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Fish Meal Replacement by Cottonseed and Soybean Meal in Diets for Juvenile Olive Flounder, *Paralichthys olivaceus*

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This study was conducted to investigate the effects of dietary supplementation of cottonseed and soybean meal on growth performance of juvenile olive flounder (*Paralichthys olivaceus*). Nine hundred fish (0.74 ± 0.11 g) in the early juvenile stage were randomly divided into 15 groups, and 3 groups were fed one of five isonitrogenous (56% CP) and isocaloric (16.3 MJ kg $^{-1}$) diets replacing 0, 10, 20, 30, and 40% of fish meal protein by equal proportion (1:1, w:w) of cottonseed and soybean meal (CS) (designated by Control, CS10, CS20, CS30, and CS40, respectively). A solvent extracted cottonseed meal containing high crude protein (44%) and low fiber content (<12%) was used in this study. After 10 weeks of feeding trial, the growth of fish fed diets CS10, CS20, and CS30 were not significantly (P>0.05) different compared to that of fish fed the control diet. However, diet CS40 exhibited significantly lower (P<0.05) growth performance than the control diet. No differences were observed in whole body composition of fish fed all the experimental diets. This study indicates that mixture of cottonseed and soybean meal with lysine and methionine supplementation can replace up to 30% fish meal protein in diet for olive founder at the early juvenile stage. However, we suggest that 20% of fish meal protein replacement by cottonseed (10%) and soybean (10%) meal can be the optimum level for commercial use in safety according to the growth performances.

Keywords: Olive flounder, Fish meal replacement, Cottonseed meal, Soybean meal

Introduction

Olive flounder (*Paralichthys olivaceus*) is currently the most important marine aquaculture species in Korea. Its aquaculture production increased from 1,037mt in 1990 to 34,533mt in 2004 (Ministry of Maritime Affairs and Fisheries, 2004). However feed costs have been identified as a major constraint for the aquaculture development in this species. Coyle et al. (2004) reported that feed costs account for over 50% of total production costs in most marine cultured species, because of the use of the expensive fish meal with a large dietary proportion. To reduce the feed costs and increase the economic benefit for fish farmers, many studies have been conducted to seek less expensive protein sources to replace fish meal (Kikuchi et al., 1997; Kikuchi, 1999; Choi et al., 2004).

Hardy (1995) reported that dietary replacement of fish meal by plant origin by products have been increasing in aquaculture industry, due to their lower price, highly market availabilities, and sufficient protein contents. Among plant origin protein sources, soybean meal has been considered as a promising protein source that can completely or partially replaces fish meal in aquatic animal diets. A number of studies have shown that soybean meal alone or in combination with other protein sources can replace fish meal from 20% up to 90% in diets for many fish species such as yellow tail (*Seriona quinqueradiata*) (Shimeno et al., 1993), red drum (*Sciaenops ocellatus*) (McGoogan and Gatlin III, 1997), sea-bass (*Lates calcarifer*) (Boonyaratpalin et al., 1998), rainbow trout (*Oncorhychus mykiss*) (Gomes et al., 1995), Australian snapper (*Pagrus auratus*) (Quantararo et al., 1998), tin foil barb (*Barbodes altus*) (Elangovan and Shim, 2000), mangrove red snapper (*Lutjanus argentimaculatus* Forsskal 1775) (Catacutan and Pagador, 2004), cobia (*Rachycentron canadum*) (Chou et al., 2004), and olive flounder (Kikuchi, 1999). However, the use of soybean meal in fish feeds is still limited because of the presence of some anti nutritional factors such as protease inhibitors, phytates, lectins, saponins, non-starch polysaccharide and high fiber content (NRC, 1993; Storebakken et al., 2000; Hendricks, 2002). In addition, the deficiency of some essential

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amino acids in soybean meal such as methionine and lysine also reduces the inclusion level of this material in fish feeds (NRC, 1993).

