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Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus*×*O. aureus*

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ABSTRACT

An 8-week feeding experiment was conducted to evaluate the effects of replacing soybean meal (SBM) with cottonseed meal (CSM) on growth, feed utilization, and hematological index of juvenile hybrid tilapia. Six isonitrogenous diets (containing ~32% crude protein) containing graded levels of cottonseed meal to replace soybean meal protein were fed to triplicate groups of fish. The diets were supplemented with lysine so that they were similar to the control diet. The results revealed that up to 60% of SBM could be replaced by CSM without causing a significant reduction in growth. Fish fed the diet highest in CSM had a significantly lower protein efficiency ratio and a significantly higher feed conversion ratio than fish fed the other diets. High survival was observed in all dietary treatments, and no significant difference among treatments was observed. The apparent digestibility coefficients (ADC) of dry matter and phosphorus significantly decreased with the increase of dietary CSM level, whereas the ADC of lipid was not affected by dietary treatment. The hepatosomatic index and condition factor were significantly affected by the replacement of SBM by CSM. No significant differences were detected in moisture, lipid, and ash content in whole body and muscle samples, but protein in whole body samples was significantly affected by CSM levels. Significant differences in hemoglobin, hematocrit, red blood cell, and white blood cell content were found in fish fed diets with different CSM levels. These results show that up to 33.76% CSM can be used to replace ~60% of SBM in diets for juvenile hybrid tilapia.

1. Introduction

Soybean meal (SBM) is one of the most nutritious of all plant protein sources (Lovell, 1988). Soybeans are the leading oilseed crop produced globally, and its production for 2004–2005 is expected to exceed 200 mmt (Gatlin et al., 2007). Because of its high protein content, high digestibility, relatively well-balanced amino acid profile, reasonable price, and steady supply, SBM is widely used as a cost-effective feed ingredient for many aquaculture animals (Storebakken et al., 2000); it is currently the most commonly used plant protein source in fish feeds (El-Sayed, 1999). However, other plant protein sources, such as cottonseed meal (CSM), generally cost less than both fish meal and SBM, thus replacing SBM with less expensive plant protein sources would be beneficial in reducing feed costs (Barros et al., 2002).

The annual production of cottonseed is more than 6 million tons in China (Luo et al., 2006). It has been used in diets for both terrestrial animals and fish due to its high protein content. Several studies have been conducted to determine the amount of CSM that can be incorporated into tilapia diets without negative effects on growth (Jackson et al., 1982; Viola and Zohar, 1984; El-Sayed, 1987, 1990; Mbahinzireki et al., 2001; El-Saidy and Gaber, 2004). Results have indicated that the amount of CSM that can be included in the tilapia diet depends mainly on the levels of gossypol, cyclopropionic acids, and available lysine (Jauncey and Ross, 1982; El-Saidy and Gaber, 2004). Viola and Zohar (1984) reported that about 50% CSM successfully replaced SBM in diets fed to hybrid tilapia reared in floating cages.

Tilapia are widely cultured in many tropical and subtropical regions of the world (more than 22 tilapia species are cultured worldwide), and they constitute the third largest group of farmed finfish (after carps and salmonids) (El-Sayed, 1999). In the past, tilapia was consumed mainly in Africa and Asia. In recent years, however, tilapia has been touted as the "new white fish" to replace the depleted ocean stocks of cod and hake (Costa-Pierce, 1997), leading to a worldwide demand for tilapia. As the international trade of tilapia products grew, tilapia aquaculture developed rapidly in China, especially in the south. Because of the high price of fish meal, it was not used or less used in commercial feeds for tilapia in China; instead, SBM was widely used as the main protein source in tilapia feeds.

This study was conducted to: (1) evaluate growth, feed utilization, and digestibility of nutrients of hybrid tilapia diets in which SBM was gradually replaced by solvent-extracted CSM; and (2) evaluate the

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effect of replacing SBM with CSM meal on body composition and hematology of juvenile hybrid tilapia.

2. Materials and methods

2.1. Diet preparation

Six isonitrogenous (containing about 32% crude protein) and isoenergetic experimental diets were formulated; proximate analysis of the diets is given in Table 1. The experimental diets were formulated to produce diets in which 0% (CSM0), 15% (CSM15), 30% (CSM30), 45% (CSM45), 60% (CSM60), and 100% (CSM100) of proteins from SBM were replaced with that from CSM. The diets were supplemented with lysine so that they were similar to the control (CSM0). Fish oil and soybean oil (V/V=1:1) were added to keep lipid and energy constant in all treatments. All of the dry ingredients were thoroughly mixed until homogenous in a Hobart-type mixer, and then water and lipid were added and thoroughly mixed. Next, 2.0 mm diameter pellets were wet-extruded, air-dried to about 10% moisture, and sealed in vacuum-packed bags and frozen (−20 °C) until feeding.

