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Effects of the Extruded Pellets and Raw Fish-Based Moist Pellet on Growth and Body Composition of Flounder, *Paralichthys olivaceus* L. for 10 Months

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Effects of the commercially available or formulated extruded pellets (EP) and raw fish-based moist pellet (MP) on growth and body composition of flounder *Paralichthys olivaceus* L. were evaluated on commercial scale for 10 months. Survivals of flounder fed the MP, EP1 and EP3 were not significantly different from those of fish fed the EP2 or EP5, but significantly ($P<0.05$) higher than that of fish fed the EP4. Weight gain of fish fed the MP was not significantly different from that of fish fed the EP3 and EP5, but significantly ($P<0.05$) higher than that of fish fed the EP1, EP2 or EP4. Improvement in weight gain of flounder fed the high lipid diets (EP1, EP2, EP3) compared to low lipid diet (EP4) in the 55% protein level, and weight gain of fish fed the high lipid diet (MP) compared to low lipid diet (EP5) in the 61% protein level indicated protein-sparing effect of lipid. FER for flounder fed the EP3, which was not significantly different from that for fish fed the EP1 or EP5 was significantly ($P<0.05$) higher than that for fish fed the EP2, EP4 and MP. FER significantly ($P<0.05$) changed over time. CF of flounder fed the MP was significantly ($P<0.05$) higher than that of fish fed EP1 or EP4. Moisture content of dorsal muscle in flounder fed the EP2 was significantly ($P<0.05$) higher than that in fish fed the EP1 or EP4. Crude protein content of dorsal muscle in flounder fed the EP1, EP4 and EP5 was significantly ($P<0.05$) higher than that in fish fed the EP2. However, crude lipid content of dorsal muscle, and moisture, crude protein and crude lipid content of liver in flounder was not significantly different among the diets. In considering performance of flounder, it can be concluded that the use of EP3 and EP5 is more recommendable than the MP, which commonly used in flounder farm.

Keywords: Flounder *Paralichthys olivaceus*, Extruded pellet, Moist pellet, Protein-sparing effect of lipid

Introduction

Flounder *Paralichthys olivaceus* L., frequently called bastard halibut, is one of the most commercially important aquaculture species in Korea. Most flounder production in Korea has been made using frozen raw fish (sardine or mackerel) or raw fish-based moist pellet (MP) composed of frozen raw fish and commercially available binder meal at a certain ratio. However, supplying frozen raw fish or raw fish-based MP for aquaculture led to several problems, such as an unstable supply and a sharp increase in price of raw fish due to a shortage of its supply, its over-catch and an extra cost for storage in frozen form for later use. Alternatively, commercially available extruded pellets (EP) that are nutritionally-balanced using least-cost formulation could be developed to efficiently produce fish in more environmentally friendly manner. However, commercially available EP are not widely used in flounder farms in Korea due to lack of information on growth performance of fish fed on EP or MP and a biased perception of superior production using MP feeds.

Recently, many nutrition studies evaluating nutrient requirements (Lee et al., 2000a; Lee et al., 2000b; Bai et al., 2001; Kim et al., 2002b; Lee et al., 2002a), feeding regime (Lee et al., 2000b; Kim et al., 2002a), substitution of fishmeal with other protein sources (Kikuchi et al., 1994a; Kikuchi et al., 1994b; Kikuchi et al., 1997; Sato and Kikuchi, 1997) and herb supplementation (Kim et al., 1998; Kim et al., 2000) in flounder have been performed. Kim et al. (2002c) reported no adverse effect of EP formulated with several protein sources compared to raw fish-based MP on growth of rockfish *Sebastes schlegeli* during a long feeding trial in net cages. However, no feeding trial on the effects of EP and MP on performance of flounder over extended periods of time has been performed.

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Therefore, the objective of this study was to evaluate the effects of the EP and raw fish-based MP on growth and body composition of flounder on a commercial scale for 10 months.