By-products of cottonseed are used in diets for both terrestrial animals (Colin-Negrete et al., 1996) and fish (Hendricks et al., 1980) because of its high protein content. Cottonseed meal (CM) has been examined in diets for fish such as channel catfish, *Ictalurus punctatus* (Dorsa et al., 1982; Robinson and Brent, 1989; Robinson and Li, 1994; Robinson and Tiersch, 1995), rainbow trout (Hendricks et al., 1980; Dabrowski et al., 2000; Lee et al., 2001; Lee and Dabrowski, 2002a; Rinchard et al., 2003), and tilapia (El-Sayed, 1990; Robinson et al., 1984; Mbahinzireki et al., 2001; Rinchard et al., 2002). Despite its high nutritional value, cottonseed contains gossypol, polyphenolic compound, which is toxic to fish (Herman, 1970; Rinchard et al., 2000) and terrestrial animals (Colin-Negrete et al., 1996; Makinde et al., 1997). Studies on the use of cottonseed meal for fish meal replacement in marine fish species are very rare. Therefore, the aim of this study was to investigate the use of cottonseed and soybean meal with methionine and lysine supplementation as a partial substitute for dietary fish meal protein in olive flounder at the early juvenile stage.

Materials and Methods

Experimental diets

Five experimental diets were formulated to be isonitrogenous and isocaloric in term of crude protein (56%) and gross energy (16.3 MJ/kg) (Table 1). The energy value of each diet was estimated on the basis of mammalian physiological fuel value, i.e., 16.7 KJ/g protein or carbohydrate and 37.7 KJ/g lipid (Lee and Putman, 1973). The diet formulation and proximate compositions are presented Table 1. The five experi-

Table 1. Formulation and proximate composition of experimental diets (% dry matter)

	Diets				
	CS0	CS10	CS20	CS30	CS40
Ingredients					
White fish meal	60.0	54.0	48.0	42.0	36.0
Soybean meal	0.0	4.4	8.7	13.1	17.5
Cotton seed meal[1]	0.0	4.7	9.4	14.1	18.8
Corn gluten meal	8.0	8.3	8.7	9.0	9.3
Wheat flour	21.8	17.8	13.8	9.8	5.8
Yeast	2.0	2.0	2.0	2.0	2.0
Mineral mix[2]	1.0	1.0	1.0	1.0	1.0
Vitamin mix[3]	1.0	1.0	1.0	1.0	1.0
Choline chloride	0.2	0.2	0.2	0.2	0.2
Squid liver oil	5.0	5.4	5.8	6.2	6.6
CMC	1.0	1.0	1.0	1.0	1.0
Lysine[4]	0.0	0.1	0.2	0.3	0.4
Methionine[5]	0.0	0.1	0.2	0.3	0.4
Proximate composition & gossypol concentration					
Dry matter, %	92.2	91.3	90.2	91.0	93.7
Protein, % DM	56.8	56.6	56.5	56.6	56.3
Lipid, % DM	12.0	12.6	12.5	12.6	12.1
Ash, % DM	10.1	9.9	9.5	9.1	8.9
Gross energy, MJ/kg DM	16.3	16.3	16.3	16.3	16.3
Total gossypol, % DM[6]	0	0.08	0.16	0.24	0.32

[1]Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, Tennessee 38108, USA.
[2]$MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3.6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.
[3]L-ascorbic acid, 121.2; DL-a 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-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.
[4]L-lysine mono-hydrochloride, Sigma, USA.
[5]L- methionine, Sigma, USA.
[6]Total gossypol in the experimental diets are calculated based on the gossypol concentration in cottonseed meal.

Table 2. The proximate composition of major ingredients used in experimental diets

Ingredients	Moisture	Protein	Lipid	NFE [1]	Ash
White fish meal	8.72	68.33	8.56	0.32	14.07
Soybean meal	7.59	46.91	2.52	36.44	6.54
Cottonseed meal [2]	11.40	43.54	3.18	34.52	7.36
Corn gluten meal	9.50	61.70	1.03	26.59	1.18
Yeast	5.49	42.15	0.49	46.25	5.62

[1]Nitrogen-free extract = 100 - (%moisture + %CP + %lipid + %ash).
[2]Cottonseed meal was purchased from Southern Cotton Oil Co., Memphis, Tennessee 38108, USA.

