



Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*

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ABSTRACT

Two consecutive feeding trials were conducted to determine the optimum dietary level of the cottonseed and soybean meal (CS) for replacement of fish meal (FM) in diets for juvenile and on-growing parrot fish with or without iron and phytase. In experiment I, juvenile parrot fish (BW: 3.17 g) were fed one of six experimental diets for 12 weeks which were formulated to replace FM protein by equal proportion (1:1, w:w) of CS at 0, 10, 20, 30, 40, or 50% (designated as CS0, CS10, CS20, CS30, CS40, or CS50, respectively). In experiment II, on-growing parrot fish (BW: 55 g) were fed one of five experimental diets for 9 weeks. The experimental diets were formulated to replace FM protein by CS at 0, 20, or 30% (designated as CS0, CS20, or CS30, respectively) and to include ferrous sulfate (0.1 and 0.2%) and phytase into the CS20 and CS30 diets (CS20 + Fe&P and CS30 + Fe&P, respectively). Results from the two feeding trials indicated that the CS could replace up to 20% FM protein in diets for juvenile parrot fish (3–22 g) and up to 30% in on-growing (55–120 g) parrot fish. The supplemental effect of iron and phytase was not significant on growth performance. Total or each gossypol enantiomer concentration in the liver increased as the level of dietary cottonseed meal increased. However, gossypol in the liver of fish fed diets supplemented with iron was not detected. The dietary supplementation of CS significantly reduced the levels of plasma triacylglycerols and total cholesterol. The present study indicates that plant protein sources are better utilized in larger fish, and that up to approximately 30% FM protein could be replaced by CS with iron and phytase in the presence of supplemental lysine and methionine.

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1. Introduction

Fish meal (FM) is a major protein source in aquafeeds especially for carnivorous fish species because it is an excellent source of essential nutrients such as indispensable amino acids, essential fatty acids, vitamins, minerals, attractants and unknown growth factors (Zhou et al., 2004). However, increasing demand, unstable supply and high price of the FM with the expansion of aquaculture made it necessary to search for alternative protein sources (FAO, 2004; Lunger et al., 2007).

Defatted soybean meal (SM) has been the most frequently studied ingredient as a FM replacer in diets for many fish species, due to its high protein content, relatively well-balanced amino acid profile, reasonable price and steady supply (Storebakken et al., 2000). Several studies have shown promising SM results in aquafeed formulation for carnivorous as well as herbivorous fish species. Data have shown that approximately 20 to 40% FM protein can be replaced in diets for

carnivorous fish species (Chou et al., 2004; Lim et al., 2004; Hernandez et al., 2007; Pham et al., 2007; Lim and Lee, 2008).

Cottonseed meal (CM) has long been used in diets for both terrestrial animals (Colin-Negrete et al., 1996) and fish (Hendricks et al., 1980) because of its high protein content, availability and low cost. CM has been tested in numerous fish species such as rainbow trout (Cheng and Hardy, 2002), channel catfish (Robinson and Li, 1994), tilapia (El-Sayed, 1990; Mbahinzireki et al., 2001), largemouth bass (Kurten et al., 1999) and sunshine bass (Rawles and Gatlin, 2000). However, when compared to FM, a mixture of CM and SM (CS) may result in several problems including lower crude protein level, suboptimal levels of certain essential amino acids, palatability issues and the presence of antinutritional factors (NRC, 1993; Storebakken et al., 2000; Imorou et al., 2007). Phytic acid content is the main limiting factor in using plant protein sources in diets for most monogastric animals including fish. Most plant protein sources used in fish diets contain phytic acid in the range of 5 to 30 g kg⁻¹ (Reddy, 2002) and approximately 70% of their total phosphorus content is bound as phytate (Lall, 1991) which is not available for fish. Supplemental phytase (Biswas et al., 2007; Pham et al., 2008) has been used to liberate free phosphorus from phytate (Albrektsen et al., 2006; Lim and Lee, 2008) in diets containing plant protein sources.

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Table 1
Formulation and proximate composition of diets used in experiment I (% dry matter).

