Growth, Feed Utilization and Nutrient Retention of Juvenile Olive Flounder (*Paralichthys olivaceus*) Fed Moist, Semi-moist and Extruded Diets

J.-D. Kim* and S.-H. Shin

Department of Feed Science & Technology, Kangwon National University, Chuncheon 200-701, Korea

ABSTRACT : In an attempt to develop an artificial diet for growing olive flounder (*Paralichthys olivaceus*), weight gain, feed utilization and nutrient retention were investigated in fish fed moist (MP), semi-moist (SMP) and extruded pellets (EP). Excretion of nitrogen and phosphorus was also estimated based on their whole body gain and intake. EP and MP composed of raw fish and SMP made of formulated powder feed with water were prepared to have the same energy contents on a dry matter basis. A total of 240 fish with an average initial weight of 120 g were randomly distributed to each (20 fish/tank) of 12 circular plastic tanks (4 tanks/treatment) and fed experimental diets for 8 weeks. Fish groups fed EP (209 g) or SMP (209 g) recorded higher final weight than those fed MP (176 g), while dry feed consumption was highest in SMP groups (106 g), followed by MP (71 g) and EP groups (62 g). As a consequence, fish groups fed EP showed much lower feed conversion ratio than the other two groups. Protein efficiency ratio was also significantly higher in fish groups fed EP (2.55) than in those fed MP (1.44) and SMP (1.31). Fish groups fed EP, which showed the highest nitrogen retention of 43.9%, resulted in the lowest excretion of nitrogen of 35.5 g per kg gain. Also, the lowest phosphorus excretion of 6.0 g per kg gain was found in the EP groups with the highest P retention (37.0%) among treatments. Although the EP groups had the lowest dietary energy intake, they retained the highest energy in the whole body among treatments. The present results showed that EP could be more advantageous than MP or SMP in terms of growth, feed utilization and excretion of nitrogen and phosphorus for olive flounder. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 5 : 720-726)***

Key Words : *Paralichthys olivaceus*, Extruded Pellet, Semi-moist Pellet, Moist Pellet, Growth, Nitrogen Retention, Phosphorus Excretion

INTRODUCTION

With the establishment of the technique for artificial seed production in 1990, the olive flounder *(Paralichthys olivaceus*) has become the major commercial marine fish cultured in Korea since 2000. More than 50,000 metric tons of olive flounder are produced every year in Korea. A wide range of feeds are employed to culture the flounder, which include extruded dry pellet, raw fish-based feed with or without compound feed and raw fish itself, such as sand lance. Even though the use of different kinds of feeds depends on the availability, cost and culture method, flounder cultured in land-based facilities are widely fed raw fish as a moist pellet (MP) with 5 to 10% of compound feed. This results in poor growth because most farmers believe that the olive flounder does not accept dry pellets well. It is estimated that about 420,000 mt of raw fish were fed to marine fish being cultured in 2001 of which two thirds were used for flounder production (Kim, 2002). However, with increasing demand the supply and quality of raw fish are declining fast, while the price is increasing. In almost all flounder farms, an extruded dry pellet is fed to the fish from weaning of live food organisms until they reach about 20 g. The dry diet is then switched to MP with which they reach market size of 800 g to 1.5 kg. However, MP feeding causes a significant waste of nutrients and water quality deterioration due to uneaten feed (Kim and Lee, 2000). Also, high mortality by direct transmission of pathogenic bacteria is observed in farms where MP is fed, especially in summer. Therefore, it is necessary to develop a diet, which is pathogen-free, water-stable, environment- friendly and highly palatable to olive flounder.

Many nutritional studies with olive flounder have been conducted to determine the requirements of protein (Kikuchi et al., 1992, 2000; Kim et al., 2002; Lee et al., 2002), lysine (Forster and Ogata, 1998), methionine (Alam et al., 2000), arginine (Alam et al., 2002), lipid and fatty acids (Furuita et al., 1998; Lee et al., 2000). However, almost all studies were concentrated on the fry and young stages. Evaluation of alternative protein sources to fish meal were also conducted for olive flounder (Kikuchi and Sakaguchi, 1997; Sato and Kikuchi, 1997; Kikuchi, 1999). Even though commercial dry pellets are now marketed for all growth stages of olive flounder in Korea, it is a common practice that the farmers themselves prefer to manufacture and feed MP. This suggests a great potential for water pollution from flounder production. A few preliminary attempts were made to resolve this problem using different types of diet (Kim and Lee, 2000; Kim, 2000; Kim et al., 2002). The purpose of this study was to develop a new type of diet, either a semi-moist pellet (SMP) or an extruded pellet (EP) to replace MP for olive flounder. For this purpose, three different types of diet were fed to olive

