietary needs. Because there is a wide variance in values of the digestibility of cottonseed flour per species, digestibility of protein and energy should be investigated for the Florida pompano. In these studies, digestibility coefficients for several cottonseed flour diets and select ingredients were determined.

Objectives

1: Evaluate the response of Florida pompano to increasing levels of cottonseed flour

2: Determine if varying cottonseed meals or flours, that differ in the methods in which gossypol was extracted or not extracted influence performance of the fish when included in diets.

3: Determine digestibility coefficients for primary protein sources used in the trails.

Materials and Methods

Growth Trials

Diet Preparation

Experimental diets were produced at Auburn University, School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn, AL, USA. Each diet was prepared by mixing pre-ground dry ingredients and menhaden fish oil in a food mixer (Hobart A200FT, Troy, OH, USA) for 15 minutes. Boiling water was then blended into the mixture to promote appropriate consistency for pelleting. Subsequently, the moist mash from each diet was passed through a 3.0-mm die in the grinder (Hobart A200FT, Troy, OH, USA). Wet diets were then placed into a forced air drying oven (<45°C) until they attained a moisture content of less than 10%. Dry diets were then stored at -20°C, and, prior to use, each diet was crumbled and sieved to an appropriate size. All experimental diets were formulated to be isonitrogenous, isoenergetic, and isolipidic, while trial 1 diets contained 40% crude protein crude protein and 8.0% lipid and trial 2 diets contained 40% crude protein and 10.0% lipid.

The basal diet for the first trial was formulated to contain 15% fishmeal, which was replaced on an iso-nitrogenous basis with a cottonseed product to produce diets with 10%, 5% and 0% fishmeal (Table 1). A 10-week growth trial was conducted with the four diets with

Table 1. Composition (g/100g as is) of practical test diets used to culture Florida pompano in two growth trials. Diets were formulated to be iso-nitrogenous and iso-lipidic and to contain 40% protein, 8% lipid for growth trial 1 and 40% protein, 10% lipid for trial 2.

	Trial 1						Trial 2			
Ingredients	FM15	FM10	FM5	FM0	FM0-LCPC	FM0-CL	BD	UCSF	ECSF	GCSF
Cottonseed meal Cotton Inc.[1]	-	6.50	13.05	19.60	19.65	19.60				
Menhaden fishmeal[2]	15.00	10.00	5.00	-	-	-				
Cottonseed Flour prior to extraction[1]							0.00	30.30	0.00	0.00
Cottonseed Flour gossypol extracted[1]							0.00	0.00	27.00	0.00
Cottonseed Flour glandless[1]							0.00	0.00	0.00	24.50
Poultry by product meal[3]	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soybean meal solvent extracted[4]	20.60	20.60	20.60	20.60	20.60	20.60	45.70	12.00	12.00	12.00
Cottonseed Oil[1]							4.23	0.00	3.02	2.64
Corn starch[5]	15.32	13.63	11.90	10.12	10.00	10.12	0.22	7.54	7.82	10.59
Whole wheat[5]	20.00	20.00	20.00	20.00	20.00	20.00	21.00	21.00	21.00	21.00
Menhaden fish oil[2]	2.82	2.56	2.29	2.02	1.99	2.02	1.60	1.60	1.60	1.60
Corn protein concentrate[6]	8.00	8.00	8.00	8.00	3.90	8.00	8.00	8.00	8.00	8.00
High lysine corn protein concentrate[6]	-	-	-	-	4.20	-				
Lysine[7]	-	-	-	-	-	0.17	0.00	0.31	0.31	0.42
ASA Trace Mineral[8]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
ASA Vitamin premix[9]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Choline cloride[5]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg[0]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic[11]	1.00	1.45	1.90	2.40	2.40	2.40	2.10	2.10	2.10	2.10
Lecethin [5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Glutamic acid[7]	0.31	0.28	0.23	0.17	0.18	0.00				
Taurine[7]	0.40	0.43	0.45	0.48	0.48	0.48	0.60	0.60	0.60	0.60
DL-Methionine[7]				0.03	0.06	0.05				
Total Lysine (%)	2.11	2.01	1.90	1.79	1.91	2.08	2.14	2.09	2.09	2.19

FM15= 15% Fishmeal diet, FM10= 10% Fishmeal diet, FM5= 5% Fishmeal diet, FM0= 0% Fishmeal diet, LCPC= Lysine Corn Protein Concentrate, CL= Crystalline Lysine, BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour.

[1] Cotton Incorporated, Cary NC, USA.

[2] Omega Protein Inc., Huston TX, USA

[3] Griffin Industries, Inc., Mobile, Al, USA.

[4] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Al, USA.

[5] MP Biomedicals Inc., Solon, Oh, USA

[6] Empyreal® 75, & LYSTO, respectively Cargill Corn Milling, Cargill, Inc., Blair, NE, USA

[7] Aldrich-Sigma, St. Louis, MO, USA,
[8] Trace mineral: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose
[9] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B12, Inositol, Vitamin A acetate, Vitamin D3, Vitamin E, cellulose
[10] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[11] J. T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA

increasing inclusion of cottonseed meal as a substitution to fishmeal, and two fishmeal-free diets with lysine supplemented by a high lysine corn protein concentrate or a crystalline source of lysine. Proximate analysis and amino acid profiles were obtained for these diets to confirm their formulation (Table 2 and 3).

The basal diet for the second growth trial was formed containing 45.7% soybean meal and 15% poultry by-product, which allowed for 0% fishmeal but not eliminating animal meal completely. The subsequent diets were made by replacing soybean meal on an iso-nitrogenous and, iso-lipidic, basis with varying cottonseed flours. Test ingredients included unextractred cottonseed flour (UCSF), cottonseed flour after the gossypol was extracted (ECSF), and GCSF which contained cottonseed flour made from glandless cotton (Table 1). The cottonseed flours were produced by Cotton Inc. using a proprietary steam dehulling 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, WI, USA) for oil extraction. The cottonseed cake produced by the press was ground in a rotary plate grinder (La Milpa, Tiffin OH, 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. Lysine and taurine were supplemented to the diets to maintain similar calculated levels of these amino acids. Proximate analysis and amino acid profiles were obtained for these diets to confirm the formulations (Table 2 and 3). Gossypol levels were also monitored for each of the included cottonseed meals or flours for correlation with results (Table 4).

Table 2. Proximate analysis of practical diets growth trial 1[1] and growth trial 2[2,3] formulated at Auburn University on an "as is" basis.

Components	Trial 1						Trial 2			
	FM15	FM10	FM5	FM0	FM-LCPC	FM-CL	BD	UCSF	ECSF	GCSF
Crude Protein (%)	38.1	38.7	39.8	38.6	40.4	43.9	42.3	41.5	41.7	42.3
Moisture (%)	7.5	9.4	7.3	7.3	7.9	7.9	5.8	5.0	4.8	4.8
Crude Fat (%)	7.9	8.4	8.6	8.9	8.9	9.1	11.1	10.9	11.3	10.7
Ash (%)	6.9	6.7	6.5	6.5	6.9	6.8	6.6	6.9	9.1	6.3
Crude Fiber (%)							2.3	2.0	2.1	1.8
Gossypol (%)							BMT	0.200	0.008	0.006

[1] Analyzed at Midwest Laboratories, Inc. (Omaha, NE, USA)

[2] Analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA)

[3] Gossypol analyzed at USDA labs (New Orleans, LA, USA)

FM15= 15% Fishmeal diet, FM10= 10% Fishmeal diet, FM5= 5% Fishmeal diet, FM0= 0% Fishmeal diet, LCPC= Lysine Corn Protein Concentrate, CL= Crystalline Lysine, BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour, BMT= Below Measurable Threshold.

