

Effect of Partial Dietary Substitution of Meat Meal for Fish Meal on the Growth and Body Composition of the Juvenile Olive Flounder *Paralichthys olivaceus*

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This study was conducted to investigate the effect of partially substituting meat meal for fish meal on the growth and body composition of juvenile olive flounder *Paralichthys olivaceus* during the winter season. Twenty-five fish (initial body weight, 23 g) were distributed into twelve 250 L flow-through tanks. Four experimental diets were prepared in triplicate: the control, MM20, MM40, and MM60 diets. Sixty percent mackerel meal was used as the primary protein source in the control diet. Meat meal was substituted for 20, 40, and 60% of the mackerel meal in the MM20, MM40, and MM60 diets, respectively. Survival was not significantly affected by the experimental diets. However, the weight gain and specific growth rate of fish fed the control, MM20, and MM40 diets were significantly higher than those of fish fed the MM60 diet ($P < 0.05$). The feed efficiency ratio of fish fed the control, MM20, and MM40 diets was significantly higher than that of fish fed the MM60 diet ($P < 0.05$). The protein efficiency ratio for fish fed the control diet was significantly higher than that for fish fed the MM40 and MM60 diets ($P < 0.05$). Moisture, crude protein, crude lipid, and ash content, and blood chemistry of the flounder at the end of the feeding trial were unaffected by the experimental diets. Considering these results, it can be concluded that up to 40% substitution of meat meal for fish meal in the diet could be implemented without a reduction in growth or deterioration of the feed efficiency of juvenile olive flounder during the winter season.

Key words: Fish meal, Meat meal, Olive flounder, *Paralichthys olivaceus*, Dietary substitution

Introduction

The olive flounder, *Paralichthys olivaceus* is the most commercially important marine fish for aquaculture in Korea due to both its high annual production and value over the past two decades (KNSO, 2003).

Fish meal is widely used as the main protein source for fish feed because it contains nutrients essential for fish, including amino and fatty acids, and minerals. However, due to overfishing of species (e.g., mackerel and sardines) used in fish meal, a reduction in the availability of these fish, and a high demand for fish meal resulting from the expansion of fish aquaculture,

the price of fish meal has increased sharply. Therefore, many studies have been conducted to find substitute protein sources for olive flounder aquaculture (Kikuchi et al., 1994a, b, c, 1997; Sato and Kikuchi, 1997; Cho et al., 2005).

Sato and Kikuchi (1997) reported that 60% of whitefish meal could be replaced with meat meal in the diet of juvenile olive flounder (initial body weight: 3 g) at 20°C. Substituting meat meal for fish meal in the diet has been reported to yield superior results in the cultivation of some marine fish (Shimeno et al., 1993a, c; Lee, 2002; Millamena, 2002).

Because water temperature affects the availability of dietary nutrients for fish, the effect of substituting

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meat meal for fish meal in the diet on fish performance could differ according to temperature. Therefore, in this study, the effect of partially substituting meat meal for fish meal on the growth and body composition of juvenile olive flounder *Paralichthys olivaceus* was examined during the winter season.

Materials and Methods

Experimental fish

Juvenile flounder were purchased from a private flounder hatchery (Kyungbook, Korea) and transported to the lab. Twenty-five fish (initial body weight: 23.0±0.08 g) were distributed into twelve 250 L flow-through tanks (water volume: 150 L) and allowed to acclimate to experimental conditions for one week. During the acclimation period, fish were hand-fed twice daily with commercial feed containing 52% crude protein and 8% crude lipid (Jeil Feed Co., Taejeon, Korea).

Preparation of the experimental diets

Four experimental diets were prepared in triplicate for this study: the control, MM20, MM40, and MM60 diets. Mackerel meal at the level of 60% was used as the primary protein source in the control diet. Meat meal was substituted for 20, 40, and 60% of the mackerel meal in the MM20, MM40, and MM60 diets, respectively (Table 1). The amino acid compositions of the ingredients used in the experimental diets are given in Table 2. The contents of lysine (Lys) and methionine + cystine (Met + Cys) in meat meal was relatively low compared to that in mackerel meal, but the amount of leucine (Leu) in the meat meal was relatively high. Wheat flour and α -starch, and squid liver oil were used as the primary carbohydrate, and lipid sources in the experimental diets, respectively. The ingredients in the experimental diets were mixed well with water at the ratio of 7:3 and pelletized using a pellet-extruder. The experimental diets were dried overnight and stored at -20°C until use.

Experimental conditions

Sand-filtered seawater was supplied to each experimental tank, and the flow rate of water into each tank was 6.6 L/min. Fish were hand-fed to apparent satiation twice daily (09:00 and 17:00 h) throughout the feeding trial. The water temperature ranged from 9.5 to 16.0°C (mean±SD: 12.3±1.75°C) throughout the feeding trial, and the photoperiod followed natural conditions. The feeding trial continued for 10 weeks.