* Corresponding Author: J.-D. Kim. Tel: +82-33-250-8634, Fax: +82-33-255-8639, E-mail: jdekim@kangwon.ac.kr
Received August 12, 2005; Accepted December 21, 2005

Table 1. Ingredient composition of the experimental diets (%)[1]

Ingredient	EP	SMP	MP
Fish meal[2]	55.0	72.0	-
Wheat flour[3]	16.0	3.48	-
Soybean meal[4]	11.5	-	-
Squid liver powder[5]	5.0	4.0	-
Corn gluten meal[6]	5.0	5.0	-
Wheat gluten[7]	-	11.5	-
Fish oil[5]	5.0	2.0	-
Finnstim[8]	1.0	0.5	-
Antioxidant[9]	-	0.02	-
Vit.-Min. mixture[10]	1.5	1.5	-
Frozen raw fish[11]	-	-	90.1
Powdered feed[12]	-	-	4.7
Others[13]	-	-	5.2

[1] EP: extruded pellet; MP: moist pellet; SMP: semi-moist pellet.
[2] El-Golfo, Chile (CP 68%).
[3] Daehan flour mills Co., Ltd., Korea (CP 14%).
[4] Shin Dong Bang Corporation, Korea (CP 46%).
[5] Korea Oil & Pets Co., Ltd., Korea.
[6] Doosan CPK, Korea (CP 60%).
[7] Roquette, France (CP 78%).
[8] Finnsugar Bioproducts, Inc., Finland (Betaine 90%).
[9] Endox, Kemin Industries Inc., USA.
[10] Contained vitamins (per kg diet: vitamin A, 80,000 IU; vitamin D, 32,000 IU; vitamin E 160 IU; vitamin K, 16 mg; vitamin B_1, 80 mg; vitamin B_2, 64 mg; vitamin B_6, 80 mg; vitamin B_{12}, 96 mg; vitamin C, 160 mg; biotine, 0.320 mg; choline, 2,000 mg; folic acid, 16 mg; niacin, 480 mg; pantothenic, 144 mg; inositol, 640 mg; BHT, 20 mg) and minerals (per kg diet: Mg, 100 mg; Mn, 80 mg; Zn, 120 mg; Fe, 40 mg; Cu, 20 mg; Se, 0.12 mg; Co, 1.9 mg; I, 5 mg).
[11] Composed of sand lance (42.7%), anchovy (28.4%) and tiny shrimp (19.0%).
[12] Commercial feed (CP 50%) supplied by Korea Special Feed mill.
[13] Composed of water (2.4%), kelp powder (2.4%) and Aqua-Vita (0.4%) supplied by Bayer, Korea.

flounder weighing 120 g for 8 weeks and growth, feed utilization and nutrient retention were compared.

MATERIALS AND METHODS

Experimental diets

Three different physical types of diet, EP, SMP and MP, were prepared. Before formulating EP and SMP, chemical analysis of MP was done. Based on protein (55%) and lipid (18%) levels of MP on dry matter basis, EP was designed to maintain the same level of protein (55%) and a half level of lipid (9) as MP, while SMP contained half the lipid (9%) of MP with a concomitant increase in protein (64%). As a feed attractant, Finnstim (Finnsugar Bioproducts Inc., Finland) was incorporated at a level of 1% for EP and 0.5% for SMP. Following the formula (Table 1), ingredients were mixed using a horizontal mixer for EP and SMP. Then, floating EP was made to two pellet sizes of 5.3 mm and 6.5 mm for the first and second 4-week feeding, respectively, using the Wenger X-185 (Kansas, USA). Ingredient mixing for both EP and SMP, as well as the extrusion processing of EP, was conducted following conventional procedures used by Korea Special Feed Mill Company (Inchon., Korea). SMP was prepared by adding 60% tap water to the mixture composed of 72% fish meal and 28% other feed ingredients. MP was composed of 90% raw fish, 5% mash feed and other additives (3%) with 2% water (Table 1). Both SMP and MP were cold-pelleted every other week using the same commercial moist pellet machine and stored in a freezer (-30°C). SMP, with a pellet size of 10 mm, was fed during the whole 8 week experimental period, while MP, of 10 mm and 15 mm, was fed for the first and second 4 weeks, respectively. During preparation, aliquots of SMP and MP were sampled to determine moisture content for the calculation of dry feed intake. Composition of the experimental diets is shown in Table 1.

