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# Effect of Various Commercial Fish Meals as Dietary Protein Sources on Growth and Body Composition of Juvenile Flounder, *Paralichthys olivaceus*

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A feeding trial was carried out to investigate the effect of various fish meals as dietary protein source on growth, feed utilization and body composition of juvenile flounder. Ten experimental diets were prepared to contain different fish meals: 4 kinds of white fish meal (WM-1, 2, 3, 4), 3 kinds of herring meal (HM-1, 2, 3), mackerel meal (MM), WM mixture and HM+MM mixture. Dietary energy and protein levels were designed to be isocaloric (3.8 kcal $g^{-1}$ diet) and isonitrogenous (46%) by adjusting the levels of fish meal, wheat flour and squid liver oil. Three replicate groups of fish (initial mean weight: 11.1 g) were hand-fed to visual satiety two times daily for 7 weeks. Survival was not significantly different among all groups. Weight gain of fish fed the HM-1 and HM-3 diets was the highest, but not significantly different from that of fish fed the either WM-2 or HM+MM diets. The lowest weight gain was found in fish fed the WM-1 and WM-3 diets. Feed efficiency and protein efficiency ratio of fish fed the WM-2, HM-1, HM-3, MM and HM+MM diets were significantly higher than those of fish fed other diets. Daily feed intake of fish fed the WM-3 diet was highest, but not significantly different from that of fish fed the either WM-4 or WMM diet. Significant differences were found in contents of moisture, crude protein, crude lipid and ash of whole body of fish. The results of this study indicated that growth of flounder can be affected by dietary fish meal source and quality, and WM-2, HM-1, HM-3 and HM+MM mixture are considered as useful dietary fish meal sources under these experimental conditions.

**Keywords:** Fish meal, Flounder, *Paralichthys olivaceus*, Growth

## Introduction

Aquaculture production of flounder has long been the highest among that of commercially important fish species in Korea. Flounder is a carnivorous marine fish and needs a high percentage of protein content in diets for optimal growth (Lee et al., 2000; Lee et al., 2002). Dietary protein sources are the most important ingredients affecting growth performance of fish and feed cost (Lovell, 1989).

Fish meal has commonly been the major protein source for fish diets due to its high nutritive values such as high protein content, well-balanced essential amino acids and fatty acids, and is the most expensive dietary component. Thus, the supply, quality and cost of fish meal are important for marine fish feed production. However, the market supply of fish meal shows great variation in quality, due to differences in freshness, kinds of raw material and processing conditions. In addition, the shortage in global fish meal production has further increased its price coupled with increased demand and competition for the use in livestock and poultry feeds.

Therefore, it is needed to evaluate their quality in relation to various fish meals for improvement of feed efficiency and reduce fish production cost. The objective of this study was to investigate the influence of various fish meals in diets on growth and body composition of juvenile flounder.

## Materials and methods

### Experimental fish meals and diets

The chemical compositions of white fish meal (WM), herring meal (HM) and mackerel meal (MM) used in this study are presented in Table 1. All the test fish meals were produced by steam-dry method. Ten isonitrogenous (46%) and isoenergetic (3.8 kcal $g^{-1}$ diet) diets were formulated (Table 2) based on the previous study (Lee et al., 2002) using different fish meals and soybean meal as protein sources, squid liver oil as the lipid source, and wheat flour and α-starch as the carbo-

