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# Nutrient Availability and Growth Rate Associated with Three Different Feed Types Used by Olive Flounder, *Paralichthys olivaceus*, Farmers in Korea

**Jinhwan Lee* and Jae-Yoon Jo[1]**

*Research Institute of Marine Science and Technology, Korea Maritime University, Dongsam-Dong, Yeongdo-Gu, Busan 606-791, Korea*

*[1]Department of Aquaculture, Pukyong National University, Busan 608-737, Korea*

We estimated the digestibility and growth rate of juvenile olive flounder (*Paralichthys olivaceus*) on three diets: raw fish-based moist pellets, moist pellets, and extruded pellets. The diets were created using commercially available methods, and a basic formulated powder. A reference diet was used to compare feed digestibility and the fish growth rate achieved using the experimental diets. The apparent digestibility coefficients (ADCs) of protein for raw fish-based moist pellets and moist pellets were significantly higher ($P<0.05$) than those for extruded pellets and the reference diet. The ADCs of nitrogen-free extracts (NFE) of extruded pellets and moist pellets were significantly higher ($P<0.05$) than those for raw fish-based moist pellets and the reference diet. Fish that were fed with the raw fish-based moist pellets showed the greatest weight gain (452.4%), which was not significantly different from that of fish fed with extruded pellets (414.4%; $P>0.05$). These results indicate that the higher protein efficiency in fish fed with extruded pellets can result in good growth performance within flounder culture systems.

Key words: Olive flounder, Digestibility, Growth, *Paralichthys olivaceus*.

## Introduction

In intensive aquaculture systems, most circulated or effluent wastes originate primarily from the feed (Cripps, 2000). For this reason, a proper understanding of digestibility for enhancing utilization of feed (Hajen et al., 1993a; Hajen et al., 1993b) and for reducing excretion rates of nitrogen (Mcgoogan and Gatlin, 1999; Webb and Gatlin, 2003), together with growth rate of the species based on the feed supplied is essential to achieve a cost-effective aquaculture. Enhancing nutrient digestibility and growth rate can support a cost-effective regime.

Flounder (*Paralichthys olivaceus*) is commercially important for aquaculture and fisheries in Korea, Japan, and China. Until recently, most flounder farmers usually used raw fish-based moist pellet feed to ensure flounder growth, and occasionally used extruded or moist pellets. These types of pellets are made in part or entirely from a basic powder compound that is commercially formulated and supplied by feed companies. However, there is little information regarding digestibility and the amount of nutrients taken up by the fish, from these feeds. Meanwhile, the desirability of using raw fish-based moist pellets at marine fish farms has been a point of contention between farmers and environmental activists. Thus, we evaluated the digestibility and growth in juvenile flounder fed on three different diets commonly used in flounder farms, with the aim of providing some data on nutrients utilization and growth performance of the fish based on the type of feed.

*Corresponding author: jinhwanlee@hhu.ac.kr

## Materials and Methods

### Experimental conditions

The experiment was conducted at the Finfish Research Center, Uljin, Gyeongbuk, Korea. Flounder purchased from a private flounder farm were allowed 3 weeks to acclimatize to the experimental conditions. During this period, the fish were fed the same type of extruded feed that was used at the previous farm. Subsequently, a series of digestibility and growth experiments were conducted using three replicates for each diet in 250-L circular PVC tanks (40 fish/tank) for 6 weeks. Growth rates were analyzed after every two weeks. The fish in each experimental tank were counted and weighed. Sand-filtered seawater was continuously pumped into the culture system. The water temperature and salinity were maintained at 19±1°C and 34 ppt, respectively, during the experiment. The juvenile fish (initial average body weight of 3.4 g) were hand-fed twice daily until satiated.

The diets contained varying amounts of formulated powder compound, from 100% in moist pellets to 65% in raw fish-based moist pellets (Table 1). The raw fish-based moist pellets were pelletized after mixing ground frozen raw mackerel with the formulated powder compound at a 1:1 wet weight ratio. The formulated powder compound and extruded pellets were manufactured and provided by the Woosung Feed Co. Ltd., Korea. Based on a report by Kim et al. (1993), a reference diet was also palletized. This reference diet was used to compare the growth and digestibility of the experimental diets. All diets contained 0.5% chromic oxide ($Cr_2O_3$).