Experimental conditions

Three hundred juvenile olive flounder, *Paralichthys olivaceus*, were selected form a big concrete tank and randomly distributed to each (20 fish/tank) of the 15 cylindro-conical fiberglass reinforced plastic tanks with water volume of 64 L (53 cm diameter, 29 cm depth) in a flow-through system. Fish were adapted to the experimental tanks for 3 weeks before starting the feeding trial. The MP was fed to all fish during the adaptation period. Following a 24 h fasting period after the adaptation, 20 fish with similar size were randomly distributed into each of 12 tanks (4 tanks/treatment) and bulk-weighed (body weight of 120 g/fish). Diets were fed to apparent satiation twice daily for 6 days per week for 8 weeks (49 feeding days). Water pumped from the sea was mixed with warm seawater from a hydroelectric power plant. Filtered and UV-treated water (salinity, 33-34 ppt) was supplied to each tank at a flow rate of 5 L/min to ensure 100% water exchange rate every 5 min. Water temperature ranged from 16 to 18°C for the whole experimental period. Water outlets of the tanks were located at the bottom with a double standing pipe design. Photoperiod was under natural conditions. Light intensity at the water surface was, however, maintained at around 80 lux during daytime by a shade. The dissolved oxygen and ammonia levels were determined once per week and averaged 5.5-6.1 mg/L and 0.03 to 0.05 ppm, respectively for the experimental periods.

Daily feed allowances, calculated at the level of 1% total fish weight, were divided into two meals in plastic containers and fed at 9 am and 3 pm. Each meal was carefully fed so that fish were not stressed. The weight of unconsumed MP and SMP feeds after every feeding were recorded and discarded. All diets fed were considered consumed. Daily feed allowances were increased weekly following estimated weight gains based on the feed conversion of 2. The number and weight of dead fish were recorded to correct weight gain and feed intakes of the affected fish group.

Table 2. Chemical composition of the experimental diets

Composition	EP	SMP	MP
Proximate composition (g or kJ/100 g DM)			
C. protein	55.7	63.2	54.3
C. lipid	10.0	9.5	17.7
C. ash	11.1	9.6	11.3
C. fiber	1.1	0.5	1.0
Ca	3.0	4.2	4.5
P	1.4	1.5	1.1
Gross energy	2,493	2,532	2,501
Essential amino acid composition (% protein)			
Lysine	6.64	6.32	6.21
Arginine	5.77	5.59	5.36
Histidine	3.02	2.91	2.39
Isoleucine	4.39	4.54	4.35
Leucine	7.93	8.26	7.10
Methionine +cystine	3.46	3.19	3.34
Phenylalanine +tyrosine	7.30	7.46	6.72
Threonine	4.02	3.97	4.17
Valine	5.47	5.40	5.46
Tryptophan	1.03	1.18	1.26

Chemical analysis

At the beginning of the feeding trial, 20 flounder were randomly sampled, killed with an overdose of tricaine methanesulfonate and thoroughly ground using a commercial chopper. At the end of the 8-week feeding, five fish per tank were sampled, sacrificed and ground in the same manner. Ground fish carcasses were frozen, lyophilized and then finely ground. These samples were then stored in air-tight containers at -30°C until analysis. Lyophilized fish carcasses and the experimental diets were analyzed using the AOAC (1990) procedures: dry matter by drying for 24 h at 110°C; crude protein (N×6.25) by the Kjeldahl method after an acid digestion; crude lipid after ether extraction following acid (4 N HCl) hydrolysis (Tecator Soxtec System, Hoeganaes, Sweden); crude ash by incineration in a muffle furnace at 550°C for 24 h; Ca by a wet ash method and titration with $KMnO_4$ and phosphorus by a spectrophotometric method using molybdovanadate reagent; crude fiber by digestion with 1.25% H_2SO_4 and 1.25% NaOH solutions. Gross energy was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA). Amino acid composition of diets was analyzed after acid hydrolysis using an automatic analyzer (Hitachi Model 835-50, Japan). Tryptophan was determined by the calorimetric method of Basha and Roberts (1977) after alkaline hydrolysis of each sample.