**Table 1.** Chemical compositions of the experimental fish meals

| | White fish meal (WM)[1] | | | | Herring meal (HM) | | | Mackerel meal (MM)[3] |
|---|---|---|---|---|---|---|---|---|
| | WM-1 | WM-2 | WM-3 | WM-4 | HM-1[2] | HM-2[2] | HM-3[3] | |
| Proximate composition (%) | | | | | | | | |
| Moisture | 5.0 | 3.4 | 7.0 | 3.0 | 5.0 | 10.6 | 7.0 | 8.5 |
| Crude protein | 65.7 | 72.8 | 59.5 | 67.5 | 65.8 | 56.2 | 65.6 | 73.3 |
| Crude lipid | 7.9 | 7.5 | 2.7 | 7.6 | 8.8 | 10.0 | 9.5 | 8.0 |
| Ash | 17.9 | 13.2 | 29.8 | 21.1 | 20.2 | 19.8 | 17.2 | 10.0 |
| Essential amino acids (% in protein) | | | | | | | | |
| Arg | 7.1 | 7.3 | 7.5 | 7.7 | 7.2 | 6.8 | 7.0 | 6.9 |
| His | 2.9 | 3.0 | 3.0 | 3.0 | 2.8 | 3.0 | 3.6 | 4.5 |
| Ile | 4.7 | 4.8 | 3.8 | 3.5 | 4.4 | 5.0 | 5.1 | 5.2 |
| Leu | 8.3 | 8.6 | 8.4 | 8.1 | 8.6 | 8.3 | 8.2 | 8.1 |
| Lys | 8.8 | 8.8 | 8.4 | 8.2 | 8.6 | 8.6 | 8.6 | 8.6 |
| Met+Cys | 3.4 | 3.6 | 3.7 | 3.2 | 3.5 | 3.3 | 3.8 | 4.3 |
| Phe+Tyr | 7.1 | 7.9 | 7.9 | 7.6 | 7.8 | 7.7 | 7.4 | 7.6 |
| Thr | 4.8 | 5.1 | 5.2 | 5.0 | 5.1 | 4.7 | 5.0 | 5.0 |
| Val | 5.4 | 5.3 | 4.5 | 4.4 | 5.0 | 5.4 | 5.5 | 5.1 |
| Essential fatty acids (% of total fatty acids) | | | | | | | | |
| 20:4n–6 | 1.2 | 1.1 | 1.3 | 1.2 | 1.0 | 1.9 | 1.1 | 1.6 |
| 20:5n–3 | 12.2 | 16.0 | 12.9 | 15.0 | 12.7 | 11.5 | 13.4 | 10.4 |
| 22:6n–3 | 17.5 | 26.5 | 21.2 | 20.0 | 26.4 | 9.1 | 23.2 | 22.7 |
| n–3 HUFA[4] | 32.9 | 44.0 | 35.6 | 36.2 | 40.0 | 24.7 | 37.4 | 37.6 |
| VBN (mg 100 $g^{-1}$)[5] | 58 | 136 | 105 | 122 | 68 | 102 | 44 | 33 |

[1]Produced by steam dry method.
[2]Imported from Russia.
[3]Imported from Chile.
[4]Highly unsaturated fatty acids (C≥20).
[5]Volatile basic nitrogen.

hydrate sources. Ingredients of the experimental diets were mechanically mixed with water at the ratio of 100 g ingredients mixture to 35~40 g water and pressure–pelleted using laboratory pellet machine, and dried at room temperature for overnight. All diets were stored at –30°C until use.

**Fish and feeding trial**

Juvenile flounder (*Paralichthys olivaceus*) were obtained from local farm (Uljin, Korea). They were acclimated to rearing conditions for 10 days before the beginning of the feeding trial. Fish (11.1±0.6 g) were allocated randomly to thirty 300 l cylindrical plastic tanks with 30 fish to each tank for feeding trial. Three replicate groups of fish were hand-fed to apparent satiation twice a day (08:00 and 17:00) for 7 weeks. Filtrated seawater was supplied at a flow rate of 5 L $min^{-1}$ in each tank, and mean water temperature and salinity were 20.3±2.6°C and 34.5±0.7 g $L^{-1}$, respectively. Photoperiod was left at natural conditions during the feeding trial. All fish in each tank were weighed collectively at the beginning and the end of feeding trial after being fasted for 24 h and anesthetized with MS222 (Tricaine methanesulfonate, Sigma, USA) at a concentration of 100 mg $L^{-1}$. Records were kept for daily feed consumption, mortalities and feeding behavior.