2.2. Animal rearing

Juvenile hybrid tilapia (*Oreochromis niloticus*×*O. aureus*) were obtained from a local tilapia breeding farm. Prior to the start of the feeding trial, fish were acclimated to the experimental conditions and fed a commercial diet (CP=32%, lipid=5.6%) for 2 weeks. At the beginning of the feeding trial, fish (initial weight 6.27±0.12 g) were weighed, measured for body length, and sorted into 18–500-l cylindrical fiberglass tanks, with 20 fish per tank. Three replicate groups of fish were used for each diet. They were provided with a continuous flow of water (2 l/min) with continuous aeration to maintain the dissolved oxygen level above saturation. Fish were fed to apparent satiety three times daily. The amount of feed consumed by the fish in each tank was recorded daily, and rations were adjusted according to feed consumed the previous day. Tanks were cleaned weekly, and the feeding trial lasted for 8 weeks. Water quality parameters were monitored daily between 09:00 and 15:00 h. During the feeding trial, temperature ranged from 28 to 30 °C, ammonia nitrogen was lower than 0.05 mg/l, and dissolved oxygen was not less than 6.0 mg/l.

Table 1
Formulation and proximate analysis of the experimental diets (% dry matter)

Content (%)	Experimental diets					
	CSM0	CSM15	CSM30	CSM45	CSM60	CSM100
Ingredients						
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal	56.00	47.60	39.20	30.80	22.40	0.00
Cottonseed meal	0.00	8.44	16.88	25.32	33.76	56.26
Wheat middlings	32.51	32.51	32.51	32.51	32.51	32.51
Fish oil/soy oil (1:1)	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin mixture[a]	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mixture[b]	0.50	0.50	0.50	0.50	0.50	0.50
Chromium oxide	0.05	0.05	0.05	0.05	0.05	0.05
Lysine	0.00	0.07	0.14	0.21	0.29	0.48
Cellulose	0.74	0.63	0.52	0.41	0.29	0.00
Proximate composition						
Dry matter	89.00	88.73	88.69	88.72	89.06	88.95
Crude protein	31.85	31.77	31.63	31.30	31.19	30.78
Crude lipid	3.58	3.66	3.74	3.81	3.89	4.09
Ash	6.22	6.14	6.15	6.73	6.49	6.63
Phosphorus	0.93	0.97	0.99	1.15	1.12	1.24
Free gossypol (mg kg^{-1})	0.00	7.01	14.02	21.04	28.05	46.74

[a] Vitamin mixture (mg or IU if mentioned/kg diet): retinylacetate, 5000 IU; cholecalciferol, 2000 IU; all-*rac*-a-tocopheryl acetate, 80 IU; menadione sodium bisulfite, 10; thiamin, 10; riboflavin, 5; pyridoxine, 10; D-calcium pantothenate, 50; niacin, 120; choline, 500; biotin, 1; folic acid, 5; myoinositol, 400; vitamin C, 50; vitamin B_{12}, 0.05.
[b] Mineral mixture (mg/kg diet): $FeSO_4 \cdot 7H_2O$, 40; $ZnSO_4 \cdot H_2O$, 150; $MnSO_4 \cdot H_2O$, 25; $GuSO_4 \cdot 5H_2O$, 3; KI, 5; Na_2SeO_3, 0.09; $CoSO_4$, 0.05.

2.3. Fecal collection techniques

Triplicate groups of fish were fed the experimental diets to visual satiety at 18:00 h daily. Two hours after feeding, uneaten feed and fecal residues were removed. Feces were then allowed to settle overnight, fecal samples were collected at 08:00 h each morning prior to the next feeding. Feces collected from the settling columns were immediately filtered with filter paper for 60 min at 4 °C and stored at −18 °C for chemical analyses. Daily fecal samples from each tank were pooled over the course of the experiment until sufficient sample was available for chemical analyses.

2.4. Sample collection and analytical methods

At the termination of the 8-week feeding trial, fish in each tank were individually weighed and sampled for tissue analysis 24 h after the last feeding. Twenty fish at the start were sampled and stored frozen (−18 °C) for analysis of whole body composition. Three to five fish from each tank were used for whole body composition analysis, and the livers and viscera of five fish per tank were weighed for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). Dorsal muscles of fish were sampled, sealed in plastic bags, and stored frozen (−18 °C) until analysis for the muscle nutrient compositions. Blood samples were drawn from the caudal vein of 5–8 fish from each tank; these samples were considered to be replicates and were used to determine blood characteristics according to the methods described by Barros et al. (2002).

Crude protein, crude lipid, moisture, and ash in diets, muscle, and whole body samples were determined following standard methods (AOAC, 1995). Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Hoganos, Sweden). Crude lipid was determined by the ether-extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Moisture was determined by oven drying at 105 °C until a constant weight was achieved. Ash content was measured after placing the samples in a muffle furnace at 550 °C for 24 h. Chromic oxide content in diets and feces was determined by acid digestion with nitric acid and perchloric acid, according to the method described by Zhou et al. (2004). Free gossypol concentration in the diets was determined by high-performance liquid chromatography (HPLC) (Luo et al., 2006).

2.5. Calculations and statistical analysis

The following variables were calculated:

$$\text{Weight gain (WG, \%)} = 100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$$

$$\text{Specific growth ratio (SGR)} = 100 \times \ln(\text{final weight} / \text{initial weight}) / \text{days of the experiment}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed consumed (g, dry weight)} / \text{weight gain (g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g)} / \text{protein intake (g)}$$

$$\text{Condition factor (CF)} = 100 \times (\text{body weight, g}) / (\text{body length, cm})^3$$

$$\text{Hepatosomatic index (HSI)} = 100 \times (\text{liver weight} / \text{whole body weight})$$

$$\text{Viscerosomatic index (VSI)} = 100 \times (\text{viscera weight, g}) / (\text{whole body weight, g}).$$