Table 3. Amino acid analysis of practical diets from growth trial 1[1] and growth trial 2[2] formulated at Auburn University[1]

	Trial 1						Trial 2			
Component	FM15	FM10	FM5	FM0	FM0-LCPC	FM0-CL	BD	UCSF	ECSF	GCSF
Alanine (%)	2.46	2.27	2.13	2.16	1.89	2.00	2.21	2.07	2.21	2.09
Arginine (%)	2.31	2.56	2.65	2.60	2.75	3.01	2.54	3.00	2.95	3.21
Aspartic acid (%)	3.12	3.18	3.51	2.98	3.24	3.15	3.79	3.36	3.37	3.47
Cystine (%)	0.52	0.52	0.60	0.63	0.63	0.61	0.58	0.61	0.62	0.62
Glutamic acid (%)	6.72	7.12	7.22	7.23	7.04	7.13	7.34	7.32	7.62	7.53
Glycine (%)	2.11	1.82	1.76	1.67	1.61	1.75	1.89	1.81	1.85	1.89
Histidine (%)	0.99	1.05	0.95	1.02	1.00	1.31	0.95	0.96	0.97	0.98
Hydroxylysine (%)							0.09	0.09	0.05	0.08
Hydroxyproline (%)							0.24	0.52	0.72	0.69
Isoleucine (%)	1.60	1.71	1.47	1.61	1.26	1.58	1.78	1.57	1.59	1.59
Leucine (%)	3.41	3.26	3.18	3.22	3.13	3.33	3.82	3.42	3.66	3.44
Total lysine (%)	2.11	2.01	1.90	1.79	1.91	2.08	2.14	2.09	2.09	2.19
Methionine (%)	0.83	0.69	0.77	0.74	0.72	0.77	0.74	0.76	0.77	0.75
Phenylalanine (%)	1.71	1.90	1.95	2.04	2.10	2.22	2.08	2.06	2.13	2.12
Proline (%)	2.59	2.54	2.48	2.38	2.38	2.44	2.57	2.29	2.46	2.30
Ornithine (%)							0.04	0.03	0.03	0.03
Serine (%)	1.85	2.07	1.98	1.74	1.79	2.15	1.68	1.50	1.57	1.53
Threonine (%)	1.61	1.57	1.66	1.44	1.44	1.45	1.53	1.36	1.42	1.39
Tyrosine (%)	1.40	1.70	1.55	1.59	1.48	1.58	1.55	1.38	1.45	1.43
Tryptophan (%)	0.32	0.38	0.32	0.29	0.30	0.31	0.55	0.52	0.50	0.52
Valine (%)	1.84	1.79	1.83	1.92	1.58	1.94	2.00	1.91	1.93	1.96
Taurine (%)	0.47	0.48	0.44	0.45	0.42	0.50	0.69	0.71	0.72	0.69

[1] Analyzed at Midwest Laboratories, Inc. (Omaha, NE, USA)

[2] Analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA)

FM15= 15% Fishmeal diet, FM10= 10% Fishmeal diet, FM5= 5% Fishmeal diet, FM0= 0% Fishmeal diet, LCPC= Lysine Corn Protein Concentrate, CL= Crystalline Lysine, BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour.

Table 4. Gossypol analysis[1] of ingredients used in practical diets in growth trial 2. Total gossypol % and % free isomers within the finalized diets.

	Total gossypol % ingredient	Total gossypol % diet	% isomer in diet
BD	BMT	BMT	BMT
GCSF	0.023	0.006	55.4
ECSF	0.027	0.008	55.8
UCSF	1.172	0.200	57.1

[1] Analyzed at USDA labs (Cary, NC, USA).

BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour**.**

Culture conditions

Juvenile pompano (~1 g mean weight) were obtained from Proaquatix in Vero Beach, FL. Fish were loaded into a hauling tank equipped with a supplemental oxygen supply system and transported to the Alabama Department of Conservation and Natural Resource's Marine Resource Division, Claude Peteet Mariculture Center (CPMC), in Gulf Shores, Alabama. At CPMC, the fish were acclimated to local conditions by slowly replacing the hauling water which had on average a temperature of 25.5 °C, dissolved oxygen of 24.7 mg L^{-1}, salinity of 28.7 ppt and pH of 7.2, with local salt water which was on average 25.7 °C, 5.6 mg L^{-1}, 30.6 ppt, and 7.8 for temperature, dissolved oxygen, salinity, and pH respectively, before being transferred into a 5-m^3 fiberglass tank with an independent biological filter, air lift pumps and supplemental aeration provided by a 3.5-hp regenerative air blower (Pentair Aquatic Eco-Systems, Inc., Apopka, FL, USA) and air diffusers. Fish remained in this tank for approximately 30 days acclimating to local conditions until adequate size for the growth trial was achieved. During this period, fish were fed to apparent satiation with a 3mm crumble feed with 40% crude protein and 12% crude fat commercial diet (EXTR 400, Rangen Inc., Angleton, TX).

Two 10-week growth trials were conducted using three or four replicate tanks containing 20 juvenile pompano per tank. Each system consisted of culture tanks, each with at least 800 L capacity, sump tanks, bead filter, biological trickle filter, circulation pump, and supplemental aeration. The experimental systems are located in greenhouses, which provided natural 14 hour light and 10 hour dark cycles with slight seasonal variation. Ventilation was adjusted to maintain a relatively constant water temperature throughout the summer and late fall. Trial one used 24 circular culture tanks. Four replicates of each diet were randomly assigned to the tanks that contained 20 juvenile pompano (12.5 g initial mean weight). For trial two, three replicates of each diet were randomly assigned to a different recirculating system of 12 circular tanks, each containing 20 juvenile pompano (4.54 g initial mean weight).

Water quality, such as dissolved oxygen, temperature, salinity, and pH, was measured from the first and last tank in the line, twice daily using an YSI 556 multi-probe meter (Yellow Spring Instruments Co., Yellow Springs, OH, USA) and total ammonia nitrogen measured twice per week using an ion selective electrode (Orion EA 940, Thermo Electron Corporation, Beverly, MA, USA). Nitrite and nitrate levels were monitored by using test kits (3354-01, 3352-01, LaMotte Comp., Chestertown, MD) once a week.

Feed management

Daily rations were divided into four feedings per day (0700, 1100, 1500, and 1900 hr), based on a fixed percentage of body weight ranging from 8.0% to 25.0%, designed to be near satiation. Every other week, the fish were weighed and counted in each tank, then the fish were treated with a chloriquine dip followed by fresh water to aide with parasite prevention. The percent weight gain (%), feed conversion ratio (FCR), survival rate, and growth per week (g) were calculated to modify the amount of feed to maintain satiation feeding. Calculating the TGC allows the growth to be standardized and more easily compared amongst treatments and species. The thermal growth coefficients were calculated by the equation set forth by Cho (1992)

$$TGC = 100 * \frac{(mean\ final\ weight^{\frac{1}{3}} - initial\ mean\ weight^{\frac{1}{3}})}{\sum temp(°C) * days}$$

Following each sampling a 25% water change was performed. In the week prior to each sampling, beginning with the second week of the trial, the system was treated with EarthTec with an active ingredient of copper sulfate pentahydrate (20%) (Earth Science Laboratories, Bentonville, AR, USA) at a dosage of 0.18 ppm as a parasitic preventative.

Muscle and liver analysis

At the final sampling of growth trial 2 and after the group weight and number were determined; five fish from each tank were selected for muscle and liver sampling. These fish were euthanized by placing them in an aerated container with 80 mg/L of buffered MS222. The livers were then carefully removed and, by grouping per tank, each liver was split into halves, with half of the samples frozen for later analysis and the remaining liver tissue placed in 3.7% formaldehyde solution. The collected samples for all the tanks were then sent for whole body proximate analysis and gossypol content analysis of the livers by the department of commodity utilization research division of the USDA (New Orleans La.). A typical sample analysis was as follows. After first freeze drying and grinding the tissue, a 50-mg sample of each was centrifuged and covered with a complexing reagent containing R-(-)-2-amino-1-propanol. The sample was then heated to allow the gossypol to form a Schiff's base complex with the amine. After cooling, the sample was mixed with a mobile phase and centrifuged. The supernatant was then subjected to high performance liquid chromatography. Derivatized gossypol forms were detected by UV light absorption at 253 nm. The second half of the liver samples, preserved in formaldehyde, were cut on a microtome at 6 microns thickness. Tissue sections were floated in a water bath and placed on silane-coated slides. Slides were air dried then placed on a 45°C hot plate overnight. Before staining, slides were placed in an oven at 60°C for 20 minutes to ensure the tissue would stay on the slide during staining. After removing slides from oven, they were cooled for 10 minutes before staining by the standard hematoxylin and eosin staining methods. These slides were viewed and interpreted by a histologist at Auburn University.