Analysis of chemical composition and blood chemistry of fish

At the beginning and end of the experiment, ten fish

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

Ingredients	Diets			
	Control	MM20	MM40	MM60
Mackerel meal ¹	60	48	36	24
Meat meal ²		15	30	45
Wheat flour	26.8	24	21.2	18.4
α -starch ³	5	5	5	5
Brewer yeast	2	2	2	2
Squid liver oil	2	1.8	1.6	1.4
Vitamin premix ⁴	2	2	2	2
Mineral premix ⁵	2	2	2	2
Choline (50%)	0.2	0.2	0.2	0.2
Nutrients (%)				
Crude protein	48.7	50.4	51.7	54.3
Crude lipid	7.5	7.0	7.4	7.9
Ash	9.2	9.4	9.8	10.4

¹Produced by steam dry method, imported from Chile.

²Provided by Shinchon Feed Co. Ltd., Incheon, Korea.

³Sigma Chemical, St. Louis, MO, USA. ⁴Vitamin premix contained the following amount that were diluted in cellulose (g/kg mix): 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. ⁵Mineral premix contained the following ingredients (g/kg mix): MgSO₄·7H₂O, 80.0; NaH₂PO₄·2H₂O, 370.0; KCl, 130.0; ferric citrate, 40.0; ZnSO₄·7H₂O, 20.0; Ca-lactate, 356.5; CuCl₂, 0.2; AlCl₃·6H₂O, 0.15; KI, 0.15; Na₂Se₂O₃, 0.01; MnSO₄·H₂O, 2.0; CoCl₂·6H₂O, 1.0.

Table 2. Proximate analysis and essential amino acid compositions of the ingredients

Proximate composition (% of DM basis)	Protein sources			
	Mackerel ¹	Meat meal ²	Wheat flour	Brewer's yeast
Moisture	8.7	5.0	10.5	3.6
Crude rotein	75.3	74.4	11.7	48.7
Crude lipid	7.9	10.8	1.0	2.0
Ash	15.7	13.2	1.0	9.1
Essential amino acids (% in protein)				
Arg	6.7	5.7	6.5	6.2
His	4.0	3.2	4.1	3.6
Ile	4.0	4.0	3.9	4.0
Leu	8.2	10.1	8.6	9.2
Lys	8.4	6.1	7.1	7.4
Met+Cys	3.0	1.8	1.6	1.6
Phe+Tyr	7.3	7.6	7.6	7.9
Thr	5.1	4.7	4.7	4.9
Val	5.6	5.7	5.6	5.5
Total	52.3	48.9	49.7	50.3

¹Produced by steam dry method, imported from Chile.

²Provided by Shinchon meat meal Ind. Co., Incheon, Korea.

were sampled and sacrificed for proximate analysis. Crude protein content was determined by the Kjeldahl method using the Auto Kjeldahl System (Buchi B-324/435/412; Buchi, Flawil, Switzerland); lipid content was assessed by the ether-extraction method; moisture content was measured by oven (drying at 105°C for 24 h); fiber content was calculated with an automatic analyzer (Fibertec, Tecator, Sweden); ash content was determined using a muffle furnace (550°C for 4 h) based on standard methods (AOAC, 1990). Blood samples were obtained from the caudal vein of five fish from each tank by using a heparinized syringe after they were starved for 24 h and anesthetized with MS-222 at a concentration of 100 ppm at the end of the feeding trial. Plasma was collected after centrifugation (3000 rpm for 10 min) and stored at -70°C as separate aliquots for analysis of protein, glucose and glutamic oxaloacetic transaminase (GOT)

Statistical analysis

One-way ANOVA and Duncan's multiple range test (Duncan, 1955) were used to analyze the significance of the difference among the means of treatments by using SAS Version 9.1 (SAS Institute, Cary, NC, USA).

Results and Discussion

Survival, weight gain (g/fish), and specific growth rate (SGR) of olive flounder fed the experimental diets for 10 weeks during the winter season are given in Table 3. Survival was not significantly ($P>0.05$) affected by the experimental diets. Similarly, the survival of flounder was not affected by different substitution ratios of meat meal for fish meal in the experimental diets (Sato and Kikuchi, 1997).

However, weight gain and SGR of fish fed the control, MM20, and MM40 diets were significantly ($P<0.05$) higher than those of fish fed the MM60 diet in this study, indicating that the growth of juvenile olive flounder fed the diet in which meat meal was substituted for fish meal at levels up to 40% was comparable to that of fish fed the fish meal-based diet during the winter season. However, Sato and Kikuchi

(1997) reported that 60% fish meal could be replaced by meat meal in the diet of juvenile olive flounder at 20°C. This difference in results may be attributable to differences in water temperature; the mean water temperature in our study was 12.3°C. The lower metabolism rate of fish at this temperature may have been a factor. Differences in the protein content of the control diet in the two studies could have been another reason. The protein level of the control diet used in Sato and Kikuchi's (1997) study was 55.6% compared to 48.7% in our study. Shimeno et al. (1993a) found that the growth of yellowtail fed diets in which meat meal was substituted for fish meal at levels of up to about 40% was comparable to that of fish fed the fish meal-based diet. Growth of juvenile flounder fed diets in which meat and bone meal were substituted for fish meal at levels up to 20% was comparable to that of fish fed the fish meal-based diet (Kikuchi et al., 1997).

