



Long Term Feeding Effects of Dietary Dehulled Soybean Meal as a Fish Meal Replacer in Growing Olive Flounder *Paralichthys olivaceus**

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ABSTRACT : This experiment was conducted to evaluate the long term effects of dehulled soybean meal (DHSM) as fish meal (FM) replacer in the diet for olive flounder, *Paralichthys olivaceus*, for 42 wk at a commercial fish farm. The four experimental diets were MP (moist pellet based on raw fish), DHSM₀ (fish meal based dry pellet), DHSM₃₀ (30% fish meal was replaced by DHSM) and COMD (commercial dry pellet). Fish were fed one of the four diets containing 50% crude protein and 16.7 kJ available energy/kg diet for 42 wk. Fish averaging 3.20±0.04 g (mean±SD) were initially distributed to each tank as a group of 6,000 fish reared in a flow-through system. At the end of the experiment, average body weight of fish was 315±9.3 g (mean±SD). Weight gain (WG) and feed efficiency (FE) of fish fed MP, DHSM₀ and DHSM₃₀ showed no significant difference among the diet treatments. However, WG and FE of fish fed MP were significantly higher than those of fish fed COMD (p<0.05). Specific growth rate (SGR), protein efficiency ratio (PER) and survival of fish fed all diets were not significantly different among the dietary treatments. Therefore, these results indicate that DHSM could replace up to 30% white fish meal with amino acid supplementation in olive flounder diets for long-term feeding practice at the commercial farm. (**Key Words** : Dehulled Soybean Meal (DHSM), Fish Meal (FM) Replacer, Long Term, Olive Flounder)

INTRODUCTION

Fish meal (FM) has traditionally been a major ingredient in fish diets because of its high protein quality and palatability, but is one of the most expensive ingredients in formulated fish diets. Lee and Bai (1997) noted that the world supply of FM increased by only about 27% during the past two decades and FM output by the major FM-producing countries actually declined. Because of limited supply of FM around the world coupled with the increasing demand, the cost of producing fish is likely to increase. For this reason, many studies have been conducted to replace or reduce FM inclusion in fish diets using less expensive alternative protein sources. Among several

protein feedstuffs, soybean meal (SM) is considered to be the most nutritious replacer because of its generally favorable protein content and amino acid (AA) profile (McGoogan and Gatlin III, 1997; Kikuchi, 1999). There have been numerous studies on the utilization of SM in the diet for various species of fish such as grass carp, yellowtail, rainbow trout, Atlantic salmon, olive flounder and Korean rockfish (Dabrowski and Kozak, 1979; Shimeno et al., 1993; Carter and Hauler, 2000; Choi et al., 2004; Lim et al., 2004). However, few studies have evaluated dehulled soybean meal (DHSM) in olive flounder feed, and few data can be found on DHSM as FM replacer for long-term feeding practice at commercial farm level.

Olive flounder is one of the commercially important fish species in Korea. Its production is topmost among the Korean mariculture finfish species. Culture of olive flounder has increased rapidly in the last 20 years in Korea from an annual production of 3 MT in 1981 to 43,724 MT in 2006 (MOMAF, 2006). Concurrently, the production of formulated feeds of olive flounder also increased to 116,659 MT in 2006 (MOMAF, 2006).

Therefore, the purpose of this study was to evaluate the long-term effects of DHSM as fish meal replacer in a practical diet for olive flounder at the commercial farm level.

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Table 1. Ingredients and proximate analysis of the experimental diets (% of dry matter basis)¹

Ingredients	Diets ¹			
	MP	DHSM ₀ ²	DHSM ₃₀ ²	COMD
White fish meal ²	-	60.0	42.0	Closed
Corn gluten meal ²	-	6.9	5.0	
Soybean meal ³	-	0.0	26.5	
Wheat meal ²	-	8.2	8.2	
Dextrin ²	-	11.9	2.55	
Methionine ²	-	0.0	0.50	
L-lysine-HCl ²	-	0.0	0.56	
Squid oil ²	-	7.5	8.2	
Vitamin premix ²	-	1.0	1.0	
Mineral premix ²	-	3.0	3.0	
CMC ⁴	-	0.0	1.0	
Cellulose ²	-	0.03	0.52	
Attractant ⁵	-	1.0	0.5	
Compound feed ⁶	20.0			
Frozen mackerel ⁶	80.0			
Proximate analysis				
Moisture	65.4	7.60	7.52	8.32
Crude protein	45.4	52.3	52.1	51.7
Crude lipid	24.4	11.2	11.9	12.3
Crude ash	19.5	10.9	9.8	10.3

¹ MP = Raw fish: powder compound feed (8:2); DHSM₀ = 100% fish meal based diet; DHSM₃₀ = 30% fish meal was replaced by DHSM; COMD = Commercial diet.