Statistical analysis of data

The results were subjected to analysis of variance; where appropriate, differences between treatment means were determined at the 5% probability level using Duncan's new multiple range test, as described by Steel and Torrie (1960).

RESULTS

Analysis of the experimental diets showed intended levels of nutrients with the same gross energy of 2,500 kJ/100 g on a dry matter basis (Table 2). It was found that lysine, methionine and arginine levels in diets were all higher than the reported requirements of 4.6% (Forster and Ogata, 1998; Kim and Lall, 2003), 2.98% (Alam et al., 2000) and 4.2% (Alam et al., 2002), respectively. All amino acids satisfied the requirements estimated based on the A/E ratios of whole body tissues of turbot (Kaushik, 1998) and flounder (Forster and Ogata, 1998). Fish adapted to the rearing tank, showed good acceptance of diets when MP was switched to either EP or SMP after the 3-week acclimation period. Numbers of mortalities were 3, 2 and 1 for fish groups fed EP, MP and SMP, respectively during the 8-week feeding trial. Weight gain of fish fed EP (88.7 g) and SMP (88.3 g) were comparable to each other, and significantly higher ($p<0.05$) than fish fed MP (56.1 g). Dry feed intake was 62.4 g, 70.6 g and 105.9 g for fish fed EP,

Table 3. Weight gain and feed utilization of olive flounder fed the experimental diets for 8 weeks[1]

	EP	SMP	MP
Initial wt. (g/fish)	120.0±1.10^{ns}	120.2±1.38	119.5±1.11
Wt. gain (g/fish)	88.7±5.52^{a}	88.3±8.18^{a}	56.1±8.37^{b}
Total feed intake (g/fish)			
As-fed	65.8±1.79^{c}	182.9±6.74^{b}	238.6±16.11^{a}
DM	62.4±1.69^{b}	105.9±3.90^{a}	70.6±4.77^{b}
FCR[2]			
As-fed	0.75±0.03^{c}	2.11±0.13^{b}	4.41±0.37^{a}
DM	0.71±0.03^{b}	1.22±0.07^{a}	1.31±0.11^{a}
PER[3]	2.55±0.14^{a}	1.31±0.11^{b}	1.44±0.19^{b}
SGR (%/day)[4]	0.99±0.05^{a}	0.98±0.07^{a}	0.69±0.08^{b}

[1] Values (means±SE of four replicate groups) in the same row sharing a common superscript were not significantly different ($p>0.05$); ns = nonsignificant.
[2] Feed conversion ratio = feed intake (as-fed or DM)/wet weight gain.
[3] Protein efficiency ratio = wet weight gain/protein intake.
[4] Specific growth rate = (ln(final wt.)-ln(initial wt.))/duration (56 days)×100.

MP and SMP, respectively. The FCR was, however, converted to 0.71, 1.22 and 1.31 for EP, SMP and MP, respectively, expressed on a dry matter basis. Even though no significant difference was observed between fish groups fed EP and SMP in weight gain, fish fed EP showed FCR significantly lower ($p<0.05$) than those fed SMP or MP. A significantly higher protein efficiency ratio (PER) of 2.55 was obtained in fish fed EP, compared to other fish groups. On the other hand, similar specific growth rates (SGR) were obtained in fish fed EP (0.99%) and SMP (0.98%), which was, however, significantly higher than that (0.69%) of fish fed MP (Table 3).

Fish groups fed SMP showed the highest nitrogen gain of 2.66 g, which was not significantly different ($p<0.05$) from fish fed EP. Fish fed MP showed the lowest nitrogen gain of 1.49 g. With the lowest N intake, fish fed EP recorded the highest nitrogen retention efficiency (NRE) of 43.9%. N excretion (g/kg weight gain), which was calculated as the difference between N intake and gain, was the highest (93.1 g) in fish fed SMP and the lowest (35.5 g) in fish fed EP. Phosphorus intakes ranged from 0.79 g to 1.61 g, while the gain ranged from 0.15 g to 0.42 g for fish fed MP and SMP, respectively. However, fish fed EP showed the highest phosphorus retention efficiency (PRE), resulting in the lowest excretion of 6.0 g among treatments. Fish fed MP consumed the highest level of dietary lipid (12.5 g) among treatments, while they gained the lowest in the body thereby showing the lowest lipid retention efficiency (LRE; 10.3%). Fish fed EP, however, consumed the lowest lipids (6.2 g), and had the highest LRE (64.9%) among treatments. On the other hand, LRE of fish fed SMP was much lower ($p<0.05$) than that of fish fed EP, although body lipid gains were not significantly different between them. Although the energy intake was the highest (2,680 kJ) in fish fed SMP and the lowest (1,551 kJ) in fish fed EP, the energy gain of fish fed SMP and EP were similar. Energy retention efficiency (ERE) of fish fed EP was significantly higher ($p<0.05$) than that of fish fed MP or SMP (Table 4).