**Sample collections and analytical methods**

A sample of 30 fish at the beginning and all fish of tank at the end of the feeding trial were collected and stored at –75°C in freezer for chemical analysis. Crude protein was determined by Kjeldahl method using Auto Kjeldahl System (Buchi B–324/435/412, Switzerland). Crude lipid was determined by extraction using Soxhlet extractor. Moisture was determined by oven drying at 105°C for 12 h. Ash was determined by muffler furnace at 550°C for 6 h. Gross energy contents of diets were analyzed using an adiabatic bomb calorimeter (Parr, Moline, IL, USA). Amino acids were analyzed using an automatic analyzer (Sykam Amino acid analyzer S433, Ger-

**Table 2.** Ingredients and nutrient contents of the experimental diets

| | Diets | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | WM-1 | WM-2 | WM-3 | WM-4 | HM-1 | HM-2 | HM-3 | MM | WMM | HM+MM |
| Ingredients (%) | | | | | | | | | | |
| White fish meal[1] | 58.0 | | | | | | | | 15.0 | |
| White fish meal[1] | | 57.0 | | | | | | | 15.0 | |
| White fish meal[1] | | | 64.0 | | | | | | 15.0 | |
| White fish meal[1] | | | | 62.0 | | | | | 15.0 | |
| Herring meal[2] | | | | | 61.0 | | | | | 15.0 |
| Herring meal[2] | | | | | | 69.0 | | | | 15.0 |
| Herring meal[3] | | | | | | | 58.0 | | | 15.0 |
| Mackerel meal[3] | | | | | | | | 54.0 | | 15.0 |
| Soybean meal | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Wheat flour | 22.9 | 23.7 | 14.6 | 19.4 | 21.7 | 14.0 | 24.4 | 28.0 | 20.4 | 22.8 |
| α–potato starch | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Squid liver oil | 2.1 | 2.3 | 4.4 | 1.6 | 0.3 | - | 0.6 | 1.0 | 2.6 | 0.2 |
| Vitamin premix[4] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Mineral premix[4] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Choline salt | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Nutrient contents (% DM) | | | | | | | | | | |
| Crude protein | 45.2 | 47.5 | 45.5 | 46.7 | 46.6 | 46.3 | 44.5 | 45.9 | 47.1 | 46.8 |
| Crude lipid | 6.3 | 7.0 | 6.2 | 7.1 | 6.1 | 7.5 | 6.7 | 6.2 | 6.8 | 6.7 |
| Ash | 12.9 | 8.4 | 18.9 | 14.1 | 12.7 | 17.2 | 12.7 | 9.7 | 13.6 | 13.3 |
| Gross energy (kcal $g^{-1}$) | 3.8 | 3.8 | 3.7 | 3.7 | 3.8 | 3.7 | 3.8 | 3.8 | 3.7 | 3.8 |

[1]Produced by steam dry method.
[2]Imported from Russia.
[3]Imported from Chile.
[4]Same as Lee et al. (2003).

many). Lipid for fatty acids analysis was extracted by mixture of chloroform and methanol (2:1, v/v) according to the method of Folch et al. (1957), and fatty acids were analyzed as described by Lee et al. (2003) using gas chromatography (HP 5890, Hewlett-Packard, USA). Volatile basic nitrogen (VBN) was determined by the method of the Conway (1950).

### Statistical analysis

The data were subjected to one–way analysis of variance (ANOVA) and if significant ($P<0.05$) differences were found, Duncan's multiple range test (Duncan, 1955) was used to rank the groups using the SPSS program Version 10.0 for Windows (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA). The data were presented as mean ± S.E. of three replicate groups.

## Results

The compositions of proximate, essential amino acids and essential fatty acids, and VBN content of the test fish meals are presented in Table 1. The moisture content of the fish meals ranged from 3.0 to 10.6%, and crude protein content of fish meals, except for WM-3 and HM-2, were over 60%. Samples of HM showed higher crude lipid content compared with WM or MM, and WM-3 showed considerably higher ash content. There were not considerable differences in essential amino acids composition among the different fish meals. The HM-2 had considerably less 22:6n–3 content compared with other fish meals. The VBN value (33~135 mg 100 $g^{-1}$) was different among experimental fish meals.