Materials and Methods

Experimental fish

Young juvenile flounder were purchased from a private flounder hatchery (Uljin, Gyeongbuk, Korea). Fish were grown to the size in which they were able to be fed commercial EP in flounder farms, by feeding MP composed of frozen mackerel and commercial binder meal for flounder at a ratio of 3:1, or commercial EP (Woosung Feed Co. Ltd, Korea) in the indoor flow-through concrete tank. A thousand juvenile fish (an initial mean body weight±SD: 87.9±0.56 g) were randomly distributed into 12 round flow-through concrete tank (460 cm in diameter and 60 cm in depth) on a commercial scale and acclimated to the experimental conditions for a week

Table 1. Ingredients, amino acid composition, volatile basic nitrogen (VBN) and thiobarbituric acid (TBA) values of the experimental diets

	Diets					
	EP1	EP2	EP3	EP4	EP5	MP
Ingredients (%)						
Whitefish meal[1]	21					
Alaska Pollack meal	14		30			
Herring meal[2]		60	30			
Herring meal[3]	20					
Mackerel						75
Commercial binder meal[4]						25
Soybean meal[5]	6	4	4			
Meat meal[5]	4					
Wheat flour[5]	22.8	14.8	19.8			
α-starch[5]		5				
Wheat gluten[5]	8	8	8			
Pollack oil[5]	1.5	1.5	1.5			
Undaria powder[5]		3	3			
Others	2.7	3.7	3.7			
Proximate analysis						
Moisture (%)	8.7	6.4	9.0	7.8	7.9	56.3
Crude protein (%, DM)	55.3	55.2	56.0	56.1	60.7	60.6
Crude lipid (%, DM)	8.1	9.0	7.4	4.6	6.1	11.0
Crude ash (%, DM)	13.4	11.4	13.0	7.8	15.1	4.8
Essential amino acids (% of protein)						
Arginine	6.3	6.6	6.5	5.4	7.7	6.6
Histidine	3.4	3.1	4.2	3.7	4.6	3.9
Isoleucine	4.2	4.9	4.9	4.0	3.5	4.4
Leucine	8.5	8.9	8.4	10.5	6.4	8.6
Lysine	7.0	8.2	8.8	6.4	8.8	7.7
Methionine+Cystein	4.0	3.3	2.9	3.4	3.6	4.3
Phenylalanine+Tyrosine	7.0	7.4	10.9	7.4	8.0	7.4
Threonine	4.5	4.7	4.0	4.4	5.1	4.6
Valine	4.8	5.4	4.7	5.0	5.2	4.8
Chemical value						
VBN (mg/100 g)	39	41	53	124	86	88
TBA (μg/g)	8.7	14.0	10.5	14.6	9.6	17.4

[1]Produced in Korea.
[2]Imported from Russia.
[3]Imported from Chile.
[4]Commercial binder meal purchased from Woosung Feed Co. Ltd, Korea.
[5]Provided by E-wha Oil and Fat Industry Co., Busan, Korea.

before the initiation of the feeding trial. During the acclimation period, fish were hand-fed commercial EP twice daily to apparent satiation.

Design of the experiment and preparation of the experimental diets

Six diets with duplication per diet were prepared for this study: two commercially available extruded pellets, three formulated extruded pellets and moist pellet (Table 1). Various ingredients were used as the primary dietary protein sources; white fishmeal, Alaska pollack meal and herring meal from Chile were used as the primary protein source in EP1, herring meal from Russia as the sole protein source in EP2, and Alaska pollack meal and herring meal from Russia in EP3, respectively. Herb Obosan was supplemented into the EP2 and EP3 to improve performance of flounder. And EP1, EP2 and EP3 were extruded with Joda Extruder Pellet Mill (Japan). Two commercially available EP diets for flounder produced in Korea (EP4) and Japan (EP5) were prepared. Besides, the MP composed of frozen mackerel and commercial binder meal for flounder at the ratio of 3:1 (wet weight basis) was prepared.