The amount of feed consumed (g/fish), feed efficiency ratio (FER), and protein efficiency ratio (PER) of olive flounder fed the experimental diets during the winter season are presented in Table 4. Feed consumption was not significantly affected by the experimental diets ($P>0.05$). However, the FER of flounder fed the control, MM20, and MM40 diets was significantly ($P<0.05$) higher than that of fish fed the MM60 diet. Similarly, the FER of yellowtail and olive flounder fed diets in which up to 40% meat meal was substituted for fish meal was comparable to that of fish fed a fish meal-based diet (Shimeno et al., 1993a; Sato and Kikuchi, 1997).

The PER for olive flounder fed the control diet was not significantly different from that for fish fed the MM20 diet, but was significantly higher than that for fish fed the MM40 and MM60 diets in this study. In the present study, the poorest PER was obtained in flounder fed the MM60 diet, PER decreased linearly fish meal by meat meal in the diets of yellowtail (Shimeno et al., 1993a).

Moisture, crude protein, crude lipid, and ash content of the whole flounder ranged from 74.1 to 74.9%, 17.4 to 17.9%, 3.1 to 3.3%, and 2.6 to 3.9%, respectively, with an increase in the substitution ratio of fish meal

Table 3. Survival, weight gain, specific growth rate (SGR) of juvenile olive flounder fed the experimental diets substituting meat meal for fish meal for 10 weeks during the winter season (mean±S.E.)

Diets	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR ¹
Control	23.1±0.05	56.1±2.75 ^a	96.0±2.31 ^{n.s.}	33.0±2.70 ^a	1.25±0.065 ^a
MM20	23.2±0.06	55.6±1.74 ^a	94.7±2.67	32.5±1.67 ^a	1.23±0.039 ^a
MM40	22.9±0.14	52.3±1.31 ^a	97.3±1.33	29.3±1.15 ^a	1.16±0.025 ^a
MM60	23.0±0.09	44.1±1.13 ^b	97.3±2.67	21.1±1.14 ^b	0.91±0.037 ^b

Different superscript letters within same columns are significantly different ($P<0.05$). n.s.: not significantly different ($P>0.05$). ¹SGR = (Ln final weight - Ln initial weight) × 100 / days of feeding.

Table 4. Amount of feed supply (g/fish), feed efficiency ratio (FER) and protein efficiency ratio (PER) for juvenile olive flounder fed the experimental diets substituting meat meal for fish meal for 10 weeks during the winter season (mean±S.E.)

Diets	Amount of feed supply	FER ¹	PER ²
Control	36.2±1.42 ^{n.s.}	0.93±0.053 ^a	1.91±0.108 ^a
MM20	36.5±0.98	0.91±0.027 ^a	1.81±0.054 ^{ab}
MM40	34.5±1.71	0.87±0.003 ^a	1.68±0.005 ^b
MM60	33.3±0.10	0.65±0.028 ^b	1.19±0.052 ^c

Different superscript letters within same columns are significantly different ($P < 0.05$). n.s.: not significantly different ($P > 0.05$). ¹FER=weight gain/dry feed fed. ²PER=weight gain/protein fed.

by meat meal. Similarly, in a previous study, PER deteriorated with an increase in substitution ratio of and was not significantly affected by the experimental diets ($P > 0.05$). Similarly, body composition was not affected by substitution of other protein sources for fish meal diets of yellowtail and flounder in other studies (Shimeno et al., 1993a, b; Kikuchi, 1999; Cho et al., 2005). However, unlike in this study, the chemical composition of fish was directly influenced by dietary nutrient composition in many other studies (Shimeno et al., 1992; Kikuchi et al., 1994a, b, 1997; Sato and Kikuchi, 1997).

Plasma protein, glucose, and glutamic oxaloacetic transaminase (GOT) of olive flounder ranged from 3.6 to 5.1 g/dL, 17.0 to 23.2 mg/dL, and 16.8 to 24.4 IU/L, respectively, and were not significantly affected by the experimental diets in this study ($P > 0.05$). Similarly, the blood chemistry of yellowtail and olive flounder was not influenced by diets in which other protein sources were substituted for fish meal (Shimeno et al., 1993a, c; Kikuchi et al., 1994b; Kikuchi et al., 1997; Sato and Kikuchi, 1997). However, the substitution of defatted soybean meal and feather meal for fish meal was shown to affect the blood chemistry of olive flounder (Kikuchi et al., 1994a; Kikuchi, 1999).

Considering these results, it can be concluded that meat meal can be substituted for fish meal at levels of up to 40% without a reduction in growth or deterioration of feed efficiency of juvenile olive flounder during the winter season.

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