Ingredients	Diets					
	CS0	CS10	CS20	CS30	CS40	CS50
White fish meal	52.0	46.8	41.6	36.4	31.2	26.0
Soybean meal	0.0	3.8	7.6	11.5	15.3	19.1
Cottonseed meal ^a	0.0	4.0	8.1	12.1	16.1	20.1
Corn gluten meal	8.0	7.7	7.4	7.1	6.8	6.5
Wheat flour	6.5	6.5	6.5	6.5	6.5	6.5
Starch	16.0	13.7	11.4	9.1	6.8	4.5
Yeast	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mix ^b	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mix ^c	1.0	1.0	1.0	1.0	1.0	1.0
Squid liver oil	11.0	11.3	11.5	11.7	12.0	12.3
Lysine ^d	0.0	0.1	0.2	0.3	0.4	0.5
Methionine ^e	0.0	0.1	0.2	0.3	0.4	0.5
Cellulose	2.5	2.0	1.5	1.0	0.5	0.0
<i>Chemical analyses</i>						
Crude protein, % DM	46.3	46.1	46.3	46.4	46.5	46.8
Crude fat, % DM	16.1	15.4	15.6	16.0	16.2	16.9
Ash, % DM	7.9	7.8	7.5	7.3	7.2	7.2
Fiber, % DM ^f	1.1	1.8	2.5	3.2	3.9	4.6
NFE, % DM ^g	28.6	28.9	28.1	27.1	26.2	24.5
Gross energy, MJ/kg DM ^h	22.2	21.9	21.9	21.9	21.9	21.9
Total gossypol ($\mu\text{g g}^{-1}$) ⁱ	Nd ^j	316	507	858	1016	1274
(+)-Enantiomer	Nd	192	317	528	623	783
(-)-Enantiomer	Nd	124	190	330	393	491

^a Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, TN, USA.

^b Mineral premix (g kg^{-1} mixture) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 80.0; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 20.0; Ca-lactate, 356.5; CuCl₂, 0.2; AlCl₃ · 6H₂O, 0.15; Na₂Se₂O₃, 0.01; MnSO₄ · H₂O, 2.0; CoCl₂ · 6H₂O, 1.0.

^c Vitamin premix (g kg^{-1} mixture) L-ascorbic acid, 121.2; DL- α -tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

^d L-lysine mono-hydrochloride, Sigma, USA.

^e L-methionine, Sigma, USA.

^f Fiber content was calculated based on fiber contents of white fish meal, soybean meal, cottonseed meal, corn gluten meal, wheat flour and yeast.

^g Nitrogen-free extract (NFE) = 100-(% protein + % lipid + % ash + % fiber).

^h Gross energy of experimental diets was calculated according to gross energy values 5.64 kcal/g crude protein, 4.11 kcal/g carbohydrate, and 9.44 kcal/g crude fat, respectively (NRC, 1993).

ⁱ Total gossypol includes free and bound gossypol.

^j nd: not detected.

Additionally, CM contains gossypol which is toxic to fish (Herman, 1970) leading to a restriction of its use as a fish feed ingredient.

Parrot fish, a subtropical marine fish, is carnivorous species and has been regarded as an emerging aquaculture species because of its high economic value, excellent meat quality and strong resistance to diseases. However, nutritional information on this species is limited (Kang et al., 1998; Wang et al., 2003) and no data are available on the dietary utilization of the CS. We have recently reported that CS with iron and phosphorus supplementation could replace up to 40% FM protein in diets for juvenile olive flounder in long-term feeding trial of over 6 months (Lim and Lee, 2008). That study indicated a potentiality of CS for FM replacement in diets for carnivorous marine fish species. Therefore, two consecutive feeding trials were conducted to determine the optimum dietary level of the CS with or without iron and phytase in the presence of supplemental lysine and methionine for the FM replacement in two different growth phases of parrot fish.

2. Materials and methods

Two consecutive feeding trials were conducted to determine the optimum dietary inclusion level of an equal mixture of cottonseed and soybean meal (CS) for replacement of FM in diets for juvenile (experiment I) and on-growing (experiment II) parrot fish with or without iron and phytase.