Digestibility

Digestibility coefficients for protein and energy of the basal and test diets were determined using chromic oxide as an inert marker. The digestibility of each ingredient was based on nutrient substitution of the basal diet with the test ingredients using a dry matter ratio of 70:30.

Diets were prepared as previously described. The reference diet was designed to contain 40% protein, 8% lipid and 1% chromium oxide as a non-digestible marker. Experimental diets were produced using 70% reference, 30% ingredient (Low gossypol CSM, fishmeal, and corn gluten meal for trial 1; cottonseed flour prior to gossypol extraction, cottonseed flour after the gossypol was extracted, and cottonseed flour made from glandless cotton for trial 2) combined on a weight basis (Table 5).

Fifteen fish, at an average initial weight of 96.17g (trial 1) and 97.14g (trial 2), that had been provided as previously described and grown out by means of another experiment were stocked into the system described previously for trial 2 of the growth trials. Water quality was monitored and maintained as described. Daily rations were divided into four feedings per day at 0700, 1100, 1500, and 1900 hours, based on a fixed percentage of body weight designed to be near satiation. Feeding continued for four days, after which the fish were manually strip-sampled for feces on the fourth day, fed another three days and then strip-sampled again to ensure adequate sample size. On the day of sampling, fish were fed on a staggered feeding schedule. Four tanks were fed per feeding block and three hours later the fish were manually stripped of feces. Each block of feeding contained one tank from each treatment to ensure that the time of day of stripping would not influence the results of a single treatment. Feeding occurred at 0600, 0800, and 1000 hours, with stripping at 0900, 1100, and 1300 hours, respectively.

Table 5. Digestibility diets formulated at Auburn University (40% protein, 8% lipid). Experimental diets contain 70% the reference diet and 30% test ingredient, Low Gossypol Cottonseed meal, Fishmeal, Corn Gluten meal, Cottonseed Flour prior to extraction, Cottonseed Flour gossypol extracted, Cottonseed Flour glandless. The reference diet was made separately for each digestibility trial.

Ingredients	RD
Menhaden Fishmeal[1]	15.00
Poultry by product meal[2]	15.00
Soybean meal solvent extracted[3]	20.60
Corn Gluten meal[4]	8.00
Corn starch[5]	12.63
Whole wheat[5]	22.00
Menhaden fish oil[1]	2.82
ASA Trace Mineral[8]	0.25
ASA Vitamin premix[9]	0.50
Choline cloride[5]	0.20
Stay C 250 mg/kg[0]	0.10
CaP-dibasic[11]	1.00
Lecethin [5]	0.50
Taurine[10]	0.40
Chromic oxide[11]	1.00

RD= Reference Diet.

[1] Omega Protein Inc., Huston TX, USA.

[2] Griffin Industries, Inc., Mobile, Al, USA.

[3] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Al, USA.

[4] Grain Processing Corporation, Muscatine, IA, USA.

[5] MP Biomedicals Inc., Solon, Oh, USA.

[6] Trace mineral: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose

[7] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B12, Inositol, Vitamin A acetate, Vitamin D3, Vitamin E, cellulose

[8] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[9] J. T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

[10] Aldrich-Sigma, St. Louis, MO, USA.

[11] Fisher Scientific, Fair Lawn, NJ, USA.

[12] Cotton Incorporated, Cary NC, USA.

Separate fecal samples from each trial were tested by analyzing excreted protein using the standard micro-Kjeldahl method (Ma & Zuazaga 1942), gross energy content using semimicro-bomb calorimeter (Model 1425, Parr Instrument Co. Moline, IL, USA), and chromic oxide content using the McGinnis and Kasting (1964) method. The absorbance of the colorimetric reaction was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy Co., Rochester, NY, USA) at 540 nm. The apparent digestibility coefficients for dry matter, protein, and energy were calculated according to Mainard & Loosli (1969) and Hardy & Barrows (2002).

$$ADMD\ (\%) = 100 - \left[100 * \left(\frac{\%\ Cr_2O_3\ in\ feeds}{\%\ Cr_2O_3\ in\ feces}\right)\right]$$

$$ADP\ and\ AED\ (\%) = 100 - \left[100 * \left(\frac{\%\ Cr_2O_3\ in\ feeds}{\%\ Cr_2O_3\ in\ feces} * \frac{\%\ nutrients\ feces}{\%\ nutrient\ feed}\right)\right]$$

The apparent digestibility coefficients of the ingredients were then calculated by using standard formulas (Cho et al., 1982) and adjusting the ingredient ADC of gross energy according to Forster (1999).

$$ADC_{ingredient} = \frac{ADC - (1 - \left(\frac{energy_{test\ diet} - (energy_{basal\ diet} * 0.7)}{energy_{\ test\ diet}}\right) * Avg(ADC_{basal\ diet})}{\frac{energy_{test\ diet} - (energy_{basal\ diet} * 0.7)}{energy_{\ test\ diet}}}$$

This allows the chromium percentages and energy levels to be taken into account rather than just assuming the 70:30 inclusion ratio was exact.

The resulting data were analyzed using one-way analysis of variance to determine significant differences in the AD, the protein retention, energy retention, and dry matter digestibility of the primary ingredients among fish fed each of the test diets. A Student-Neuman-Keuls multiple comparison test was conducted to define significant differences among the means of the treatments. All statistical analyses were conducted using SAS (V9.1 SAS Institute, Cary, NC, USA).

Data Analyses

Data, including the mean final weight, survival, feed conversion ratio, and thermal growth coefficients of the Florida pompano were statistically analyzed using one-way analysis of variance to determine significant differences among the test diets. To determine if significant differences existed among the means of the treatments, Student-Neuman-Keuls multiple comparison test was used. Additionally, Dunnett's test was used to compare the test diets to a control and pair wise contrast comparing pre-determined test diets to one another. The TGC values were compared to the percent of cottonseed included in the diets of growth trial 1 and the TGC values of treatments in growth trial 2 were compared to the gossypol levels in the diets of growth trial 2, in both linear and quadratic regression analysis to illustrate any potential correlation. All statistical analyses were conducted using SAS (V9.1 SAS Institute, Cary, NC, USA).

Results

Growth Trial 1

The overall mean water quality parameters: dissolved oxygen (5.8 ± 0.4 mg L^{-1}), temperature (27.6 ± 1.9 °C), salinity (29.8 ± 5.1 ppt), pH (7.8 ± 0.2), and total ammonia nitrogen (0.18 ± 0.29 mg L^{-1}) were typical for these systems. The values of all water quality parameters were consistent and within acceptable ranges for pompano production. (Watanabe 1995; Weirich & Riche 2006).

At the conclusion of the 10-week trial, the final mean weight of the fish that had been fed FM15 was 60.3 g, with the percent mean weight gain being 387.3%; for FM10 the values were

66.0 g and 427.4%, for FM5 62.7 g and 401.6%; and FM0 57.0 g and 354.7%, respectively. The final mean weight of the fish that were fed the FM0-LCPC was 71.6 g while the mean weight gain percent was 475.9%, while the fish fed FM0-CL was 61.3 g and 396.8%. The thermal growth coefficients (TGC) were calculated and ranged from 0.082 to 0.099. The TGC values for fish fed diet FM15 was 0.087, for FM10 0.093, for FM5 0.089, and for FM0 0.082. The fish fed diet FM0-LC had a TGC value of 0.088, and FM0-LCPC had a value of 0.099 (Table 6). The overall mean FCR value for fish in growth trial 1 was 1.89± 0.09 with no significant differences found amongst treatments. Mean survival rates for the treatments ranged from 50.0% to 90.0% (Table 7). Fish from treatments fed FM0, FM0-LCPC and FM0-LC had survival rates of 50.0%, 55.0%, and 56.3%, respectively. Fish from treatments fed FM15, FM5, and FM10 had mean survival rates of 75.0%, 78.8%, and 90.0%, respectively.

