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Effects of Dietary Protein Sources on Growth and Body Composition in Olive Flounder (*Paralichthys olivaceus*)

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A 6-week feeding trial was conducted to investigate the effects of six different dietary animal protein sources on growth and body composition of olive flounder, *Paralichthys olivaceus* in recirculating system. White fish meal (WFM), flounder muscle (FLM), carp muscle (CM), blood meal (BM), squid liver powder (SLP) and casein (CA) were used as the main animal protein sources in the six experimental diets. Fish averaging 2.9 ± 0.03 g (mean ± SD) were distributed to each aquarium as a group of 15 fish and were fed one of the six experimental diets to each treatment of triplicate groups. After 6-week of the feeding trial, fish fed white fish meal (WFM) and flounder muscle (FLM) diets showed a significant higher weight gain (WG%) ($P<0.05$) than those of fish fed the CM, BM, SLP and CA diets. Fish fed BM diet showed the lowest WG among all the dietary treatments. Feed conversion ratio (FCR) and protein efficiency ratio (PER) showed the similar trend as WG. Hematocrit and hemoglobin were not affected by the dietary treatments. Fish fed the FLM and CM diets showed significant higher survival rate than those of fish fed BM diets, and there was no significant difference in survival of fish fed WFM, FLM, CM, SLP and CA diets. These results indicated that WFM and FLM are the best dietary protein sources tested in olive flounder.

Key words: Animal protein sources, Growth, Body composition, Olive flounder

Introduction

Olive flounder (*Paralichthys olivaceus*) is a commercially important aquaculture fish species in Korea; its production is the top among the Korean mariculture finfish species (Statistical Yearbook of Agriculture Forestry and Fisheries, 1998). Raw fish-based diets are now used in the most of the flounder culture farms in Korea. This feeding method causes many problems in aquaculture including disease, water pollution, high production costs and unbalanced nutrient supply. Therefore it is necessary to develop the formulated diets for this species.

Dietary protein sources have profound effects on growth, feed utilization and whole body composition of acuacultured fish, it can provide indispensable amino acids that fish cannot synthesize and supply dispensable amino acids or amino nitrogen to enable the fish to synthesize them. Also the protein component is typically one of the most expensive ingredients in formulated feeds. Fishmeal was the main protein source traditionally used in formulated fish feed. However, the supply of fishmeal is decreasing and the availability of high quality fishmeal is uncertain. Many researches have been done trying to use some other sources of protein such as soybean meal, feather meal, meat meal, etc. in rainbow trout (Tacon et al., 1983; Murai et al., 1989), carp (Viola et al., 1982; Abel et al., 1984), tilapia (Shiau et al., 1987; Shiau et al., 1990), catfish (Mohsen and Lovell, 1990; Webster et al., 1992), and flounder (Kikuchi et al., 1994).

Therefore, the objective of this experiment was to investigate the effects of different dietary protein sources on growth and body composition in juvenile olive flounder (*Paralichthys olivaceus*).

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Materials and Methods

Experimental design and diets

Composition of the experimental diets was shown in Table 1. White fish meal (WFM), flounder muscle (FLM), carp muscle (CM), blood meal (BM), squid liver powder (SLP) and casein (CA) were used as the main animal protein sources in the six experimental diets. The proximate composition of the six experimental animal protein sources was shown in Table 2. In the control diet, WFM was used as the main protein sources; and in each of the other five diets, 50% WFM protein was replaced by the other five protein sources on protein equivalent basis. Six experimental diets were formulated to be *isonitrogenuous and isocaloric to contain 50%* crude protein and 19.5 KJ of gross energy/g diet. Gross energy content was calculated according to the gross energy content of each ingredient (excluding cellulose) that was measured by bomb calorimeter (PARR 1351, USA). After all the dry ingredients were mixed completely, fish oil and 30% water were added and mixed further in a commercial food mixer, the mixture were pelleted by forcing it through 2 mm diameter module as described by Kim and Bai (1997). After processing, all the diets were kept −20℃ until use.