² Dong-Sun Co., Seoul, Korea. ³ American Soybean Association.

⁴ Carboxymethyl cellulose.

⁵ Feeds and Foods Nutrition Research Center, Pukyong National University, Busan, Korea.

⁶ Dongyang fish-farm Co., Pohang, Korea.

MATERIALS AND METHODS

Experimental design

Four experimental diets (Table 1) were formulated as follows: MP (raw fish based moist pellet); DHSM₀ (fish meal (FM) based dry pellet); DHSM₃₀ (30% FM was replaced by dehulled soybean meal); COMD (commercial dry pellet) to contain 50% crude protein (CP) and 16.7 kJ available energy/kg diet. Estimated available energy values (Garling and Wilson, 1976) of experimental diets were adjusted to 16.7, 16.7 and 37.7 kJ/kg for protein, carbohydrate and lipid, respectively. Proximate and essential amino acid (EAA) composition of FM and DHSM are shown in Table 2. White FM, DHSM and corn gluten meal served as the major protein sources. Squid liver oil and wheat flour were used as lipid and carbohydrate sources, respectively. Cellulose was also included in the diets to match CP and energy levels. Dehulled soybean meal was obtained from ASA (American Soybean Association, Korea). DHSM was composed of 50.4% crude protein, 2.1% crude lipid and 7.1% ash on a dry-matter basis.

Fish and feeding trial

Juvenile olive flounder were obtained from Gochang,

Table 2. Proximate analysis and essential amino acid (EAA) composition of white fish meal and dehulled soybean meal used in the experimental diets (% of dry matter basis)¹

	White fish meal	Dehulled soybean meal
	Crude protein	72.2
Crude lipid	6.8	2.1
Crude ash	19.4	7.1
Arginine	4.21	3.84
Histidine	1.34	1.36
Lysine	4.53	3.22
Leucine	4.52	3.85
Isoleucine	2.67	2.50
Methionine	1.68	0.80
Phenylalanine	2.34	2.57
Threonine	2.57	1.96
Tryptophan	0.60	0.56
Valine	3.02	2.33

¹ Amino acids were analyzed at Feeds and Foods Nutrition Research Center at Pukyong National University, Busan, Korea.

Table 3. Calculated essential amino acid (EAA) composition of four experimental diets (% of dry matter basis)¹

Ingredients	Diets ²			
	MP	DHSM ₀	DHSM ₃₀	COMD
Arginine	4.02	3.71	3.56	3.65
Histidine	1.67	1.27	1.32	1.30
Isoleucine	3.07	2.53	2.34	2.44
Leucine	5.22	4.92	5.21	5.09
Lysine	4.95	3.86	3.34	3.64
Met+cyst	2.82	2.08	1.98	2.01
Phe+tyr	4.31	4.17	4.17	4.26
Threonine	3.12	2.38	2.26	2.31
Tryptophan	0.79	0.55	0.55	0.58
Valine	3.32	2.87	2.74	2.82

¹ Amino acids were analyzed at Feeds and Foods Nutrition Research Center at Pukyong National University, Busan, Korea.

² Refer to the Table 1 foot note 1.

Korea. Prior to the feeding trial, the fish were fed the DHSM₀ diet for 2 wk to adjust to the experimental diets and conditions. Feeding trials were conducted in 4.5×4.5×0.8 m tanks. Supplemental aeration was also provided to maintain dissolved oxygen levels near 6.5±0.5 ppm. The sea water temperature was maintained at 16±3°C throughout the experimental period. The salinity was maintained at 31±1 g/L (mean±SD) and the pH at 7.8±0.3 (mean±SD). At the beginning of the experiment, a group of 6,000 fish averaging 3.2±0.04 g (mean±SD) was randomly assigned to each diet treatment. At 12 wk, fish were weighed and a group of 1,500 fish was selected from the 6,000 fish. At 24 wk, a group of 600 fish was selected from the 1,500 fish and at 36 wk, a group of 500 fish was selected from the 600 fish to adjust the rearing density to 12 kg/m² for optimum growth. Each diet was fed to a single group to apparent satiation 2 times a day at the rate of 6% to 1% (6% of wet body weight per day at the beginning and continuously decreased to 1% of wet body weight per day at the end of

Table 4. Growth performance and survival of olive flounder fed four different experimental diets for 42 wk¹