Regression analysis was used to evaluate TGC values compared to the amount of cottonseed meal in each diet, which was 0 in FM15, 6.5% in FM10, 13.1% in FM5, and 19.6% in FM0, FM0-LCPC, and FM0-CL. Linear regression and quadratic regression analyses were performed. There was no linear or quadratic relationship between the percent of cottonseed meal and the TGC values for each treatment (Table 6).

Means separation indicated that mean weight gain for fish fed FM0-LCPC was not significantly different from those of fish fed any diet containing FM or lysine supplementation, but did have significantly different mean weight gain from the fish fed the diet containing 0 FM. There were no significant differences in FCR among treatments.

Table 6. Thermal growth coefficients of growth trial 1 compared to the percent of cottonseed meal included in the diets to determine if the relationship fits either linear or quadratic regression. Thermal growth coefficients of growth trial 2 compared to the percent of total gossypol included in the diet for both linear and quadratic regressions.

Diet	Cottonseed Meal inclusion (%)	TGC	P Value	
			Linear Regression	Quadratic Regression
FM15	0.0	0.087^{ab}		
FM10	6.5	0.093 ^{ab}		
FM5	13.1	0.089 ^{ab}	0.9996	0.9863
FM0	19.6	0.082^{b}		
FM0-LCPC	19.6	0.099^{a}		
FM0-Cl	19.6	0.088 ^{ab}		
	Total gossypol % diet			
BD	0.00	0.104		
GCSF	0.01	0.113	0.6257	0.8194
ECSF	0.01	0.091		
UCSF	0.20	0.095		

FM15= 15% Fishmeal diet, FM10= 10% Fishmeal diet, FM5= 5% Fishmeal diet, FM0= 0% Fishmeal diet, LCPC= Lysine Corn Protein Concentrate, CL= Crystalline Lysine, BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour, TGC= Thermal Growth Coefficients.

Table 7. Response of Florida pompano juveniles (12.5g initial mean weight) to diets with varying levels of cottonseed flour over a 10-week growth trial 1 in a saltwater recirculating tank system. Values represent the means of four replicates. Means within a column and with different superscripts are significantly different based on Student Newman Keuls analysis (P= 0.05).

	Final Mean Weight (g)	Mean Weight Gain (%)	FCR	Survival (%)
FM15	60.3± 6.5^{ab}	387.3± 48.9^{ab}	1.97± 0.14^{a}	75.0± 15.8^{a}
FM10	66.0± 6.6^{ab}	427.4± 58.3^{ab}	1.92± 0.18^{a}	90.0± 9.1^{a}
FM5	62.7± 6.2^{ab}	401.6± 49.1^{ab}	1.94± 0.11^{a}	78.8± 7.5^{a}
FM0	57.0± 4.8^{b}	354.7± 32.1^{b}	1.95± 0.13^{a}	50.0± 4.1^{b}
FM0-LCPC	71.6± 4.7^{a}	475.9± 44.7^{a}	1.72± 0.11^{a}	55.0± 9.1^{b}
FM0-CL	61.3± 4.4^{ab}	396.8± 33.7^{ab}	1.87± 0.11^{a}	56.3± 7.5^{b}
P Value	0.0286	0.0283	0.1683	<0.0001
PSE	1.15	9.26	0.03	1.95

PSE= Pooled Standard Error
FM15= 15% Fishmeal diet, FM10= 10% Fishmeal diet, FM5= 5% Fishmeal diet, FM0= 0% Fishmeal diet, LCPC= Lysine Corn Protein Concentrate, CL= Crystalline Lysine.

Growth Trial 2

Trial parameters were as follows, dissolved oxygen (5.5 ± 0.3 mg L^{-1}), temperature (27.6 ± 1.5 °C), salinity (29.8 ± 3.0 ppt), pH (7.6 ± 0.2), total ammonia nitrogen (0.08 ± 0.15 mg L^{-1}), nitrite (0.13 ± 0.14 mg L^{-1}), and nitrate (12.25 ± 2.55 mg L^{-1}). The water quality was maintained at acceptable levels for rearing of juvenile pompano according to Watanabe (1995) and Weirich & Riche (2006).

The diets were analyzed for the final gossypol total and percent of free isomers. The diet containing unextracted CSF (UCSF) had a gossypol content of 0.2% and a free isomer percentage of 57.1%. The diet made with extracted cotton seed flour (ECSF) replacing SBM (soybean meal) contained 0.008% total gossypol and a free isomer content of 55.8%, while the diet which contained cottonseed flour of a glandless variety (GCSF) had .006% total gossypol and 55.4% free isomer (Table 4).

Using pairwise comparisons, significant differences in growth were observed amongst the fish fed diets containing the varying cottonseed flours. When fish fed the diets containing traditional cottonseed flour (UCSF) or gossypol extracted flour (ECSF) were compared to fish fed the diet containing genetically low gossypol cottonseed flour (GCSF), the latter showed significantly higher mean final weights and percent weight gain than those of fish fed either of the other diets. Average mean final weight and percent weight gain for fish fed the basal diet (BD) was 50.1 g and 983.8%; fish fed the UCSF, 43.2 g and 831.0%; fish on the ECSF, 40.8 g and 776.1%: and fish fed GCSF, 57.9 g and 1145.9%. The TGC value for the fish fed BD was 0.10, and 0.09 for the fish fed both UCSF and ECSF. Fish fed GCSF had a TGC value of 0.11. These values were compared to the gossypol levels within each diet by linear and quadratic regressions and, there was no linear or quadratic relationship between the gossypol levels in the diets and the TGC values for each treatment (Table 6). The average FCR for the BD, UCSF, ECSF, and GCSF, were 1.88, 1.86, 2.07, and 1.71 respectively. The mean survival rates showed no significant differences among treatments, 85%, 88%, 93%, and 92% survival for diets BD, UCSF, ECSF, and GCSF, respectively (Table 8). No significant differences were found when comparing the treatments for mean FCR or survival.

The livers were analyzed for gossypol content by the USDA laboratory (New Orleans, LA). The fish that were fed BD and GCSF had total liver gossypol below measurable threshold; however, the UCSF treatment averaged 79.73 ± 9.58 µg/g and ECSF liver content was decreased to 21.77 ± 8.54 µg/g (Table 9). Statistical analysis could only be run between the two treatments with numerical data UCSF, and ECSF. The amount of gossypol present in the livers of fish fed UCSF was found to be statically higher in than those that of ECSF.

Liver slides were reviewed by Dr. Joseph Newton, a histologist at Auburn University. When the slides were analyzed, there were no significant differences found in the cells or in the organization of the cells among treatments. There was mild vacuolization of the pancreatic tissues in the samples, but it was not severe or consistent amongst any treatments. There was slight to moderate pigmentation increase around vessels and triad centers within the liver tissue samples from tank systems that were fed UCSF. As could be seen by the slides, there was no necrosis of any tissues and most samples had normal vacuolation of the hypatocytes, and glycogen had standard vacuolization.

The proximate and amino acid analyses of whole fish from growth trial 2 showed that there were no significant differences amongst the treatments in regards to crude protein, moisture, ash, fiber, or amino acids with the exception of tryptophan in which fish in the BD has a higher value (Table 10).

Table 8. Growth response of Florida pompano (4.64g initial mean weight) to dietary treatments in saltwater recirculating tank systems over 10 weeks from growth trail 2.

Treatments	Final Mean Weight (g)	Mean Weight Gain (%)	FCR	Survival (%)
BD	50.1± 8.7	983.8± 187.9	1.9± 0.3	85.0± 17.3
UCSF	43.2± 2.4	831.0± 40.7	1.9± 0.1	88.3± 5.8
ECSF	40.8± 8.5	776.1± 185.5	2.1± 0.3	93.3± 7.6
GCSF	57.9± 6.8	1145.9± 153.4	1.7± 0.2	91.7± 7.6
P Value	0.0670	0.0697	0.4045	0.7809
PSE	2.04	44.47	0.07	3.06
Contrast (P Value)				
UCSF vs. ECSF	0.6969	0.6740	0.3239	0.5796
UCSF vs. GCSF	0.0338	0.0367	0.4716	0.7103
GCSF vs. ECSF	0.0181	0.0187	0.1084	0.8522

BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour.