## Chemical analysis

Samples of feed and fecal matter were also used for the analysis of chromic oxide (Furukawa and Tsukahara, 1966), protein (Kjeldahl, Gerhardt Co. Ltd., VAPODEST-5, No 6550, Germany), lipids (soxhlex extraction), fiber (Fibertec automatic analyzer, Tecator, Hoganas, Sweden), and ash (at 550°C for 6 h in a muffle furnace). The nitrogen-free extract (NFE) was calculated by means of difference (Aksnes et al., 1997).

## Calculations

The apparent digestibility coefficients (ADC) for the nutrients and the energy content of the diets were determined using the following equations:

ADC of nutrients or energy (%) = [1–(dietary $Cr_2O_3$/fecal $Cr_2O_3$)×(fecal nutrient or energy/dietary nutrient or energy)]×100

Dry matter of fecal solids (%) = [nutrients and

Table 1. Ingredients and chemical analysis of the experimental diets

| | Diets[1] | | | |
|---|---|---|---|---|
| | EP | RMP | MP | RD |
| Ingredients (g/100 g) | | | | |
| White fish meal | 67.26 | | 68.42 | 70.00 |
| Hydrolyzed fish protein | 2.00 | | 2.03 | - |
| Wheat flour | 8.71 | | 8.86 | 15.43 |
| Corn gluten meal | 5.00 | | 5.09 | - |
| Soybean meal | 5.00 | | 5.09 | - |
| Yeast | 3.00 | | 3.05 | - |
| Fish oil | 4.00 | | 2.28 | 10.00 |
| Choline Chloride 50% | 1.16 | | 1.18 | - |
| $CaHPO_4H_2O$ | 1.00 | | 1.02 | - |
| Carboxymethyl cellulose | - | | - | 3.00 |
| Vitamin mix[2] | 1.22 | | 1.22 | 1.00 |
| Mineral mix[3] | 1.00 | | 1.02 | 0.05 |
| $Cr_2O_3$ | 0.50 | | 0.50 | 0.50 |
| Proximate Composition (%, dry matter basis) | | | | |
| Crude protein | 50.75 | 59.30 | 54.17 | 53.27 |
| Crude lipid | 9.51 | 8.70 | 5.41 | 15.11 |
| Crude ash | 18.20 | 17.66 | 20.20 | 13.47 |
| Crude fiber | 1.72 | 1.61 | 1.51 | 0.51 |
| N-free extract[4] | 19.82 | 12.75 | 18.71 | 17.64 |
| Gross energy (kJ/g)[5] | 19.14 | 19.62 | 18.14 | 21.57 |
| Digestible protein[6] | 44.88 | 54.80 | 49.82 | 45.35 |
| Digestible lipid[6] | 8.26 | 7.98 | 4.57 | 10.44 |
| Digestible NFE[6] | 12.65 | 6.09 | 10.81 | 4.74 |
| Digestible energy (kJ/g)[6] | 15.68 | 17.39 | 15.18 | 15.68 |

[1]EP, extruded pellet; RMP, row fish-based moist pellet; MP, moist pellet; RD, reference diet.
[2]Vitamin mixture in RD was used Grobig-fish (Bayer Ltd, Seoul).
[3]As described by Kim et al. (2001).
[4]Calculated by difference (Aksnes et al., 1997).
[5]Calculated using combustion values for protein, lipid and NFE of 23.6, 39.5 and 17.2 MJ/kg, respectively (Aksnes and Opstvedt, 1998).
[6]Calculated based on Table 2.

fiber consumed in feed×(1−ADC of nutrients and fiber)]/nutrients and fiber in feed×100

Dry matter of metabolites (%) = [(nutrients consumed in feed×ADC of nutrients) −nutrients retained in fish body]/nutrients consumed in feed×100

Nitrogenous metabolites (%) = [(nitrogen consumed in feed × ADC of nitrogen) − nitrogen retained in fish body]/nitrogen in feed×100

**Statistical analysis**

One-way ANOVA (analysis of variance) and Duncan's multiple range tests were used to analyze the significance of differences among the test groups at *P*<0.05 using the SAS program, version 6.12 (SAS Institute Inc., Cary, North Carolina, USA).