2.1. Experimental diets

In experiment I, six experimental diets were formulated to replace FM protein by equal proportion (1:1, w:w) of cottonseed and soybean meal (CS) at 0, 10, 20, 30, 40, or 50% (designated as CS0, CS10, CS20, CS30, CS40, or CS50, respectively). The CS containing diets were supplemented with L-methionine and L-lysine to meet their estimated dietary requirements (NRC, 1993). The dietary formulation, proximate composition and gossypol content are presented in Table 1. All diets were formulated to be isonitrogenous (46% crude protein) and isocaloric (22 MJ/kg diet). Solvent extracted cottonseed meal was provided by Southern Cotton Oil Co., Memphis, TN, USA. Its protein and fiber content were 43.5% and <12% on a dry matter basis, respectively. All dry materials were thoroughly mixed with 30% double distilled water, extruded through a meat chopper machine (SMC-12, Kuposlice, Busan, Korea) at 5 mm in diameter, freeze-dried at -40°C for 24 h and stored at -20°C until use.

In experimental II, diets were formulated to replace FM protein by equal proportion (1:1, w:w) of CS at 0, 20, or 30% (designated as CS0, CS20, or CS30, respectively) and formulated with supplementation of ferrous sulfate (0.1 and 0.2%) and phytase in the CS20 and CS30 diets (designated as CS20 + Fe&P and CS30 + Fe&P). Microbial phytase at 1000 FTU kg diet⁻¹ was used in the diets as described by Cheng and Hardy (2003) and Yoo et al. (2005). The CS containing diets were also supplemented by L-methionine and L-lysine, the same levels as in the experiment I (Table 2). The diets were formulated to be isonitrogenous (46% crude protein) and isocaloric (22 MJ kg diet⁻¹).

Table 2
Formulation and proximate composition of diets used in experiment II (% dry matter).

Ingredients	Diets				
	CS0	CS20	CS30	CS20 + Fe&P	CS30 + Fe&P
White fish meal	52.0	41.6	36.4	41.6	36.4
Soybean meal	0.0	7.7	11.5	7.7	11.5
Cottonseed meal ^a	0.0	8.1	12.1	8.1	12.1
Corn gluten meal	8.5	7.9	7.6	7.9	7.6
Wheat flour	6.5	6.5	6.5	6.5	6.5
Starch	18.3	13.6	11.3	13.6	11.3
Mineral mix ^b	1.0	1.0	1.0	1.0	1.0
Vitamin mix ^c	1.0	1.0	1.0	1.0	1.0
Squid liver oil	11.0	11.5	11.8	11.5	11.8
Lysine ^d	0.0	0.2	0.3	0.2	0.3
Methionine ^e	0.0	0.2	0.3	0.2	0.3
Ferrous sulfate 7H ₂ O	0.0	0.0	0.0	0.1	0.2
Phytase ^f	0.0	0.0	0.0	0.01	0.01
Cellulose	1.7	0.7	0.2	0.6	0.0
<i>Chemical analyses</i>					
Crude protein, % DM	46.0	45.7	45.8	45.5	46.1
Crude fat, % DM	15.8	16.0	16.1	15.9	15.9
Ash, % DM	8.0	7.5	7.3	7.6	7.8
Fiber, % DM ^g	1.1	2.4	3.1	2.4	3.1
NFE, % DM ^h	29.1	28.4	27.7	28.6	27.1
Gross energy, MJ kg ⁻¹ DM ⁱ	22.1	22.0	21.9	21.9	21.8
Total gossypol (mg/kg) ^j	Nd ^k	461	661	439	695
(+)-Enantiomer	Nd	319	461	303	477
(-)-Enantiomer	Nd	142	200	136	218

^a Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, TN, USA.

^b See the footnote in Table 1.

^c See the footnote in Table 1.

^d L-lysine mono-hydrochloride, Sigma, USA.

^e L-methionine, Sigma, USA.

^f Phytase (10,000 FTU/g) was purchased from Easy Bio System, Inc., Seoul, Korea.

^g Fiber content was calculated based on fiber contents of white fish meal, soybean meal, cottonseed meal, corn gluten meal and wheat flour.

^h Nitrogen-free extract (NFE) = 100-(% protein + % lipid + % ash + % fiber).

ⁱ Gross energy of experimental diets was calculated according to gross energy values 5.64 kcal/g crude protein, 4.11 kcal/g carbohydrate, and 9.44 kcal/g crude fat, respectively (NRC, 1993).

^j Total gossypol includes free and bound gossypol.

^k nd: not detected.

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