Conditions of the feeding trial

During the feeding trial, fish were hand-fed to apparent satiation twice daily at 09:00 and 17:00 h based on Lee et al. (2000b)'s study. Water was aerated continuously and flow rate of water in each tank had 12-15 turnovers/day. Water temperature ranged from 8.0 to 22.0°C (mean±SD; 14.6±4.09°C) throughout the feeding trial. During the experimental period, photoperiod was left as natural conditions. Pellet size of feeds was adjusted as fish grew. Over 80% of water in each tank was daily drained out within 1 h after the early feeding schedule. Dead or severely disease-infected fish were daily removed and counted as dead fish.

Weight gain of fish was measured in 7-week intervals after the initiation of the feeding trial. After a 24 h starvation period, 50 fish from each tank were randomly sampled and collectively weighed. Three randomly chosen fish from each sample were sacrificed for proximate analysis and regarded as dead fish. After weighing fish, broad spectrum antibiotic (Oxymycin Aqua, Samu Chemical Ind. Co. Ltd, Korea) were added to each tank at a concentration of 150 ppm for 2 h to prevent disease infection. During the summer season, outbreak of disease like parasite (*Cryptocaryon irritans*) or *Edawardsiella tarda* infection occurred, thus fish were immersed in formaldehyde solution and antibiotics at the concentrations of 200 and 250 ppm, respectively for consecutive 3-7 days. While immersed, fish were starved or fed once daily after completion of immersion. However, high mortality of fish was observed during this period. The feeding trial was ended 45 weeks after the initiation of the experiments since viral disease and *Scutica* sp. infection of fish was observed.

Chemical composition of the experimental diets and samples

Three fish randomly chosen from each sample and ten fish at the end of the feeding trial were used for proximate analysis, hepatosomatic index (HSI) and condition factor (CF). Proximate analysis was conducted based on AOAC (1990) methods. Protein (Kjeldahl method), lipid (ether-extraction method), ash (muffle furnace, 600°C for 3 h), and moisture contents (dry oven, 105°C for 24 h) were determined for the whole body samples. Amino acids of the experimental diets were analyzed using an automatic analyzer (Pharmacia Biochrom 20, Li+ type high performance ultra pack, UK). Volatile basic nitrogen (VBN) and thiobarbituric acid (TBA) values of the experimental diets were measured based on Conway (1950)'s and Witte et al. (1970)s methods, respectively.

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 test groups by using SAS program version 6.12 (SAS Institute Inc., Cary, North Carolina, USA).

Results and Discussion

Survival, weight gain (g/fish), total amount of dry feed supply (kg/tank), feed efficiency ratio (FER), hepatosomatic index (HSI) and condition factor (CF) of flounder fed the extruded pellets and moist pellet for 10 months are given in Table 2. Survivals of flounder fed the MP, EP1 and EP3 were not significantly (P>0.05) different from those of fish fed the EP2 or EP5, but significantly (P<0.05) higher than that of fish fed the EP4. Weight gain of fish fed the MP was not significantly (P>0.05) different from that of fish fed the EP3 and EP5, but significantly (P<0.05) higher than that of fish fed the EP1, EP2 and EP4. Significant improvement in weight gain of flounder fed the high lipid diets (EP1, EP2, EP3) compared to low lipid diet (EP4) in the 55% protein level, and weight gain of fish fed the high lipid diet (MP) compared to low lipid diet (EP5) in the 61% protein level indicated "protein-

Table 2. Survival (%), weight gain (g/fish), total feed supply (kg/tank), feed efficiency ratio (FER), hepatosomatic index (HSI) and condition factor (CF) of flounder fed the extruded pellets (EP) and moist pellet (MP) for 10 months (Mean±SE)