Whole body composition of fish at the beginning and end of the feeding trial is shown in Table 5. Moisture content of fish fed MP was higher ($p<0.05$) than the other two groups. Body protein was higher in fish fed SMP (18.4%) than in fish fed EP (17.7%) or MP (17.6%). Compared to the initial fish, lipids increased from 1.9% to

Table 4. Utilization of nutrients and energy by olive flounder fed the experimental diets for 8 weeks[1]

	EP	SMP	MP
Nitrogen			
Intake (g/fish)	5.55 ± 0.15^{b}	10.71 ± 0.39^{a}	6.14 ± 0.41^{b}
Gain (g/fish)	2.44 ± 0.17^{a}	2.66 ± 0.24^{a}	1.49 ± 0.24^{b}
NRE (%)[2]	43.9 ± 2.19^{a}	24.7 ± 1.57^{b}	23.8 ± 2.42^{b}
Excretion (g/kg wt. gain)	35.5 ± 2.74^{b}	93.1 ± 7.62^{a}	87.2 ± 9.78^{a}
Phosphorus			
Intake (g/fish)	0.84 ± 0.02^{b}	1.61 ± 0.06^{a}	0.79 ± 0.05^{b}
Gain (g/fish)	0.31 ± 0.03^{a}	0.42 ± 0.05^{a}	0.15 ± 0.03^{b}
PRE (%)[3]	37.0 ± 2.34^{a}	25.6 ± 2.17^{b}	18.9 ± 3.05^{b}
Excretion (g/kg wt. gain)	6.0 ± 0.32^{b}	13.9 ± 1.25^{a}	11.9 ± 1.33^{a}
Lipid			
Intake (g/fish)	6.20 ± 0.24^{c}	10.06 ± 0.52^{b}	12.50 ± 1.19^{a}
Gain (g/fish)	4.04 ± 0.73^{a}	2.96 ± 0.51^{a}	1.34 ± 0.57^{b}
LRE (%)[4]	64.9 ± 10.76^{a}	29.5 ± 4.80^{b}	10.3 ± 3.63^{c}
Energy			
Intake (kJ/fish)	1551 ± 42.2^{b}	2680 ± 98.8^{a}	1766 ± 119.3^{b}
Gain (kJ/fish)	739 ± 31.6^{a}	735 ± 69.6^{a}	392 ± 76.2^{b}
ERE (%)[5]	47.6 ± 0.82^{a}	27.2 ± 1.65^{b}	21.7 ± 3.00^{b}

[1] Values (means±SE of four replicate groups) in the same row sharing a common superscript were not significantly different ($p>0.05$).
[2] Nitrogen retention efficiency = N gain/N intake×100.
[3] Phosphorus retention efficiency = P gain/P intake×100.
[4] Lipid retention efficiency = L gain/L intake×100.
[5] Energy retention efficiency = E gain/E intake×100.

Table 5. Whole body composition of olive flounder fed the experimental diets (% or kJ/100 g)

Diet	Moisture	Protein	Lipid	Ash	Ca	P	GE
EP	74.3 ± 0.33^{b}	17.7 ± 0.10^{b}	3.0 ± 0.24^{a}	3.1 ± 0.14^{b}	1.28 ± 0.04^{a}	0.51 ± 0.01^{b}	702 ± 5.3^{a}
SMP	74.5 ± 0.13^{b}	18.4 ± 0.08^{a}	2.5 ± 0.14^{ab}	3.2 ± 0.11^{b}	1.17 ± 0.01^{b}	0.57 ± 0.01^{a}	700 ± 8.0^{a}
MP	75.8 ± 0.24^{a}	17.6 ± 0.09^{b}	2.0 ± 0.17^{b}	3.8 ± 0.08^{a}	1.21 ± 0.01^{ab}	0.52 ± 0.01^{b}	633 ± 13.1^{b}
Initial	75.1±0.10	18.1±0.01	1.9±0.11	4.5±0.08	1.24±0.10	0.63±0.02	605±1.5

[a, b] Values (means±SE of four replicate groups) in the same column sharing a common superscript were not significantly different ($p>0.05$).