	Diets ²				Pooled SEM ³
	MP	DHSM ₀	DHSM ₃₀	COMD	
Initial wt. (g)	3.2	3.2	3.2	3.2	0.02
Final wt. (g)	323.1	301.4	317.5	317.2	4.66
WG (%) ⁴	10,669 ^a	10,473 ^{ab}	10,484 ^{ab}	9,948 ^b	110
FE (%) ⁵	81.4 ^a	79.3 ^a	79.6 ^a	76.7 ^b	1.12
SGR (%) ⁶	1.42	1.40	1.41	1.41	0.01
PER ⁷	2.29	2.52	2.48	2.45	0.14
HSI ⁸	2.12 ^b	3.16 ^a	2.21 ^b	3.32 ^a	0.72
CF ⁹	1.26 ^b	1.41 ^{ab}	1.67 ^a	1.32 ^b	0.16
Survival (%) ¹⁰	83.7	86.8	88.3	87.1	1.47

¹ Means of three sampling batches from each tank; values in the same row with different letters are significantly different ($p < 0.05$).

² Refer to Table 1. ³ Pooled standard error of mean: SD/\sqrt{n}

⁴ Weight gain: $((\text{final wt.} - \text{initial wt.})/\text{initial wt.}) \times 100$. ⁵ Feed efficiency: $(\text{wet weight gain}/\text{dry feed intake}) \times 100$.

⁶ Specific growth rate: $((\ln \text{ final wt.} - \ln \text{ initial wt.})/\text{days}) \times 100$. ⁷ Protein efficiency ratio: $\text{wet wt. gain}/\text{protein intake}$.

⁸ Hepatosomatic index: $(\text{Liver weight} \times 100/\text{body weight})$. ⁹ Condition factor: $(\text{Wet weight}/\text{total length}^3) \times 100$.

¹⁰ Survival (%): Average of four different checking periods.

Table 5. Whole body proximate composition of olive flounder fed four different experimental diets for 42 wk¹

Diet ²	Moisture	Crude protein	Crude lipid	Crude ash
MP	73.9	17.9	5.02 ^a	3.82
DHSM ₀	74.1	18.8	3.82 ^b	3.61
DHSM ₃₀	74.2	18.8	4.17 ^b	3.21
COMD	74.6	18.4	4.02 ^b	3.17
Pooled SEM ³	0.29	0.26	0.35	0.24

¹ Means of three sampling batches from each tank; values in the same column with different letters are significantly different ($p < 0.05$).

² Refer to Table 1. ³ Pooled standard error of mean.

the feeding trial) on a dry matter basis. Total fish weight in each tank was determined every 6 wk, and the amount of diet fed to fish was adjusted accordingly.

Sample collection and analysis

At the end of the feeding trial, fish were anesthetized with MS-222 (100 ppm), then weighed and counted to calculate weight gain (WG), feed efficiency (FE), specific growth rate (SGR) and protein efficiency ratio (PER). Three batches of samples taken from each tank were considered as the triplicate treatment groups of each experimental diet. Three fish from each sampling batch were randomly selected to determine hepatosomatic index (HSI) and condition factor (CF). Analyses of crude protein, moisture and ash were performed by the standard procedures of AOAC (1995). Crude fat was determined by ether extraction (Soxtec system 1046, Foss, Hoganas, Sweden) after freeze drying samples for 24 h. Blood samples were obtained from the caudal vein with a syringe. Haematocrit (PCV) was determined on three fish randomly selected from each batch of samples by the microhematocrit method (Brown, 1980), and hemoglobin (Hb) was measured with the same fish by the cyan-methemoglobin procedure using Drabkins solution. Hb standard prepared from human blood (Sigma Chemical, St. Louis, Missouri, USA) was used.

Statistical analysis

Data were subjected to ANOVA test using Statistix 3.1

(Analytical Software, St. Paul, MN, USA). When a significant treatment effect was observed, a Least Significant Difference test was used to compare means. Treatment effects were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Average weight gain (WG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), hepatosomatic index (HSI), condition factor (CF), hematocrit (PCV), hemoglobin (Hb) and survival (%) of the experimental fish during the 42 wk experiment are shown in Table 4 and 6. Average WG and FE of fish fed MP, DHSM₀ and DHSM₃₀ were not significantly different ($p > 0.05$). However, WG and FE of fish fed MP were significantly higher than of fish fed COMD. In order to improve the palatability of diets, amino acids such as glycine, betaine, etc., and some kinds of fatty acids have been tested in sea bream *Pagrus major* (Fuks et al., 1981; Shimizu et al., 1990) and chinook salmon (Hughes, 1985); effective results were found when attractants were added (Metailler et al., 1983; Mackie and Adroa, 1985; Takii et al., 1986; McGoogan and Gatlin III, 1997). In our previous study (Choi et al., 2004) we also found that amino acid supplementation can increase the palatability of the 30% soybean meal diet. Certain factors must be considered when soybean is used as a diet ingredient. Firstly, there are several anti-nutrition elements in full fat or dehulled