mental diets were formulated to replace 0, 10, 20, 30, and 40% of fish meal protein by equal proportion (1:1, w:w) of cottonseed and soybean meal (CS) (designated by Control, CS10, CS20, CS30, and CS40, respectively). The CS diets were supplemented by L-methionine and L-lysine to meet their dietary requirements (NRC, 1993). The cottonseed meal was provided from Southern Cotton Oil Co., Memphis, TN, USA, and its protein content was 43.5% in dry mater basis (Table 2). The proximate compositions of other protein sources used in this study are given in Table 2. The cottonseed meal were solvent extracted meals and total gossypol concentrations were 1.65%. Experimental diets were cold-pelleted through the meat chopper machine (SMC-12, Korea) in 3.0 mm diameter size, freeze dried to approximately 5% moisture, crushed into desirable particle sizes (0.4–2.0 mm), and stored at –20°C until use.

Fish, facility and feeding trial

Olive flounder juveniles were transported from a private hatchery in Jeju Island to Marine and Environmental Research Institute, Cheju National University. The fish were fed with a commercial diet for 4 weeks to allow adapting to experimental conditions. Then, total 900 fish at the early juvenile stage were randomly distributed into 15 plastic circular tanks (35 L) at a density of 60 fish/tank (initial body weight 0.74±0.11 g). Each experimental diet was fed to triplicate groups of the fish with the feeding rates ranging from 5% of fish weight at the beginning to 3% at the end of feeding trial. The fish were fed twice (9:00 and 17:00) a day, 7 days a week, for 10 weeks. The feeding trial was conducted in a flow through system supplied with sand filtered seawater at a flow rate of 2–3 L/min. Supplemental aeration was also provided to maintain dissolved oxygen levels near the saturation. The growth of fish was measured every two weeks and feeding rate was adjusted accordingly. Feeding was stopped 24 h prior to weighing.

Sample collection and analysis

At the end of feeding trial, all fish were weighed and counted to calculate percent body weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), specific growth rate (SGR). Three fish from each tank (9 fish per diet) were sampled and stored at -20°C for whole body proximate analysis. Analyses of crude protein, moisture, and ash were performed by the standard procedures (AOAC, 1995). Lipids were determined according to the method described by Folch et al. (1957) with some modifications.

Gossypol content in the cottonseed meal was determined by High Performance Liquid Chromatography (HPLC) according to the method described by Kim and Calhoun (1995) with some modifications (Lee and Dabrowski, 2002b). Briefly, the cottonseed meal was weighed, and 5–10 volumes of complexing reagent added to obtain the 2-amino-1-propanol derivatives of gossypol. The complexing reagent was composed of 2 ml 2-amino-1-propanol (Sigma Chemical, St. Louis, MO), 10 ml glacial acetic acid (Sigma Chemical) and 88 ml N, N-dimethylformamide (Sigma Chemical). The samples were homogenized in complexing reagent for 30 sec, heated at 95°C for 30 min, cooled on ice, and then centrifuged at 1500×g for 5 min. After centrifugation, an aliquot of the supernatant was diluted with mobile phase to obtain a desirable concentration, centrifuged again at 1500×g for 5 min, and filtered through a syringe filter (0.45 μm, Whatman Inc., Clifton, NJ, USA) before injection to HPLC.

Statistical analysis

Data were subjected to one-way ANOVA in SPSS version 11.0. The significant differences between group means were compared using Duncan's multiple test. Data presented are means±standard deviations. The percentage data of weight gain and specific growth rate were arcsine transformed before the ANOVA analysis. Differences were considered significant at $P<0.05$.