	Diets					
	EP1	EP2	EP3	EP4	EP5	MP
Initial weight (g/fish)	88.3±0.07	87.4±0.35	87.6±0.14	88.7±0.28	87.5±0.07	87.8±0.71
Final weight (g/fish)	462.1±29.42	466.8±13.22	495.4± 6.15	405.9±22.56	511.6±13.79	525.7±16.19
Survival (%)	55.0±0.08[a]	42.9±0.09[ab]	47.0±0.01[a]	24.2±0.02[b]	41.0±0.11[ab]	60.5±0.10[a]
Weight gain (g/fish)	373.9±29.49[c]	379.4±12.91[bc]	407.8± 6.01[abc]	317.2±22.84[d]	424.1±13.86[ab]	437.9±16.90[a]
Feed supply (kg/tank)	276.2±10.19[bc]	258.9±31.79[c]	269.2±41.24[bc]	281.8±26.26[bc]	338.5±20.89[ab]	398.5±26.87[a]
FER[1]	0.91±0.015[ab]	0.87±0.070[b]	0.96±0.007[a]	0.69±0.022[c]	0.91±0.010[ab]	0.75±0.006[c]
HSI[2]	1.42±0.206	1.58±0.285	1.38±0.013	1.50±0.002	1.15±0.118	1.21±0.111
CF[3]	1.24±0.032[c]	1.37±0.006[ab]	1.36±0.007[ab]	1.31±0.056[bc]	1.34±0.016[ab]	1.40±0.040[a]

Different superscripts in rows show significantly different values ($P<0.05$).
HSI and CF of flounder at the initiation of the feeding trial were 0.73±0.315 and 1.16±0.380, respectively.
[1]FER=weight gain/total feed fed (dry)
[2]HSI=liver weight×100/weight of fish
[3]CF=(wet weight of fish/total length^3)×100

sparing effect of lipid" in this study. Similarly, protein-sparing effect of lipid was observed in other fish (Helland and Grisdale-Helland, 1998; Company et al., 1999; McGoogan and Gatlin, 1999; Lee et al., 2002b). Unlike this study, however, Lee et al. (2000a) reported that excessive lipid content in the 30, 40 and 50% protein diets resulted in reduction in growth of juvenile flounder. The poorest survival and weight gain was observed in flounder fed the EP4 in this study. Since no information on feed ingredients in the EP4 is known, it is difficult to explain the reason why the EP4 achieved the worst fish performance. However, it could have resulted from high values of VBN and TBA (Table 1), which were toxic protein and oxidized degenerates present in the feed. It was reported that removal of both toxic protein and oxidized lipid degenerates in the feed was essential for red sea bream and fermentation and steaming were effective to minimize their adverse effect (Yone et al., 1986; Hossain et al., 1988). Similarly, Kim et al. (2002c) reported that weight gain of grow-out rockfish fed the MP was higher compared to that of fish fed the EP, but lowest survival during the 30-week feeding trial in net cages although weight gain of fish was not significantly different among the diets. So they recommended that the use of EP for grow-out rockfish because of low survival in fish fed the raw fishmeal-based MP.

Since feeding flounder with EP over long period of time usually leads to yellowish liver which is reddish color in normal condition health statue and disturbance in digestion of EP in digestive organ of fish, and results in high mortality and poor growth (several flounder farmers, personal communication), MP is preferred to EP in flounder farms in Korea. Therefore, these problems should be solved for EP to be widely accepted in flounder farms and more studies on physiological or nutritional side-effect of feeding flounder with EP over long periods of time are highly needed.

Total feed supply for flounder with the MP was not significantly ($P>0.05$) different from that for fish with the EP5, but significantly ($P<0.05$) higher than that for fish with the EP1, EP2, EP3 or EP4. This might result into poor FER in flounder fed the MP compared to that for fish fed the EP1, EP2 and EP3. Total feed supply for fish with the EP2 was lowest. FER for flounder was relatively acceptable, ranged from 0.69 to 0.96 in this study, which is within acceptable ranges on a commercial scale. FER for flounder fed the EP3, which was not significantly ($P>0.05$) different from that for fish fed the EP1 or EP5, was significantly ($P<0.05$) higher than that for fish fed the EP2, EP4 and MP. FER for flounder fed the EP2 was significantly ($P<0.05$) higher than that for fish fed the EP4 or MP. FER for flounder in this study was relatively high compared to that for rockfish on commercial scale in the 30-week feeding trial (Kim et al., 2002c) and comparable to that for flounder on pilot scale (Forster and Ogata, 1998; Lee et al., 2000b).