Table 9. Liver analysis[1] for growth trial 2.

	BD	GCSF	ECSF	UCSF	PSE	P Value
Total Gossypol, µg/g	BMT	BMT	21.8	79.7	3.71	0.001
%(+)-Isomer	BMT	BMT	63.8	60.8		

[1] Analyzed at USDA labs (Cary, NC, USA).
PSE= Pooled Standard Error
BMT= Below Measurable Threshold
BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour.

Table 10. Proximate and amino acid analyses of whole fish from growth trial 2 fed dietary treatments in saltwater recirculating tank systems over 10.

Component	BD	UCSF	ECSF	GCSF	P value	PSE
Alanine (%)	1.08 ± 0.02	1.08 ± 0.06	1.12 ± 0.08	1.11 ± 0.03	0.7507	0.0154
Arginine (%)	1.05 ± 0.01	1.04 ± 0.05	1.06 ± 0.07	1.06 ± 0.03	0.9284	0.0130
Aspartic acid (%)	1.50 ± 0.03	1.50 ± 0.03	1.48 ± 0.04	1.50 ± 0.06	0.8753	0.1326
Cystine (%)	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.2869	0.0020
Glutamic acid (%)	2.09 ± 0.03	2.09 ± 0.08	2.08 ± 0.07	2.09 ± 0.08	0.9878	0.0193
Glycine (%)	1.28 ± 0.11	1.25 ± 0.12	1.44 ± 0.20	1.34 ± 0.05	0.3591	0.0380
Histidine (%)	0.36 ± 0.02	0.36 ± 0.02	0.34 ± 0.01	0.36 ± 0.02	0.4947	0.0042
Hydroxylysine (%)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.1631	0.0014
Hydroxyproline (%)	0.28 ± 0.03	0.27 ± 0.05	0.35 ± 0.07	0.30 ± 0.04	0.3409	0.0150
Isoleucine (%)	0.68 ± 0.03	0.69 ± 0.03	0.65 ± 0.02	0.68 ± 0.03	0.4678	0.0075
Leucine (%)	1.17 ± 0.05	1.18 ± 0.04	1.13 ± 0.04	1.17 ± 0.05	0.5707	0.0127
Total lysine (%)	1.29 ± 0.04	1.29 ± 0.05	1.24 ± 0.04	1.28 ± 0.06	0.6056	0.0129
Methionine (%)	0.44 ± 0.01	0.45 ± 0.02	0.43 ± 0.01	0.44 ± 0.02	0.7643	0.0046
Phenylalanine (%)	0.66 ± 0.02	0.66 ± 0.03	0.65 ± 0.03	0.66 ± 0.02	0.9000	0.0066
Proline (%)	0.77 ± 0.05	0.74 ± 0.07	0.83 ± 0.10	0.79 ± 0.03	0.4295	0.0188
Ornithine (%)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.0000	0.0000
Serine (%)	0.62 ± 0.01	0.62 ± 0.03	0.62 ± 0.03	0.63 ± 0.03	0.9368	0.0068
Threonine (%)	0.72 ± 0.02	0.72 ± 0.03	0.71 ± 0.02	0.72 ± 0.03	0.8450	0.0069
Tyrosine (%)	0.50 ± 0.02	0.49 ± 0.01	0.48 ± 0.03	0.49 ± 0.03	0.9000	0.0062
Tryptophan (%)	0.16 ± 0.02	0.13 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.0150	0.0033
Valine (%)	0.78 ± 0.05	0.79 ± 0.02	0.76 ± 0.03	0.78 ± 0.03	0.7374	0.0076
Taurine (%)	0.38 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	0.7597	0.0033
Total AA (%)	16.00 ± 0.11	15.95 ± 0.69	16.11 ± 0.73	16.12 ± 0.56	0.9776	0.1672
Protein (%)	16.99 ± 0.19	16.77 ± 1.50	16.82 ± 0.51	17.03 ± 0.61	0.9754	0.2463
Moisture (%)	72.55 ± 1.13	71.58 ± 1.82	74.47 ± 0.18	72.27 ± 0.51	0.0595	0.3188
Fat (%)	7.09 ± 0.91	7.72 ± 1.43	5.13 ± 0.32	7.26 ± 0.05	0.0270	0.2492
Fiber (%)	0.02 ± 0.03	0.12 ± 0.14	0.10 ± 0.12	0.07 ± 0.09	0.7037	0.0297
Ash (%)	3.08 ± 0.15	3.17 ± 0.15	3.22 ± 0.20	2.90 ± 0.11	0.1315	0.0447

[1] Analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

PSE= Pooled Standard Error

BD= Basal Diet, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour.

Digestibility Trial 1

The mean apparent dry matter digestibility (ADMD) for the reference diet (RD) was 39.96 ±4.68 %, for diet CSM (the diet including cotton seed meal) ADMD was 49.35 ±2.58 %, the diet FM (testing fishmeal) ADMD was 49.52 ±1.59 %, and the diet CGM (testing Empyreal 75) ADMD was 50.6 ±1.6%. The mean apparent energy digestibility (AED) for the diets were 55.24±4.11, 64.58±1.59, 64.67±0.65, and 62.4 ±3.9 percent for RD, CSM, FM, and CGM, respectively. The mean apparent protein digestibility (ADP) for RD, which served as the basal diet, was 64.12 ±3.80 %, for CSM the ADP was 75.37 ±3.61 % for FM the ADP was 72.16 ±1.88 %, and for CGM the ADP was 68.8 ±3.6%.

These values based on the diet as a whole were then calculated into ADMD, AED, and ADP of the ingredient based on the 70:30 inclusion. For CSM, the ADMD was 71.26±8.59 %, the AED is 84.87±5.04 %, and the APD is 93.24±1.83 %. For FM, the values were 71.82±5.31, 87.40±2.20, and 91.52±6.40 percent for the ADMD, AED, and APD, respectively. CGM had ADMD of 75.6 ±5.5%, AED of 77.4 ±12.6%, and APD of 78.7 ±11.2%. There were no statistical differences found amongst ADMD, AED, or APD for any of the ingredients tested (Table 11).

Digestibility Trial 2

The ADMD for the reference diet (RD) was 42.46± 2.5 %. The RD with GCSF, which included a glandless variety of cottonseed flour had a ADMD of 52.57±2.83 %. The RD with cottonseed flour prior to extraction (UCSM) had a ADMD of 47.42±3.11 % and the extracted diet (ECSF), testing a cottonseed flour with the gossypol extracted, was 42.52±7.88%. The AED

Table 11. Digestibility coefficients for primary ingredients utilized in test diet for the Florida pompano Growth trials.

	Diets			Ingredients		
Trial 1	ADMD	AED	APD	ADMD	AED	APD
RD	40.0 ± 4.7	55.2 ± 4.1	64.1 ± 3.8			
CSM	49.4 ± 2.58	64.6 ± 1.6	75.4 ±3.6	71.3 ± 8.6	84.9 ± 5.0	93.2 ± 1.8
FM	49.5 ± 1.6	64.7 ± 0.6	72.2 ±1.9	71.8 ± 5.3	87.4 ± 2.2	91.5 ± 6.4
CGM	50.6 ± 1.6	62.4 ± 3.9	68.8 ± 3.6	75.6 ± 5.5	77.4 ± 12.6	78.7 ± 11.2
P-value				0.7794	0.3095	0.1942
PSE				2.42	2.28	2.74
Trial 2	ADMD	AED	APD	ADMD	AED	APD
RD	42.5 ± 2.5	56.2 2.4	76.6 ± 1.8			
GCSF	52.6 ± 2.8	66.7 ±2.3	86.1 ± 4.3	76.2 ± 9.5	90.0 ± 7.5	96.4 ± 2.1
UCSF	47.4 ±3.1	63.5 1.2	85.7 ± 1.7	59.0 ± 10.4	80.4 ± 3.9	97.2 ± 0.8
ECSF	42.5 ±7.9	57.8 ±6.8	73.0 ±12.9	57.3 ± 9.8	76.7 ± 7.7	86.1 ± 3.3
P-Value				0.1346	0.1351	0.0301
PSE				3.50	2.24	0.95

The following values were excluded from statistics due to contamination of sample, CGM ADMD 51.5, AED 48.2, APD 50.7; CSM APD 113.0; GCSF APD 117.7; UCSF APD 104.4; ECSF ADMD 13.4, AED 33.4, APD 30.2

PSE= Pooled Standard Error

RD= Reference Diet CSM= Cottonseed Meal, FM= Fishmeal, CGM= Corn Gluten Meal, GCSF= Glandless Cottonseed Flour, UCSF= Unextracted Cottonseed Flour, ECSF= Extracted Cottonseed Flour .