Table 1. Composition of the experimental diets

Ingredients	Experimental diets					
	WFM	FLM	CM	BM	SLP	CA
White fish meal[1]	50.0			25.0	25.0	25.0
Flounder muscle		39.2				
Carp muscle			44.0			
Haemoglobin				17.6		
Squid liver powder					34.0	
Casein[2]						17.2
Gelatin[2]	14.6	14.7	14.5	14.6	15.75	14.5
Dextrin[2]	8.85	9.06	9.35	7.79	2.91	7.88
Wheat flour	9.0	8.5	10.0	10.0	5.5	10.0
Squid liver oil[3]	5.0	5.0	5.0	5.0	3.0	5.0
Corn oil[3]	2.5	4.8	0.3	4.85	0.0	4.8
EPA+DHA[3]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[4]	3.0	3.0	3.0	3.0	3.0	3.0
Mineral mix[5]	3.0	3.0	3.0	3.0	3.0	3.0
CMC[2]	2.0	2.0	2.0	2.0	2.0	2.0
Cr_2O_3	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose[2]	1.0	0.99	0.0	1.11	1.09	1.37
Calcium monophosphate	0.0	8.7	7.8	5.0	3.7	5.2
Proximate composition (dry matter basis)						
Crude protein	50.0	50.2	50.2	50.1	49.9	50.3
Crude lipid	13.2	12.9	13.0	12.8	13.3	13.1
Crude ash	9.6	9.4	9.4	9.5	9.6	9.5

[1]White fish meal (WFM), Hanchang Co., Busan, Korea.
[2]United States Biochemical, Cleveland, Ohio 441 22.
[3]E-Wha oil Co., Ltd., Busan, Korea.
[4]Contains (as mg/kg in diets): Ascorbic acid, 300; dL-Calcium pantothenate, 150; Choline bitatrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine · HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dL-α-Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B12, 0.06.
[5]Contains (as mg/kg diet): Al, 1.2; Ca, 5000; Cl, 100; Cu, 5.1; Co, 9.9; Na, 1280; Mg, 520; P, 5000; K, 4300; Zn, 27; Fe, 40.2; I, 4.6; Se, 0.2; Mn, 9.1.

Table 2. Proximate composition of the six animal protein sources
(% of dry matter basis)[1]

	Diets[2]					
	WFM	FLM	CM	BM	SLP	CA
Crude protein	68.6	87.5	77.9	97.1	48.4	99.5
Crude fat	9.6	6.4	16.5	0.3	20.6	0.5
Crude ash	19.1	5.7	4.6	3.2	4.9	2.3

[1]Values are means from duplicate samples of in-gredients.
[2]See Table 1.

Experimental fish and feeding trial

Juvenile olive flounder (*Paralichthys olivaceus*) were obtained from Keo-Jae hatchery (Research station branch of the National Fisheries Research and Development Agency) in Korea. Prior to the start of the feeding trial, fish were fed the FM diet for 1 week to adjust to the experimental diets and conditions. The feeding trial was conducted by using a recirculating system with 30 L aquaria receiving filtered sea water at a rate of 0.8 L/min. Supplemental aeration was provided to maintain dissolved oxygen near saturation. Water temperature was kept at 17 ± 1℃ (mean ± SD) by using a heater, the salinity was maintained at 31 ± 1 g/L (mean ± SD), and the pH was maintainedat 7.5 ± 0.3 (mean ± SD). Experimental fish averaging 2.98 ± 0.06 g (mean ± SD) were randomly distributed into each aquarium in groups of 15 fish. A photoperiod of 12 h light: 12 h dark (06:00 to 18:00) was used. Each diet were fed to triplicate groups to apparent satiation three times per day at a rate of 2.5% dry matter basis diet of wet body weight. Total fish weight in each aquarium was determined every 3 weeks, and the amount of diet fed to fish was adjusted accordingly. The inside of each aquarium was cleaned during fish weighing.