## Results

The ADC of protein in fish fed with extruded pellets was significantly lower than that of fish with fed raw fish-based moist pellets or moist pellets (*P*< 0.05; Table 2). However, the ADC of NFE in fish fed with extruded pellets was significantly higher than that of fish fed with raw fish-based moist pellets or the reference diet (*P*<0.05; Table 2). The ADC of lipids did not differ among diets (*P*>0.05). However, significantly low ADCs for lipids and gross energy were produced in fish fed on the reference diet (*P*< 0.05; Table 2), which contained 15.1% dietary lipids.

Table 2. Apparent digestibility of nutrients in juvenile flounder fed on experimental diets for six weeks

| Diets[1] | Apparent digestibility coefficient (%) | | | |
|---|---|---|---|---|
| | Crude protein | Crude lipid | NFE[2] | Gross energy |
| EP | 88.4 ± 0.15$^{b}$ | 86.9 ± 0.78$^{a}$ | 63.8 ± 0.53$^{a}$ | 81.9 ± 0.61$^{b}$ |
| RMP | 92.4 ± 0.15$^{a}$ | 91.8 ± 4.25$^{a}$ | 48.1 ± 3.27$^{b}$ | 88.6 ± 0.26$^{a}$ |
| MP | 92.0 ± 0.49$^{a}$ | 84.4 ± 5.99$^{a}$ | 57.7 ± 3.73$^{a}$ | 83.7 ± 0.49$^{b}$ |
| RD | 85.1 ± 1.14$^{c}$ | 69.1 ± 2.57$^{b}$ | 26.9 ± 2.55$^{c}$ | 72.7 ± 1.09$^{c}$ |

[1]EP, extruded pellet; RMP, raw fish-based moist pellet; MP, moist pellet; RD, reference diet.
[2]NFE, nitrogen-fee extract.
Values (mean ± SD of three replications) with differ-rent superscripts within the same column are signi-0ficantly different (*P*<0.05).

The highest weight gain in juvenile flounder was achieved in fish fed on raw fish-based moist pellets, but this was not significantly different from the weight gain in fish fed on extruded pellets (*P*>0.05; Table 3). The feed efficiency of fish fed on raw fish-based moist pellets was significantly higher than that of fish fed on extruded pellets, moist pellets, or the reference diet (*P*<0.05; Table 3). However, the protein efficiency ratio (PER) for fish fed on extruded pellets (2.8) was significantly higher (*P*<0.05) than that of fish fed on raw fish-based moist pellets (2.7), moist pellets (2.6), or the reference diet (2.4). The weight gain of flounder fed on extruded pellets (44.9% of digestible protein, 15.7 kJ/g of digestible energy) was higher than that of fish fed on moist pellets (49.8% of digestible protein, 15.2 kJ/g of digestible energy) or the reference diet containing the same digestible energy (15.7 kJ/g) and similar digestible protein levels (45.4%). The survival rate of flounder fed on the reference diet was significantly lower than that of fish fed on other experimental diets (*P*<0.05).

## Discussion

Nutrient digestibility was strongly correlated with fish growth. Fish fed with extruded pellets showed lower protein ADCs than fish fed with raw fish-based moist pellets. Moon et al. (1994) demonstrated that the heating process used to make extruded pellets denatures proteins, making them less available for protein digestion. However, Akimoto et al. (1992) and Robaina et al. (1999) reported that the protein ADC in rainbow trout and sea bass was not affected by these feed production methods (pelleted and extruded). Physiological differences in enzyme activity and dietary amino acid profiles among fish species are likely responsible for these conflicting reports (Gomez-Requeni et al., 2003); however, the effect of feed process on protein ADC is still a matter for debate. Fish fed on extruded pellets had a higher NFE ADC than fish fed on raw fish-based moist pellets. The extrusion process can improve the availability of dietary starch for both marine (Jeong et al., 1991) and freshwater fish (Bergot and Breque, 1983; Takeuchi et al., 1990; Takeuchi et al., 1994). However, the high wheat flour content in the reference diet, which was not fully gelatinized, may have decreased its NFE digestibility (Grisdale-Helland and Helland, 1998). As noted in other studies of marine flatfish, such as flounder (Lee et al., 2002), turbot (Regost et al., 2001), and Atlantic halibut (Berge and Storebakken, 1991), flounder cannot efficiently use a high dietary lipid level as an energy source. None of these studies found any additional beneficial effects of a high lipid diet on fish growth performance.