ADMD= Apparent Dry Matter Digestibility, AED= Apparent Energy Digestibility, APD= Apparent Protein Digestibility for the RD, GCSF, UCSF, and ECSF were 56.21±2.43, 66.72±2.32, 63.49±1.16, and 57.82±6.82 percent, respectively. The ADP of the diets were 76.64±1.81 % for RD, 86.14±4.38 % for GCSF, 85.65±1.66 % for UCSF , and 73.00±12.86 % for ECSF.

Based on the 70:30 diet ingredient inclusion, the previous results were calculated into ADMD, AED, and ADP for the ingredient. For GCSF, the ADMD was 76.17±9.45 %, AED 89.99±7.46 %, and APD 96.42±2.14 %. For UCSF, using regular CSF, the values were 58.98±10.36, 80.45±3.88, and 97.16±0.75 percent for the ADMD, AED, and APD, respectively. ECSF, which utilized an extracted variety of CSF, had ADMD value of 57.30±9.77 %, AED of 76.66±7.72%, and APD of 86.13±3.34% (Table 11).

There were no significant differences found amongst the diets with respect to ADMD and AED for ingredients. The diet including EtOH extracted Weldon meal (ECSF) was found to be significantly lower in APD for ingredients. Some of the samples included data points that were quite different from the other data points collected. These were omitted from statistical analysis. Although outliers are inevitable, it is likely that these were due to methods used obtaining the APC or contamination of the fecal samples by blood or urine Cho and Slinger, 1979; Cho and Kaushik, 1990; Hajen et al., 1993).

Discussion

When FM is replaced by higher inclusion of alternative protein sources, there tends to be a drop off in performance of the Florida pompano as shown by Rossi and Davis (2012) when replacing fishmeal with poultry by-product meal or replacing fishmeal with either cottonseed meal, soybean meals, or soy protein isolate (Riche and Williams 2011). The depression in performance is shown here as well. This reduction in performance could be due to a lack of required amino acids. Typically, the most limiting amino acids in pompano feeds have been lysine and methionine, and more recently shown to be of importance is taurine (Rossi and Davis 2012).

As the following studies confirm, there seems to be a limit to the amount of fishmeal that can be replaced in diets before a reduction in performance is found. In a study of vundu catfish, when inclusion of CSM into the diets reached 30%, with about 30% fishmeal remaining in the diet, growth performance began to decrease significantly; however the survivals did not vary amongst treatments (Toko *et al*. 2008). When examining the growth of tilapia, Mbahinzireki *et al*. (2001) found that CSM should replace no more than 50% of fishmeal protein, in this case rendering a diet with roughly 30% CSM, before growth is negatively impacted. Similar limits are also defined for silver crucian carp with growth depression at an inclusion of 40% CSM (Cai *et al*. 2011). Both chinook salmon (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) were found to grow equally well on diets containing 34% glanded CSM and 22% glanded CSM, respectively, as they had on diets that had contained 37% fishmeal (Fowler 1980). Channel catfish showed a decrease in growth when their diet included nearly 30% CSM, even when lysine is supplemented. However, by varying cottonseed meals and amino acid supplementations, it was found that other protein sources, such as soybean meal, could replace as much as 100% of fishmeal in the diet (Robinson and Li, 2008, Robinson 1991).

In this study, fish fed diets with the highest level of cottonseed flour and reduced FM levels produced poor results, which was mediated by the addition of lysine. This confirmed that there was in fact a deficiency of lysine in the FM0 diet of growth trial 1. A deficiency of lysine when replacing fishmeal was also seen in pompano when 40% of the fishmeal was replaced by alternative proteins such as CGM, SBM, and soy protein isolate (Riche and Williams, 2011).

CSM with lysine supplementation could be used in diets for channel catfish replacing up to 50% of the main source of protein, SBM, without affecting the performance (Robinson and Li, 2008). FM0-LCPC was not significantly different in mean weight gain from any diet containing FM or lysine-supplemented diets but it was significantly different from the diet containing 0 FM and no lysine, which does confirm a deficiency. Although FM0-LCPC and FM0-CL were not found to be significantly different (Table 7), further study is needed to determine if the apparent higher final mean weight and lower FCR may actually be significantly better for the treatment with added LCPC, which in a commercial setting could prove to be more efficient. Based on this study's results, the inclusion of 19.6% cottonseed flour and 0% fishmeal does not inhibit performance of the fish if there is a lysine supplementation by crystalline lysine. Although the diet contained 0% fishmeal it should be noted that the diets also contained poultry by-product meal and soybean meal as additional protein sources. The FCR showed no significantly statistical difference for any treatment.

Mean survivals were low, but the growth exhibited is typical for this cohort of fish. There could be many explanations for the lower survival, various diseases, bacterial, or parasitic infections, but it is primarily due to a disease outbreak of *Amyloodinium sp.* which is common in this area, early in the study. There was a statistical difference found between the diets contained FM and those, which did not include any FM. The treatments in which diets contained any level of FM had mean percent survival significantly greater than those without. However, in a study of adult rainbow trout fed diets where CSM was used to fully replace fishmeal a promising result showed normal performance and survival after a 6-month grow out trial (Blom *et al*, 2001).

Balancing the essential amino acids is but one facet of ingredient substitution: other factors such as anti-nutrients like gossypol must also be considered. Gossypol included in diets has been shown to reduce growth, cause organ abnormalities especially when looking at the liver on a cellular level, and increase mortality in fish (Berardi and Goldblatt, 1980). Processing cottonseed flour differently might reduce the amount of gossypol or alter availability within the diet. Furthermore, genetic manipulations via standard breeding or genetic modification to reduce the levels of gossypol are other options to improve the quality of the product. When testing the performance of fish using cottonseed flour that underwent different processing techniques, all test diets were found to be acceptable substitutes when compared to the basal diet containing soybean meal. However, it seems that the glandless variety of CSM is a better choice when formulating practical diets for Florida pompano. When fish fed with cottonseed flours processed in different manners were compared to one another, fish fed the genetically low gossypol cottonseed flour (GCSF) had significantly higher final mean weights and percent weight gain. When the diets were tested for gossypol content and free isomer percentage, GCSF had 0.006% gossypol and 55.4% free isomer content. These values were not drastically different from those of the diet made with cottonseed flour in which the gossypol was extracted ECSF; however, they were much lower than those of the unextracted cottonseed flour diet, UCSF. This could explain the difference between the glandless variety and the non-extracted CSM. Warnick (1966) found that glandless varieties of CSM were more nutritious than their cohorts by containing more accessible lysine and methionine, which tend to be early limiting factors in diets in general. Robinson and Rawles (1983) showed that supplementation of lysine did not improve the stunted growth of fish fed diets containing 44.6 or 54.4% glandless cottonseed flour, suggesting that the glandless variety contains sufficient lysine for channel catfish growth. Lysine supplementation was needed for diets containing glanded CSM, which contains a higher gossypol content. This could explain the difference found between the glandless and extracted meals and the lack of difference between the extracted and non-extracted.

A study on channel catfish reported that although there were areas of necrosis scattered through liver samples there was no significant differences found amongst any treatments ranging from 0 to 1500 µg/g of dietary gossypol (Evans *et al*. 2010). When diets containing 642 µg/g of free gossypol and one containing 560 µg/g gossypol CSM were fed to crucian carp and the livers examined, there was no significant difference found when the hepatic cells were examined (Cai *et al.* 2011). When the livers from this study were examined, there was no indication that there was any structural damage to the livers that would result in any problems for the fish. There was vacuolization throughout the livers but it was consistent through all treatments including the livers of the basal diet, indicating that gossypol was not the cause of the vacuolization. There was no evidence of necrosis in the liver cells or the pancreatic tissue; however, there was a slight increase in pigmentation around vessels or triad centers although this occurred in all treatments.