The growth performance of flounder fed the diets containing various fish meals is presented in Table 3. Survival was not significantly different among all groups. Weight gain of fish fed the HM-1 and HM-3 diets was the highest, but not significantly different from that of fish fed the WM-2 or HM+MM diets. The lowest weight gain was found in fish fed the WM-1 and WM-3 diets. Feed efficiency and protein efficiency ratio of fish fed the WM-2, HM-1, HM-3, MM and HM+MM diets were significantly higher than those of fish fed other diets ($P<0.05$). Daily feed intake of fish fed the WM-3 diet was highest, but not significantly different from that of fish fed

**Table 3.** Growth performance of juvenile flounder fed the diets containing various fish meals for 7 weeks[1]

| Diets | Initial weight (g fish$^{-1}$) | Survival (%) | Weight gain (g fish$^{-1}$) | FE (%)[2] | PER (%)[3] | DFI (%)[4] |
|---|---|---|---|---|---|---|
| WM-1 | 10.9±0.49 | 97.7±2.33 | 30.3±1.46$^{a}$ | 95.7±3.18$^{ab}$ | 2.12±0.070$^{b}$ | 2.46±0.031$^{bc}$ |
| WM-2 | 11.0±0.30 | 95.3±2.33 | 39.4±1.91$^{cd}$ | 108.3±3.48$^{c}$ | 2.28±0.069$^{c}$ | 2.40±0.069$^{ab}$ |
| WM-3 | 11.1±0.07 | 95.7±2.96 | 30.0±2.00$^{a}$ | 89.0±2.89$^{a}$ | 1.96±0.063$^{a}$ | 2.62±0.009$^{d}$ |
| WM-4 | 11.2±0.70 | 92.3±4.67 | 36.2±1.67$^{bc}$ | 97.7±1.20$^{b}$ | 2.10±0.024$^{ab}$ | 2.54±0.026$^{cd}$ |
| HM-1 | 11.0±0.42 | 98.0±1.00 | 42.5±1.75$^{d}$ | 112.3±2.33$^{c}$ | 2.41±0.046$^{c}$ | 2.40±0.053$^{ab}$ |
| HM-2 | 11.4±0.07 | 95.7±1.33 | 31.2±1.00$^{ab}$ | 98.3±1.76$^{b}$ | 2.13±0.035$^{b}$ | 2.37±0.025$^{ab}$ |
| HM-3 | 11.1±0.40 | 94.7±3.93 | 41.5±0.95$^{d}$ | 107.3±2.67$^{c}$ | 2.38±0.063$^{c}$ | 2.46±0.030$^{bc}$ |
| MM | 11.5±0.09 | 100.0±0.0 | 35.4±1.56$^{bc}$ | 106.3±2.03$^{c}$ | 2.32±0.039$^{c}$ | 2.32±0.042$^{a}$ |
| WMM | 11.0±0.17 | 94.7±3.93 | 33.4±0.42$^{ab}$ | 95.3±1.67$^{ab}$ | 2.02±0.034$^{ab}$ | 2.57±0.050$^{cd}$ |
| HM+MM | 10.8±0.20 | 98.0±1.00 | 38.8±1.15$^{cd}$ | 110.0±0.58$^{c}$ | 2.34±0.012$^{c}$ | 2.38±0.027$^{ab}$ |

[1]Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different (P<0.05).
[2]Feed efficiency=weight gain of fish×100/feed intake (dry matter).
[3]Protein efficiency ratio=weight gain of fish×100/protein intake.
[4]Daily feed intake=feed intake (dry matter)×100/[(initial fish wt.+final fish wt.+dead fish wt.)/2×days fed].

**Table 4.** Proximate composition of the whole body of flounder fed the diets containing various fish meals for 7 weeks[1]