The weight gain and feed efficiency obtained were comparable to those reported by Kikuchi et al. (1994) and slightly higher than those reported by Kikuchi et

Table 3. Growth performance of juvenile flounder fed on experimental diets for six weeks

| Diets[1] | Av. body wt. ± SD (g) | | Weight gain[2] (%) | FE[3] (%) | PER[4] | Survival (%) |
|---|---|---|---|---|---|---|
| | Initial | Final | | | | |
| EP | 3.2 ± 0.49[a] | 17.0 ± 0.23[b] | 414.4 ± 40.43[ab] | 144 ± 1.5[b] | 2.8 ± 0.03[a] | 99 ± 1.9[a] |
| RMP | 3.5 ± 0.52[a] | 19.4 ± 0.40[a] | 452.4 ± 21.60[a] | 158 ± 4.2[a] | 2.7 ± 0.07[b] | 100 ± 0.0[a] |
| MP | 3.4 ± 0.61[a] | 16.7 ± 0.17[b] | 394.7 ± 16.45[b] | 142 ± 2.3[b] | 2.6 ± 0.04[b] | 98 ± 1.9[a] |
| RD | 3.5 ± 0.66[a] | 14.6 ± 0.31[c] | 311.8 ± 12.55[c] | 127 ± 2.4[c] | 2.4 ± 0.05[c] | 80 ± 3.3[b] |

[1]EP, extruded pellet; RMP, row fish-based moist pellet; MP, moist pellet; RD, reference diet.
[2][(Final fish weight - Initial fish weight + Fish loss weight) × 100] / Initial fish weight.
[3]Feed efficiency (%) = [(Final fish weight - Initial fish weight + Fish loss weight)×100] / Feed intake (dry weight).
[4] Protein efficiency ratio = weight gain (g) / protein intake (g).
Values (mean ± SD of three replications) with different superscripts within each column are significantly different ($P<0.05$).

al. (1993), who studied the replacement of fish meals with other protein sources in flounder. The greater weight gain of fish fed on raw fish-based moist pellets was probably caused by the higher dietary protein (59.3%) and digestible energy (17.4 kJ/g) contents of the diet. Similarly, increased dietary protein levels (from 41 to 61.8%) improved the growth and feed efficiency of young halibut, but excessive dietary protein levels (from 63.3 to 71.9%) did not further improve in fish growth (Aksnes et al., 1996). According to Kikuchi et al. (2000), both feed efficiency and protein efficiency ratios were improved in flounder fed on a 55% protein diet, with an increase in the dietary energy level from 17.3 to 19.5 kJ/g. The dietary energy levels were similar to that reported in fish fed on extruded pellet and raw fish-based moist pellet diets. However, the dietary protein level here was lower than that in the raw fish-based moist pellet diet and higher than that in the extruded pellet and reference diets. Due to differences in the sizes of fish used, a simple comparison of growth rates with those reported by other studies is difficult. These results may indicate that juvenile flounder both require and can use a considerable amount of protein for growth. Hebb et al. (2003) reported that the best growth performance of juvenile flounder grown from 0.9 to 5 g was achieved in fish fed on a 50% protein diet with a dietary protein/dietary energy ratio of 26 mg/kJ. The weight gain of flounder fed on extruded pellets was similar to this growth enhancement.

We evaluated the digestibility and growth associated with three feed types used by flounder farmers. The results indicate that extruded pellets can be substituted for raw fish-based moist pellets while still maintaining a good growth rate and better protein efficiency. The results after a six-week duration suggest that further research into the biological and nutritional improvement of the feed formulation of extruded pellets could improve the cost-effeciency of flounder culture by increasing fish growth and improving water quality.

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