The diets tested here contained 0.006- 0.2 % gossypol and the trial spanned 10 weeks; livers of fish fed UCSF contained an average of 79.7 µg/g gossypol and ECSF had values of 21.8 µg/g, and livers of the fish that had been fed GCSF had levels below the detectable limits. These results can be compared to the work of Dorsa *et al.* (1982) where channel catfish were fed diets containing 0-29% cottonseed meal. Their diets with CSM inclusion over 11.6% yielded livers with gossypol levels significantly higher (26.1-56.4 µg/g) than those of the basal diet. Rainbow trout fed diets containing 30% cottonseed meal from various suppliers for a minimum of one week showed liver total gossypol levels between 78.6 and 192.4 µg/g of wet weight liver; however, the gossypol levels in the muscle tissues were near 0 (Cheng and Hardy, 2002). As compared to the data for Florida pompano found in this study, the values found in channel catfish were similar; however, the values of gossypol found in rainbow trout were much higher those that found in this study. These results can be used as guides when developing cottonseed based diets for other species.

Conducting digestibility studies of possible ingredients provides information which will help produce formulations of practical diets that will meet nutritional requirements for the given species, while being as cost efficient as possible (Cho *et al.* 1982). With fishmeal being one of the most expensive ingredients in a typical diet the evaluation and comparison of ingredients such as CSM and CGM (corn gluten meal) will promote a nutritional yet low cost alternative to the currently available commercial diets. The ADMD values for fishmeal for red drum are 76.79% and for Nile tilapia 88% (Mcgoogan and Reigh 1996, Fontainhas-Fernandes *et al*. 1999); the Florida pompano show a value of 71.8% which is roughly in line with these reported values.

Fishmeal APD values have consistently been high which explains its success as a primary protein source in diets. Red drum have APD values of 95.87, channel catfish 85%, hybrid striped bass 88%, Japanese sea bass 83.96%, and Nile tilapia having values of 96.9% (Mcgoogan and Reigh 1996, Tucker and Robinson 1990, Wilson 1991, Sullivan and Reigh 1995, Chang *et al*. 2005, and Fontainhas-Fernandes *et al*. 1999), respectively. The APD value of 91.5% found for Florida pompano is comparable to the others reported, and is in fact higher than values reported for some species, such as channel catfish and various bass.

AED of fishmeal is 92, 95, and 91.5% for channel catfish, hybrid stripped and Nile tilapia, respectively, (Wilson 1991, Sullivan and Reigh 1995, Fontainhas-Fernandes *et al*. 1999). The AED for Florida pompano was 87.4%, which is slightly lower but still comparable to the other reported values.

In one study of Florida pompano, corn gluten meal was found to have an average APD of 82.65% APD and an AED of 77.4% when tested in low and high salinity (Riche and Williams 2010). The APD values in this study are considerably higher than those reported in the literature while the apparent digestibility coefficients for AED were comparable. Lech and Reigh (2012) reported APD values of 57.2% and AED values at 57.1% for Florida pompano, which are lower than the values found in this study. It has been reported for silver perch that the ADMD, APD, and AED are 50.5%, 83.0%, and 53.1% respectively (Allen *et al.* 2000). On the whole, the digestibility coefficients are lower for CGM even though it seems to contain a very low percent of fiber and no anti-nutritional factors, which should make it a good alternative (Lech and Reigh, 2012).

Cheng and Hardy (2002) ran cottonseed meal digestibility in diets for rainbow trout using four CSMs which had been provided to them by various suppliers. The protein digestibility ranged from 87.7 to 81.6%, which was found comparable to other oilseed meals. For red drum, the ADMD value was 39.42, APD 76.35, AED 22.11% (Mcgoogan and Reigh, 1996). When the digestibility of various ingredients were tested in Japanese sea bass, CSM was found to have the lowest AED, which was 16.99% (Chang *et al.* 2005). A study of Australian silver perch was found to have an ADMD of 50.5%, an APD of 83.0%, and an AED of 53.1%, (Allen *et al.* 2000), which are considerably lower than those found in this study, whereas, the ADP for the Australian silver perch and red drum APD's were comparable to those found in this study.

Comparison of the test ingredients in this study showed no difference amongst the variables, ADMD, APD and, AEP, supporting that substitution of plant proteins such as cottonseed meal and corn gluten meal for fishmeal is suitable. This supports that when looking at substitutions for fishmeal, plant proteins such as cottonseed meal and corn gluten meal are suitable. The amount of protein and energy that the fish retains from the plant protein sources are comparable to those of fishmeal. Our study showed cottonseed meal could be a suitable substitute for fishmeal in practical diets for the Florida pompano. When cottonseed meal was tested for digestibility with rainbow trout, the ADC values were found to be comparable to those of other oilseed meals, indicating that cottonseed meal may be used to replace up to 80% of the typical fishmeal used in practical diets.

Although no significant differences were found amongst the diets containing the different cottonseed flours when examining ADMD and AED, the mean APD for the ECSF was significantly lower than those obtained from the other two flours. Mbahinzireki *et al*. (2001) found that the digestibility of crude protein decreased as the total gossypol level in a diet increased, but this result is not supported by the data in this study. The UCSF diet had 0.2% free gossypol while ECSF contained .008% yet in this study the ADC value for crude protein was higher in the UCSF than the diet containing ECSF, which is inverse of the previously reported data.

Conclusion

Results of the present study demonstrate that inclusion of cottonseed meal, up to 20%, with supplementation of lysine may be used for potential practical diet development culminating in good growth of Florida pompano. The upper limit of gossypol inclusion or long-term effects on Florida pompano has yet to be established, but the above data suggests that at these inclusion levels it is a suitable alternative protein source. The cottonseed meal (CSM) digestibility and the digestibility of corn gluten meal (CGM), which is another additive to practical diets, were not statistically different from that of fishmeal (FM); supporting that practical diets can be produced with these ingredients.

Fish fed diets including genetically low gossypol CSM resulted in significantly higher mean weight gain and percent weight gain when compared to those of fish fed the other test diets. No differences were found in the cells of the liver samples or organization of the liver cells amongst any of the treatments, which indicates that either there was not a high enough gossypol content in these diets to produce any visible adverse effects in fish fed these diets or that this species is more tolerant to gossypol than others. There was no significant difference found amongst any ingredient with respect to ADMD and AED, but the extracted CSM showed significantly lower APD.

Overall, cottonseed flour is an acceptable substitute for fishmeal based on the performance of fish fed the test diets and digestibility results. From the comparison of performance of fish fed the test diets amongst the varying cottonseed flours, the glandless variety of cottonseed flour was shown to give significantly higher results in terms of mean final growth and percent weight gain. Although further studies are needed, cottonseed meal, especially of the glandless variety, seems to be a good alternative to fishmeal when making practical diets for Florida pompano.

Literature Cited

Allen, L.A., Parkinson, S., Booth, M. A., Stone, D. A. J., Rowland, S. J., Frances, J. & Warner Smith, R. (2000). Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, 186, 293-310.

Anderson, J.O. & Warnick, R.E., (1966). Sequence in which essential amino acids become limiting for growth of chicks fed rations containing cottonseed meal. *Poultry Science*, 45, 84-89.

Blom, J.H., Lee, K.J., Rinchard, J., Dabrowski, K. & Ottobre, J., (2001). Reproductive efficiency and maternal-offspring transfer of gossypol in rainbow trout (*Oncorhynchus mykiss*) fed diets containing cottonseed meal. *J. Anim. Sci*., 79, 1533-1539.

Cai, C., Li, E., Ye, Y., Krogdahl, A., Jiang, G., Wang, Y. & Chen, L., (2011). Effect of dietary graded levels of cottonseed meal and gossypol on growth performance, body composition and health aspects of allogynogenetic silver crucian carp, *Carassius auratus gibelio*♀ x *Cyprinus carpio*♂. *Aquaculture Nutrition*, 17, 353-360.