Sample collection and analysis

Weight gain (WG), feed efficiency ratio (FER), protein efficiency ratio (PER), hepatosomatic index (HSI), condition factor (CF), hematocrit (PCV), hemoglobin (Hb) and survival rate were calculated and measured at the end of the feeding trial. After the final weighing, 6 fish were randomly removed from each aquarium; blood samples were obtained using a heparinized syringe from the caudal vein and pooled for hematocrit (Brown, 1980) and hemoglobin determination. Crude protein, moisture and ash of diets were analyzed by Association of Official Analytical Chemists (AOAC, 1995) methods. Crude protein content was determined by the Kjeldahl method using the Auto Kjeldahl System (Tecator, Hoganas, Sweden). Crude fat was determined using the Soxtec system 1046 (Foss, Hoganas, Sweden) after freeze drying samples for 12 h.

Statistical analysis

All data were analyzed by one-way ANOVA (Statistix 3.1, Analytical Software, St. Paul, MN, USA) to test for differences among treatments. When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered with the significant level at $P<0.05$.

Results and Discussion

Growth performance was shown in Table 3. After 6 weeks of feeding trial, fish fed white fish meal (WFM) and flounder muscle (FLM) diets showed significantly higher percent weight gain (WG) ($P<0.05$) than those of fish fed carp muscle (CM), blood meal (BM), squid liver powder (SLP) and casein (CA) diets. Fish fed BM diet showed the lowest WG among all the dietary treatment. Generally protein efficiency ratio (PER) showed similar trend as WG, fish fed WFM and FLM diets showed significant higher PER than those of fish fed CM, BM, SLP and CA diets ($P<0.05$). And there was no significant difference existed in PER of fish fed CM, SLP and CA diets. Feed conversion ratio showed negative relation with WG and PER, fish fed the BM diet showed significant higher FCR among all the dietary treatments ($P<0.05$), meanwhile fish fed WFM and FLM diets showed significant lower FCR than the other groups. These results indicated that white fish meal and lyophilized olive flounder muscle resulted in superior growth of olive flounder when compared with the other dietary protein sources tested in this experiment. WFM and FLM have a balanced amino acid that more closely meets the requirements of fish and thus support the good growth rate of fish (Moon and Gatlin, 1994; Kim and Bai, 1997). Moon (1992) indicated that red drum showed excellent growth and feed efficiency with diets containing lyophilized fish muscle regardless of its origin from freshwater or seawater species. However, the fish fed carp muscle (CM) diet in the present experiment showed significant lower WG than those of fish fed WFM and FLM diets ($P<0.05$). The amino acid and/or fatty acid profile in marine fish and freshwater fish might be different and then resulted in different growth performance in experimental fish. Further research of

Table 3. Effects of different dietary protein sources in olive flounder for 6 weeks[1]

	Diets						Pooled
	WFM	FLM	CM	BM	SLP	CA	SEM[2]
WG (%)[3]	180^{a}	194^{a}	83^{b}	36^{d}	67^{c}	85^{b}	12.1
FCR[3]	0.97^{d}	0.99^{d}	1.75^{c}	2.47^{a}	2.08^{b}	1.72^{c}	0.14
PER[3]	2.06^{a}	2.01^{a}	1.14^{b}	0.81^{c}	0.96^{bc}	1.15^{b}	0.13
CF[3]	0.72^{b}	0.81^{a}	0.69^{bc}	0.60^{d}	0.69^{bc}	0.73^{b}	0.02
PCV (%)[3]	22.3^{a}	22.7^{a}	18.2^{ab}	17.5^{b}	21.0^{a}	19.7^{ab}	0.70
Hb (g/dL)[3]	4.4	4.4	3.8	3.5	4.0	4.0	0.16
Survival	86.7^{ab}	93.3^{a}	93.3^{a}	75.6^{b}	82.2^{ab}	84.4^{ab}	2.28

[1]Values are means from triplicate groups of fish where the means in each row with a different superscript are significant different ($P<0.05$).