Results and Discussion

In this experiment, the crude protein (56% DM) and energy content (16.3 MJ/kg DM) of diets were formulated based on the protein and energy requirement of olive flounder juveniles suggested by Kim et al. (2002) and Choi et al. (2004). The growth and feed utilization of fish fed experimental diets are presented in Table 3. After 10 weeks of feeding trial, the growth of fish fed diets CS10, CS20, and CS30 was not significant different compared to that of fish fed control diet. However, CS40 diet exhibited significantly lower growth performance than the control diet. The same trend was observed in feed conversion ratio. Meanwhile, there were no significant differences in specific growth rate, protein efficiency ratio, feed intake, and nitrogen retention of fish fed all experimental diets. The survivals of fish fed all experimental diets were over 80% and not significantly different. In the present study, it seemed that dietary incorporation of cottonseed (14%) and soybean meal (13%) with L-lysine and L-methionine do not affect the dietary palatability and thereby do not impair the growth of juvenile olive flounder. This finding is in agreement with another studies on a mixture of cottonseed and soybean meal with animal by-products as fish meal substitutes (Lee et al., 2002; Chou et al., 2004). However, the dietary inclusion level of cottonseed meal in the present study was lower than that reported by Mbahinzireki et al. (2001). In the 16 weeks of feeding trial, Mbahinzireki et al. (2001) found that up to 50% fish meal protein could be replaced by cottonseed meal in tilapia diets without any adverse effect on fish growth performance. Whereas, Cheng and Hardy (2002) reported that only 5% to 10% of fish meal protein can be replaced by cottonseed meal in diet for rainbow trout fingerlings (initial body weight, 11.2 g). It is apparent that level of cottonseed meal inclusion in fish diets widely varies among fish species. In addition, the incorporation level of cottonseed meal in fish diet also depends on the developmental stage of the fish. Lee et al. (2002) reported that only 15% fish meal can be replaced by cottonseed meal in diets for rainbow trout fingerlings (initial body weight, 0.96 g). Meanwhile, adult rainbow trout fed a diet replacing 50% fish meal protein by cottonseed meal did not show any significant differences compared to fish fed fish meal based diet (Blom et al., 2001). Therefore, long-term feeding trials on the use of cottonseed meal and soybean meal in olive flounder diet are necessary.

In the present study, the lower growth performances of fish fed CS40 diet compared to control diet might be a consequence of some anti-nutritional factors present in cottonseed meal and soybean meal such as gossypol, protease inhibitors, and phytic acid. Gossypol, a yellow pigment found in the gland of cottonseed, which has been demonstrated to be toxic for many fish species (Dorsa et al., 1982; Dabrowski et al., 2000; Lee et al., 2002; Garcia-Abiado et al., 2004). The toxicity of gossypol depends on several factors including the form of gossypol (free or bound), the amount of consumption, and varieties of the cottonseed meal. In this experiment, the total gossypol content in diet CS40 (18.8% dietary cottonseed meal) might be higher than the tolerant level of gos-

Table 3. Growth performance of juvenile olive flounder fed different experimental diets for 10 weeks[*]

	Diets				
	CS0	CS10	CS20	CS30	CS40
Initial body weight (IBW, g)	0.74 ± 0.11	0.74 ± 0.11	0.74 ± 0.11	0.74 ± 0.11	0.74 ± 0.11
Final body weight (FBW, g)	11.25 ± 0.94[a]	10.33 ± 2.12[ab]	10.63 ± 0.51[ab]	9.46 ± 0.62[ab]	8.47 ± 0.60[b]
Weight gain (WG)[1]	1419 ± 226[a]	1296 ± 286[ab]	1337 ± 69[ab]	1179 ± 84[ab]	1045 ± 81[b]
Specific growth rate (SGR)[2]	1.15 ± 0.04	1.06 ± 0.14	1.06 ± 0.0.9	1.01 ± 0.03	0.99 ± 0.04
Nitrogen retention (NR)[3]	27.02 ± 2.20	25.58 ± 0.69	26.51 ± 0.58	24.86 ± 1.65	24.35 ± 0.10
Protein efficiency ratio (PER)[4]	1.64 ± 0.11	1.56 ± 0.03	1.58 ± 0.05	1.53 ± 0.10	1.52 ± 0.01
Feed conversion ratio (FCR)[5]	1.02 ± 0.14[a]	1.09 ± 0.12[a]	1.12 ± 0.14[a]	1.24 ± 0.13[a]	1.36 ± 0.06[b]
Feed intake, (g/g BW)[6]	0.91 ± 0.05	0.92 ± 0.06	0.92 ± 0.02	0.91 ± 0.01	0.91 ± 0.01
Survival (%)	81.1 ± 5.85	85.5 ± 0.71	86.1 ± 3.47	88.3 ± 1.67	90.0 ± 4.71

[*]Values are presented as mean ± sd. Value in the same row having different superscript letters is significantly different ($P<0.05$).
[1]WG (%) = 100 x (final mean body weight - initial mean body weight)/ initial mean body weight.
[2]SGR (%) = [(loge final body weight - loge initial body weight)/days] × 100.
[3]NR (%) = 100 x (FBW x final CP - IBW x initial CP)/ CP intake.
[4]PER = wet weight gain/ total protein given.
[5]FCR = dry feed fed/ wet weight gain.
[6]FI (g/g body weight) = dry feed consumed (g)/ body weight (g).