Table 6. Serological characteristics of olive flounder fed four different experimental diets for 42 wk¹

	Diets ²				Pooled SEM ⁹
	MP	DHSM0	DHSM30	COMD	
Hb (g/100 ml) ³	6.21	5.12	5.04	6.36	0.31
GOT (IU/L) ⁴	13.3 ^c	19.1 ^{bc}	23.1 ^{bc}	38.2 ^a	2.93
GPT (IU/L) ⁵	6.03	5.02	5.04	5.02	0.31
TG (mg/dl) ⁶	228 ^b	171 ^e	206 ^{bc}	300 ^a	15.1
TP (g/dl) ⁷	5.61 ^a	4.47 ^b	4.17 ^b	4.33 ^b	0.19
PCV (%) ⁸	31.1	30.3	30.6	32.5	0.04
Glucose (mg/dl)	42.0	39.7	49.7	37.3	2.26
Calcium (mg/dl)	48.6	38.4	39.9	43.2	2.25
Phosphorus (mg/dl)	2.87	2.73	2.93	3.25	0.11

¹ Means of three sampling batches from each tank; values in the same column with different letters are significantly different ($p < 0.05$).

² Refer to Table 1. ³ Hb: Hemoglobin.

⁴ GOT: Glutamic oxaloacetic transaminase. One unit is defined as the amount of enzyme causing the transamination of 1.0 μ mol of L-aspartate per minute at 25°C and pH 7.4.

⁵ GPT: Glutamic pyruvic transamination. One unit is defined as the amount of enzyme causing the transamination of 1.0 μ mol of L-alanine per minute at 25°C and pH 7.4.

⁶ TG: Triglyceride. ⁷ TP: Total protein. ⁸ PCV: Hematocrit. ⁹ Pooled standard error of mean.

soybean meal such as hemagglutinin, goitrogen and protease inhibitor that could inhibit the activity of trypsin and chymotrypsin (Dabrowski and Kozak, 1979). Secondly, 0.6% phosphorous in soybean meal is in the form of phytin that fish cannot digest. Fish can only utilize approximately 1/3 of the phosphorous that is contained in soybean; therefore, phosphorous must be supplemented as the percentage of soybean meal used in fish feeds increases (Lovell, 1982). Thirdly, with increased use of soybean meal in fish diets, the balance of essential amino acids should be considered and amino acids must be supplemented in the diets (Viola et al., 1983). Many studies have been conducted to determine the optimum amino acid supplementation in soybean meal-containing diets (Murai et al., 1989); to remove the anti-nutrition elements in full fat and dehulled soybean meal (Wee and Shu, 1989); to determine the utilization of full fat, solvent extracted and refined soybean meal (Pongmaneerat and Watanabe, 1993); and to evaluate phosphorous utilization and discharge (Satoh et al., 1993). SGR and PER of fish from all treatments were not significantly different ($p > 0.05$). HSI of fish fed DHSM₀ and COMD were significantly higher than those of fish fed MP and DHSM₃₀ ($p < 0.05$). CF of fish fed DHSM₃₀ was significantly higher than of fish fed MP and COMD ($p < 0.05$).

Whole body proximate composition is shown in Table 5. Crude lipid of fish fed MP was higher than of fish fed DHSM₀, DHSM₃₀ and COMD ($p < 0.05$). However, there was no significant difference in moisture, crude protein and crude ash among fish fed the four experimental diets. Zeitler et al. (1984) and Nandeeshya et al. (1995) reported that whole body composition correlated with fish species, water temperature, weight gain, feeding and the formulated diets. Also Murai et al. (1985) reported that the content of crude protein and crude ash decreased with the increase of crude lipid content.

Serological characteristics of flounder are shown in Table 6. Hb of all treatments was not significantly different ($p > 0.05$). The average hemoglobin value was 5.68 ± 0.15 g/100 ml in olive flounder; this result was similar to the value reported by Kikuchi et al. (1994). It was reported that the hemoglobin of healthy fish should be approximately 10 g/100 ml (Post, 1983), although the normal value of hemoglobin has not been proved in flounder yet. However, many researchers found that Hb varied according to the deficiency of essential nutrients, fish species, environmental conditions and growth stage (Garrido et al., 1990). GOT and TG in fish fed COMD were significantly higher than in fish fed MP, DHSM₀ and DHSM₃₀ ($p < 0.05$). TP of fish fed MP was higher than of fish fed DHSM₀, DHSM₃₀ and COMD ($p < 0.05$). PCV, GPT, glucose, calcium and phosphorus of all treatments were not significantly different ($p > 0.05$).

Therefore, these results indicate that DHSM could replace 30% fish meal in commercial feeds for olive flounder reared in commercial farms for 42 wk.

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