3.0% (EP) and ash decreased from 4.5% to 3.1% (EP) in all fish groups. Among treatments, the highest whole-body lipid content was found in fish fed EP, while the highest ash content was found in fish fed MP. Calcium content ranged from 1.17% to 1.28%, which was not greatly different from values (1.24%) for the initial fish. Phosphorus was higher ($p<0.05$) in fish fed SMP than in fish fed EP or MP. The P levels in all fish groups were lower than in the initial fish. Energy content increased from 605 kJ for initial fish to 633 kJ and 702 kJ for fish fed MP and EP.

DISCUSSION

Moist feeds were used for salmon and trout production due to their better acceptance with soft texture and relatively low cost compared to dry diet (Ghittino, 1979). Even though moist feeds are still widely used for fish culture like yellowtail and flounder, there is no longer any merit in using them because of potential water pollution from left-over feed and decrease in quality and quantity of raw fish as a feed ingredient.

In an olive flounder farm with production of 100 metric tons of fish per year, feed cost makes up 40% of total production cost and more than 90% of the feed cost is put into the rearing stage from grower (>50 g) to market size (1 kg), during which feeds are fed in the form of moist pellets (Kim, 2000). However, there is little published information on growth and feed utilization for the species at this size. The dry diet is also more expensive than MP. However, the present study showed that flounder accepted dry EP and grew well. It is generally observed that flounder take the pellet and spit it out when dry pellets were fed. Bromly (1980b) reported that dietary water content did not significantly influence the growth of juvenile turbot. However, a retarded growth was found when dry pellet was fed to fish above 50 g (Person-Le Ruyet and Noel, 1982). Bjornsson et al. (1992) suggested that spitting out dry pellet seemed not to be due to dried texture but low palatability of the feed. Good acceptance of EP in the present experiment might be due to increased palatability through the addition of an attractant (Hara, 1994; Fredette et al., 2000).

In a previous study, Kim and Lee (2000) compared moist pellet (MP) to a semi-moist pellet (SMP) and a commercial extruded pellet (EP) as a diet for olive flounder weighing 209 g. In an 8-week feeding trial, they found that weight gain and SGR of fish fed SMP were comparable to those of fish fed MP, although fish fed EP showed the lowest gain and SGR among treatments. However, the present study revealed that weight gain and SGR of fish were significantly higher ($p<0.05$) in fish fed EP or SMP than in fish fed MP. Also, the lowest FCR was obtained in fish fed EP, while the FCR was not significantly different between fish groups fed MP and SMP. These results may suggest that olive flounder could be grown well with either dry or semi-moist diet. Contrary to the finding by Sato (1999) that growth of flounder depends on dietary protein level regardless of energy content, fish fed the lower protein level showed the best weight gain in present study. This may suggest a significant wastage of diet fed to the MP and SMP groups. It is generally accepted that waste from unconsumed feed by practical feeding of MP amounts to more than 30% total feed offered (Kim, 2002). Even though careful feeding was done for every meal in the present feeding trial, some amount of MP might have been wasted. More waste of SMP than MP might also have occurred due to its higher specific gravity. This could explain that despite higher weight gains, the FCR of fish fed SMP was not significantly improved in comparison to MP.

In general, most protein requirement studies are concentrated on the fry stage of a target fish fed either a purified, semi-purified or practical diet. The results obtained from such a study are extrapolated to practical feed formulations for the same species of fish. Once the requirements are determined for small fish, it is general practice that the requirements of bigger fish are estimated under the hypothesis that protein needs decrease with increase in age or body weight (Bowen, 1987). However, bearing in mind that olive flounder culture is conventionally conducted by feeding MP and that MP based on raw fish contains more than 50% protein, a dry diet should be developed prior to determining the protein requirements for the fish. Also, given the feed waste due to specific gravity and different physical characteristics, careful consideration is needed for the practical use of such data obtained using semi-moist diets in protein requirement studies (Lee et al., 2000, 2002; Kim et al., 2002). One should bear in mind that feed preparation methods can profoundly affect nutrient availability and feed quality. It is therefore essential that nutrient requirement studies be conducted in a similar manner to on-farm practices (Tacon, 1995).