Chang, Q., Liang, M., Wang, J. & Zhai, Y., (2005). Apparent digestibility coefficients of various feed ingredients for Japanese sea bass (*Lateolabrax japonicus*). Acta Hydrobiol. Sin., 29:2, 172-176.

Cheng, Z.J. & Hardy, R.W., (2002). Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 212, 361-372.

Cho, C.Y. (1992). Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. *Aquaculture*, 100, 107-123.

Cho, C.Y., Kaushik, S.J. (1990). Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). *World Rev. Nutr*. 61, 132–172.

Cho, C.Y., Slinger, S.J. (1979). Apparent digestibility measurement in feedstuffs for rainbow trout. *Finfish Nutrition*, 2. 239–247.

Cho, C.Y., Slinger, S.J. & Bayley, H.S., (1982). Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comparative Biochemistry and Physiology*, 73B, 25-41.

Dorsa, W.J., Robinette, H.R., Robinson, E.H. & Poe, W.E., (1982). Effects of Dietary Cottonseed Meal and Gossypol on Growth of Young Channel Catfish. *Transactions of the American Fisheries Society*, 111:5, 651-655.

Evans, J.J., Pasnik, D.J., Yildirim-Aksoy, M., Lim, C. & Klesius, P.H., (2010). Histologic changes in channel catfish, *Ictalurus punctatus* Rafinesque, fed diets containing graded levels of gossypol-acetic acid. *Aquaculture Nutrition*, 16:4, 385-391.

Fonta'inhas-Fernandes, A., Gomes, E., Reis-Henriques, M.A. & Coimbra, J., (1999). Replacement of fish meal by plant proteins in the diet of Nile tilapia: digestibility and growth performance. Aquaculture International, 7, 57-67.

Forster, I., 1999. A note on the method of calculating digestibility coeficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquac. Nutr. 5, 143-145.

Fowler, L.G., (1980). Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and coho salmon. *Prog. Fish-Cult.*, 42, 87-91.

Francis, G., Makkar, H.P.S. & Becker, K., (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199, 197-227.

Gaylord, T. G. & Gatlin III, D. M., (1996). Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). Aquaculture 139, 303-314.

Guimaraes, I. G., Pezzato, L. E., Barros, M. M., (2008). Amino acid availability and protein digestibility of several protein sources for Nile Tilapia, *Oreochromis niliticus*. Aquaculture Nutrition, 14, 396-404.

Hajen, W.E., Beames, R.M., Higgs, D.A., Dosanjh, B.S. (1993). Digestibility of various feedstuffs by post-juvenile chinook salmon (*Onchorynchus tshawytscha)*. in sea water: 1. Validation of technique. *Aquaculture* 112, 321–332.

Lech, G.P., & Reigh R.C., (2012). Plant Products Affect Growth and Digestive Efficiency of Cultured Florida Pompano (*Trachinotus carolinus*) Fed Compounded Diets. *PLoS ONE* 7(4): e34981. doi:10.1371/journal.pone.0034981

Lee, S. M., (2002). Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish(*Sebastes schlegeli*). Aquaculture 207, 79-95

Lui, H., Wu, X., Zhao, M., Xue, M., Guo, L., Zheng, Y., Yu, Y., (2009). Nutrients apparent digestibility coefficients of selected protein sources for juvenile Siberian sturgeon (*Acipenser baerii* Brandt), compared by two chromic oxide analysis methods. Aquaculture Nutrition, 15, 650-656.

Main, K.L., Rhody, N., Nystrom, M. & Resley, M., (2007). Species Profile- Florida Pompano. *Southern Regional Aquaculture Center*. 7206.

McGoogan, B.B., & Reigh, R.C., (1996). Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. *Aquaculture*, 141, 233-244.

Mbahinzireki, G.B., Dabrowski, K., Lee, K.J., El-Saidy, D., & Wisner, E.R., (2001). Growth feed utilization and body composition of tilapia (*Oreochromis* sp.) fed with cottonseed meal- based diets in a recirculating system. *Aquaculture Nutrition*, 7, 189-200.

National Research Council (NRC). (2011). Nutrient Requirements of Fish. National Academy Press,Washington, DC, USA.

Nengas, I., Alexis, M. N., Davies, S. J., Petichakis, G., (1995). Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* L. Aquaculture Res. 26,185-194.

Riche, M., & Williams, T.N., (2010). Apparent digestible protein, energy and amino acid availability of three plant proteins in Florida pompano, *Trachinotus carolinus* L. in seawater and low-salinity water. *Aquaculture Nutrition*, 16, 223-230.

Riche, M., & Williams, T., (2011). Fish meal replacement with solvent extracted soybean meal or soy protein isolate in a practical diet formulation for Florida pompano (Trachinotus carolinus, L.) reared in low salinity. *Aquaculture Nutrition*, 17:4, 368-379.

Robinson, E.H. (1991). Improvement of cottonseed meal protein with supplemental lysine in feeds for channel catfish. *Journal of Applied Aquaculture*, 18,101-106.

Robinson, E.H., & Brent, J.R. (1989). Use of cottonseed meal in channel catfish feeds. *Journal of the World Aquaculture Society*, 20, 250-255.

Robinson, E.H., & Li, M.H. (2008). Replacement of Soybean Meal in Channel Catfish, *Ictalurus punctatus*, Diets with Cottonseed Meal and Distiller's Dried Grains with Solubles. *Journal of the World Aquaculture Society*, 39:4, 521-527.

Robinson, E.H., & Rawles, S.D., (1983). Use of defatted, glandless cottonseed flour and meal in channel catfish diets. *Proceedings of the South-Eastern Association of Fish and Wildlife Agencies*, 37, 358-363.

Rossi Jr., W., & Davis, D.A., (2012). Raplacement of fishmeal with poultry by-product meal in the diets of Florida pompano *Trachinotus carolinus L. Aquaculture*, 338–341, 160–166.

SAS Institute Inc., SAS 9.1, Cary, NC: SAS Institute Inc., 2000-2004.

Serrano, J.A., Nematipour, G.R., & Gatlin, D.M., (1992). Dietray protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. *Aquaculture*, 41, 21-30.

Smith, R.R., Winfree, R.A., Rumsey, G.W., Allred, A. & Peterson, M., (1995). Apparent digestion coefficients and metabolizable energy of feed ingredients for rainbow trout *Oncorhynchus mykiss. Journal of the World Aquaculture Society*, 26, 432-437.

Sullivan, J.A. & Reigh, R.C., (1995). Apparent digestibility if selected feedstuffs in diets for hybrid stripped bass (*Morone saxatilis*♀ x *Morone chrysops*♂). *Aquaculture*, 138, 313-322.

Toko, I.I., Fiogbe, E.D., & Kestemont, P., (2008). Growth, feed efficiency and body mineral composition of juvenile vundu catfish (*Heterobranchus lingifilis*, Valenciennes 1840) in relation to various dietary levels of soybean or cottonseed meals. *Aquaculture Nutrition*, 14, 193-203.

Tucker, C.S. & Robinson, E.H., (1990). Channel Catfish Farming Handbook. Van Nostrand Reinhold, New York, NY, 454.

Watanabe, W.O., (1995). Aquaculture of the Florida pompano and other jacks ((Family Carangidae) in the Western Atlantic, Gulf of Mexico, and Caribbean basin: status and potential. *Culture of High-value Marine Fishes* (Main, K.L., Rosenfeld, C. eds), 185-205. Oceanic Institute, Honolulu, HI, USA.

Weirich, C.R. & Riche, M., (2006). Acute tolerance of juvenile Florida pompano, *Trachinotus carolinus* L. to ammonia and nitrite at various salinities. *Aquac. Res*., 37, 855-861.

Wilson, R.P., (1991). Channel catfish, *Ictalurus punctatus*. In: R.P. Wilson (Editor), Handbook of Nutrient Requirements of Finfish, CRC Press, Boca Raton, FL, 35-53.

Wilson, R. P. & Poe, W.E. (1985). Apparent digestibility of protein and energy in feed ingredients for channel catfish. Prog. Fish Culture., 47, 154-158.