HSI of flounder ranged from 1.15 to 1.58, but was not significantly ($P>0.05$) different among the diets in this study. However, HSI of flounder at the end of the feeding trial was almost as double as that of fish at the initiation of this study. CF of flounder fed the MP, which was not significantly ($P>0.05$) different from that of fish fed the EP2, EP3 or EP5, was significantly ($P<0.05$) higher than that of fish fed EP1 or EP4, probably indicating that flounder fed the MP became

Table 3. Proximate composition (%) of the dorsal muscle and liver in flounder fed the extruded pellets (EP) and moist pellet (MP) for 10 months (Mean±SE)

Diets	Dorsal muscle			Liver		
	Moisture	Crude protein	Crude lipid	Moisture	Crude protein	Crude lipid
Initial	76.9	23.1	0.2	73.2	13.1	7.2
EP1	75.3±0.45[a]	22.2±0.73[b]	0.4±0.14	65.3±0.02	11.8±0.27	14.7±3.88
EP2	77.4±0.48[c]	20.6±0.15[a]	0.3±0.06	62.4±7.41	11.0±1.65	16.8±1.24
EP3	76.9±0.37[bc]	21.3±0.36[ab]	0.4±0.01	59.4±4.79	11.1±1.07	20.6±0.37
EP4	76.1±0.21[ab]	22.5±0.30[b]	0.7±0.25	65.8±5.54	12.1±0.57	13.5±4.05
EP5	76.2±0.30[abc]	22.7±0.40[b]	0.4±0.29	61.6±0.92	12.6±0.42	15.3±0.57
MP	77.0±0.09[bc]	21.4±0.16[ab]	0.5±0.28	65.2±2.06	12.6±1.37	17.4±3.88

Different superscripts in columns show significantly different values ($P<0.05$).

fattier than fish fed on the EP1 or EP4. CF of flounder in this study was comparable to that for flounder obtained in 48-week feeding trial (Kim et al., 1998).

Proximate composition of dorsal muscle and liver in flounder fed the extruded pellets and moist pellet is shown in Table 3. Moisture content of dorsal muscle in flounder fed the EP2 was not significantly ($P>0.05$) different from that in fish fed the EP3, EP5 or MP, but was significantly ($P<0.05$) higher than that in fish fed the EP1 or EP4. Crude protein content of dorsal muscle in flounder fed the EP1, EP4 and EP5 was not significantly ($P>0.05$) different from that in fish fed the EP3 or MP, but significantly ($P<0.05$) higher than that in fish fed the EP2. However, crude lipid content of dorsal muscle, moisture, crude protein and crude lipid content of liver in flounder was not significantly ($P>0.05$) different among the diets. In this study, unlike Lee et al. (2000a, b)s studies, body composition of flounder did not well reflect dietary nutrient composition.

Since effluent discharged from fish farms is one of the most severe water pollution sources, minimizing a potential pollution source in effluents by adapting proper feed type should be achieved. Therefore, supply of EP leading to less pollution effluent is more recommendable than that of MP for flounder farms. Although water quality was not monitored in this study, it appeared to be severely polluted after supplying MP to flounder throughout the feeding trial. Not only survival of flounder fed the extruded pellets except for EP4, but also weight gain of fish fed the EP3 and EP5 was comparable to that of fish fed the MP on commercial scale for 10 months. Besides, FER for flounder fed the EP1, EP2, EP3 and EP5 was superior to that for fish fed the MP. Therefore, in considering results of survival, weight gain and efficiency of feed, it can be concluded that the use of EP3 and EP5 is more recommendable than the MP for flounder production.

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