Table 4. Whole body composition of juvenile olive flounder fed different experimental diets for 10 weeks*

	Initial	Diets				
		CS0	CS10	CS20	CS30	CS40
Moisture, %	81.9 ± 0.21	76.5 ± 1.55	78.7 ± 1.63	77.6 ± 0.26	78.6 ± 0.62	78.7 ± 0.17
Protein, % DM	61.5 ± 0.00	67.1 ± 0.72	69.1 ± 0.11	69.2 ± 0.84	68.4 ± 1.38	67.5 ± 0.76
Lipid, % DM	7.18 ± 0.78	9.52 ± 1.13	8.12 ± 0.98	9.05 ± 0.69	8.02 ± 0.95	8.54 ± 0.08
Ash, % DM	21.7 ± 0.76	16.6 ± 0.63	15.8 ± 0.04	15.8 ± 0.06	16.1 ± 0.46	16.9 ± 1.53

*Values are presented as mean±sd. Value in the same row having different superscript letters is significantly different ($P<0.05$).

sypol of olive flounder at the early juvenile stage and might have resulted in the decrement of fish growth. Moreover, phytic acid, IP 6-inositol hexaphosphate, presents in both cottonseed and soybean meal also has been reported as a major anti-nutrient factor that limits the utilization of the ingredients in fish diets (Bransden and Carter, 1999; Lee et al., 2002; Barual et al., 2004; Riche and Garling JR, 2004). Owning to its unique chemical structure, phytic acid has ability to combine with other minerals, such as calcium, magnesium, and zinc, which reduce biological availability of these nutrients in mono-gastric animals including fish (NRC, 1993; Storebakken et al., 2000). It is well known that the gossypol molecule can easily combine with lysine resulting in the deficiency of lysine. In the present study, L-methionine and L-lysine were supplemented in the experimental diets containing cottonseed and soybean meal to meet the amino acids requirement of fish. The lower growth performances of fish fed the CS40 diet could be attributed to deficiency of some minerals, such as zinc, iron, and phosphorus. Therefore, studies on the supplementation of the minerals in the diets containing both cottonseed and soybean meal are recommended in olive flounder.

Recently, the use of mixture of plant protein meals has been reported to be superior to single one. The essential amino acid profile in multiple plant protein might be able to meet their requirements in many cultured fish species. In addition, the level of anti-nutritional substances in individual plant protein source can be reduced during feed processing (Riche et al., 2001). Pongmaneerat and Watanabe (1993) reported that mixture of soybean meal and corn gluten meal can replace up to 63% fish meal protein in rainbow trout diet. Tilapia fingerlings (initial body weight, 3.7 g) were fed diets containing 100% protein from plant meals (El-Saidy and Gaber, 2003). After sixteen weeks, the authors concluded that the plant protein mixture consisting of 25% soybean meal, 25% cottonseed meal, 25% sunflower meal, 25% linseed meal and 0.5% both methionine and lysine supplementation can completely replace fish meal without any significant differences in growth performance.

Whole body compositions of fish fed experimental diets are given in Table 4. There were no significant differences in moisture, crude protein, crude lipid, and ash contents of whole body of fish fed all the experimental diets after ten-week feeding period. The result indicated that up to 40% fish meal protein replaced by both cottonseed and soybean meal by equal proportion (1:1, w:w) with L-methionine and L-lysine supplementation did not affect the whole body composition of olive flounder at the early juvenile stage.

In conclusion, the mixture of cottonseed and soybean meal with L-methionine and L-lysine supplementation can replace up to 30% fish meal protein in diet for olive flounder at the early juvenile stage based on growth performance, survival, and whole body composition during 10 week feeding trial. However, we suggest that 20% of fish meal protein replacement by cottonseed (10%) and soybean (10%) meal protein could be the optimum level for commercial use in safety according to the trends in growth performances.

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