| Diets | Moisture (%) | Crude protein (%) | Crude lipid (%) | Ash (%) |
|---|---|---|---|---|
| WM-1 | 74.9±0.02$^{bcde}$ | 17.8±0.21$^{a}$ | 1.4±0.35$^{a}$ | 3.9±0.14$^{bc}$ |
| WM-2 | 75.4±0.28$^{de}$ | 17.9±0.20$^{ab}$ | 2.4±0.26$^{ab}$ | 3.6±0.19$^{ab}$ |
| WM-3 | 74.7±0.21$^{abcde}$ | 18.0±0.12$^{ab}$ | 2.4±0.19$^{ab}$ | 4.1±0.05$^{c}$ |
| WM-4 | 73.9±0.48$^{a}$ | 18.3±0.29$^{abc}$ | 2.9±0.07$^{b}$ | 3.6±0.09$^{ab}$ |
| HM-1 | 75.2±0.55$^{cde}$ | 18.0±0.25$^{abc}$ | 1.5±0.38$^{a}$ | 3.4±0.03$^{a}$ |
| HM-2 | 74.3±0.23$^{abc}$ | 18.3±0.12$^{abc}$ | 1.5±0.10$^{a}$ | 4.0±0.08$^{c}$ |
| HM-3 | 75.6±0.27$^{e}$ | 18.5±0.13$^{bc}$ | 1.6±0.45$^{a}$ | 3.8±0.04$^{bc}$ |
| MM | 74.2±0.15$^{ab}$ | 18.6±0.29$^{c}$ | 2.3±0.59$^{ab}$ | 3.8±0.11$^{bc}$ |
| WMM | 74.1±0.05$^{ab}$ | 17.7±0.04$^{a}$ | 1.9±0.33$^{ab}$ | 3.9±0.07$^{bc}$ |
| HM+MM | 74.6±0.18$^{abcd}$ | 18.5±0.08$^{bc}$ | 1.4±0.08$^{a}$ | 3.7±0.06$^{ab}$ |

[1]Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different (P<0.05).

the either WM-4 or WMM diets. The fish fed the MM diet showed the lowest daily feed intake. Proximate composition of fish fed the diets containing various fish meals are presented in Table 4. Significant differences were found in contents of moisture, crude protein, crude lipid and ash of whole body.

## Discussion

Fish meal is a major ingredient as protein source in feed for flounder because availability of plant protein sources to flounder is relatively low in comparison with that in fresh water fish (Kim et al., 2000; Choi et al., 2004). In this study, the growth and feed efficiency of fish were affected by different fish meal sources. Similar results have been reported for other fish species (Aksnes and Mundheim, 1997; Aksnes et al., 1997). Previous studies reported that the use of high quality fish meal in diets improves growth and feed utilization of fish (Anderson et al., 1993; Vergara et al., 1999). These studies showed a clear effect of fish meal quality on growth and feed utilization of fish. In addition, the quality of fish meal depends on protein digestibility and freshness of the raw material used for processing, and the processing conditions in the manufacturing of fish meal. Different processing techniques (e.g. drying process) may have an effect on the quality of the fish meal. Considering the effect of fish meal quality on growth and feed utilization of cultured fish, use of the proper fish meal in diet is important for fish growth and for economical aquaculture production. In several studies, quality of fish meal has been evaluated by in vitro assays such as total volatile nitrogen, biogenic amines, and available lysine, and by growth studies (Anderson et al., 1993; Romero et al., 1994; Ricque-Marie et al., 1998; Caballero et al., 1999; Vergara et al., 1999). In this study, weight gain and feed efficiency of flounder

were not correlated with VBN values of fish meals. Anderson et al. (1993) suggest that biological testing of a feedstuff to determine protein quality is recommend as the final method of comparison rather than in vitro assay.

This study showed a significantly different daily feed intake among the dietary groups. Similar results have been reported for Atlantic salmon (*Salmon salar*) and Atlantic halibut (*Hippoglossus hippoglossus*) (Anderson et al., 1993; Aksnes and Mundheim, 1997). Different quality of fish meal may affect feed intake and digestibility, and consequently have an impact on growth performance of fish (Aksnes and Mundheim, 1997). In this study, the exact reason of different growth performance of juvenile flounder is not clear, but it may be due to differences in the contents of nutrient such as protein and essential fatty acids (20:5n-3 and 22:6n-3), raw material sources, and processing conditions of fish meal. Poor growth of fish fed the WM-1, WM-3 and HM-2 diets may be due to the lower contents of protein or essential fatty acid such as 22:6n-3 or higher ash content compared with other fish meals.

Proximate composition of whole body of fish was significantly affected by dietary fish meals. Similar results have been reported for Atlantic salmon (Anderson et al., 1993) in contrast other studies in which dietary fish meals did not influence on the body compositions of fish (Lee et al., 1996; Aksnes et al., 1997).

The results of this study indicated that growth of flounder can be affected by dietary fish meal kinds and quality, and WM-2, HM-1, HM-3 and HM+MM mixture are considered as useful dietary fish meal sources under these experimental conditions.

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