[2]Pooled standard error of mean.

[3]WG (percent weight gain) = (final weight-initial weight) × 100/initial weight.

FCR (feed conversion ratio) = dry feed intake (g) /weight gain (g).

PER (protein effeciency ratio) = weight gain/dietary protein intake.

CF (condition factor) = (wet weight/total length3) × 100.

PCV: haematocrit.

Hb: hemoglobin.

the effects of lyophilized fish muscle originated from marine fish and freshwater fish is necessary in the future.

Although some studies have showed that BM can replace up to 50% FM protein without any negative effects on growth in some freshwater fish species (Åsgard and Åstreng, 1986; Luzier et al., 1995; Lee and Bai, 1997), olive flounder fed BM diet in which 50% of WFM was replaced by BM in the present study showed the lowest WG, PER, and FCR among all the dietary treatments including the SLP group. However, Bai and Kim (1997) reported that Korean rockfish fed BM diet showed significant higher WG than those fed SLP diet, and the WG of fish fed BM diet showed significant lower WG than those of fish fed WFM and FLM diets ($P < 0.05$). The digestibility of BM in different fish species might be different. Also the reason of those fish fed BM diet showing poor growth rate in olive flounder is not well understood. However, BM is produced through spray drying in low temperature, there is no biological degradation of amino acids (Harimex, 1992). The detailed study of the utilization of BM in marine fish species is needed in the future.

In order to develop a basal diet for vitamin studies, CA was also included in this experiment. WG of fish fed CA diet showed significant lower value than those of fish fed WFM and FLM diets ($P < 0.05$), less than half of the value got from WFM and FLM groups (Table 3). However, casein based diet was extensively used in vitamin researches at present (Wilson et al., 1989; Lee et al., 1998).

Fish fed FLM diet showed significant higher condition factor (CF) among all the dietary treatments ($P<0.05$), meanwhile those fish fed BM diet showed significant lower CF among all the dietary treatments ($P<0.05$). There is no significant difference existed in haematocrit (PCV), hemoglobin (Hb) and survival among all the dietary treatments.

Whole body composition data were shown in Table 4. Fish fed WFM and FLM diets had the lowest moisture and crude ash than those of fish fed CM, BM, SLP and CA diets ($P<0.05$). Meanwhile fish fed WFM and FLM showed significant higher crude protein and crude fat than those of fish fed BM diet ($P<0.05$). These results are in accordance with the results of Kim and Bai (1997).

Table 4. Effects of different dietary protein sources on whole body composition in olive flounder for 6 weeks[1]

	Diets						Pooled
	WFM	FLM	CM	BM	SLP	CA	SEM
Moisture	78.6^{b}	78.4^{b}	79.5^{ab}	80.5^{a}	79.1^{ab}	79.1^{ab}	0.18
Crude protein	15.6^{a}	15.8^{a}	15.1^{ab}	14.2^{b}	14.7^{ab}	15.0^{ab}	0.14
Crude lipid	4.3^{a}	4.3^{a}	4.0^{b}	3.5^{cd}	3.7^{c}	3.7^{c}	0.08
Ash	4.4^{d}	4.3^{d}	4.7^{cd}	5.4^{a}	5.0^{b}	4.9^{bc}	0.10

[1]Values are means from triplicate groups of fish where the means in each row with a different superscript are significantly different ($P<0.05$).

Based on these results, we concluded that WFM and FLM are the best animal protein sources for olive flounder among the tested animal protein sources under our experimental conditions. When CM, BM and SLP were used as the alternative protein sources to replace WFM in marine fish aquaculture, the detailed replacement experiment should be done to determine the proper replacement levels. When CA was used as the main protein source in vitamin studies, the low growth rate arose from CA diet should be considered.

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