Kim et al. (2002) mentioned that the protein requirements for olive flounder observed by Lee et al. (2000) and by Kikuchi et al. (1992, 2000) were confusing. Kikuchi et al. (2000) concluded that the optimal protein level for maximum growth was 51% in a diet containing 17 kJ/kg energy. The energy used in their study was calculated based on 16.7, 37.6 and 16.7 kJ/g for protein, lipid and carbohydrate, respectively. The energy calculation used for channel catfish by Garling and Wilson (1976) was also made in the protein studies by Lee et al. (2000; 2002). Given the different digestive physiology between flounder and channel catfish, the requirements obtained with such isocaloric diets remain to be re-evaluated.

Water pollution by fish feeding is caused largely by increasing biological oxygen demand, as well as nitrogen and phosphorus loading through unconsumed feed and feces (Falke and Kautsky, 1989). From an on-farm experiment with flounder, Kim and Lee (2000) reported that the excretion of nitrogen (N) ranged from 48 g to 70 g and phosphorus (P) from 10 g to 12 g per kg weight gain. However, under practical feeding conditions, flounder excreted much higher N of 114 g and P of 28 g per kg weight gain, suggesting a substantial waste of feed (Kim et al., 2002). In the present study, fish fed EP excreted 36 g N and 6 g P per kg weight gain, however more than two-fold higher levels of N and P were excreted in fish fed MP and SMP (Table 4). The loads excreted from fish fed EP in this study were comparable to those (38 g N and 8 g P/kg weight gain) in carp fed an EP (Kim et al., 1998). From an environmental point of view, these results suggest that EP rather than MP or SMP should be recommended for flounder culture. The moisture content of EP used in this experiment was 5.5%, while that of MP and SMP was 70.4% and 42.1%, respectively. This difference in moisture content resulted in the differences in size, consumption time and specific gravity which, in turn, might increase feed waste. Despite higher N intake, fish fed MP and SMP had significantly lower ($p<0.05$) NRE than EP. This could be explained by both an increase in feed waste and the fact that an increase in dietary protein intake results in a decrease in protein utilization efficiency (Lee and Putnam, 1973; Bromly, 1980a; Kim and Lall, 2001). However, lower P gain and PRE obtained in fish fed MP may be explained by their lower weight gain in this study than fish fed EP or SMP.

Fish like rainbow trout (Kim and Kaushik, 1992) and ayu (Takeuchi, 1978) show LRE higher than 100%. This means that lipid retention of the fish surpasses its intake. However, olive flounder are lean fish (Sato et al., 1986) and have a limited capacity for whole-body lipid deposition (Oku and Ogata, 2000). The highest LRE was found in fish fed EP which was, however, only 64.9% in present study. Regost et al. (1999) reported that the LRE of turbot fed different diets with the same 12% level of lipid ranged from zero to 32%. Oku and Ogata (2000) reported that the increase in dietary lipid from 11% to 21% for olive flounder weighing 3 g resulted in an increase in lipid intake from 1.1 g to 2.3 g, although LRE decreased from 42% to 23%. Lower lipid retention in turbot has been explained by no change in hepatic lipogenic enzymes in response to dietary lipid level in turbot, reflecting the high protein requirements (Regost et al., 2001). However, it remains to be clarified that such a mechanism observed in turbot would occur in olive flounder also. Lipid levels of 2.0 to 3.0% in the whole body of olive flounder (Table 5) were comparable to those found by Oku and Ogata (2000), Lee et al. (2000, 2002) and Choi et al. (2004), but much lower than those (4.6% to 6.2%) found by Kim et al (2002). On the other hand, ERE of fish fed MP (21.7%) and SMP (27.2%) obtained in the present study were comparable to those reported by Kim and Lee (2000), although fish fed EP had a much higher ERE of 47.6%.

Present results obtained in fish fed EP suggest that lipid or protein levels higher than optimum requirements have no effects on growth and feed utilization of olive flounder and that a dry diet could be fed without any detrimental effect. However, a study under practical farming conditions should be conducted because it is uncertain if results similar to those in the present study would be obtained. It is also recommended that an on-farm feeding trial be conducted until fish reach market size to evaluate the cost efficiency of production and pollution loads of fish fed different physical types of diets.

ACKNOWLEDGEMENTS

The research project was funded by the Ministry of Marine Affairs of Korea. We thank J. S. Shin, president of Hangdo flounder farm, for providing moist pellet and experimental fish.

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