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Effects of Protein and Lipid Levels in Extruded Pellets on the Growth and Body Composition of the Olive Flounder *Paralichthys olivaceus* during the Summer and Winter Seasons

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Feeding trials were performed at two different water temperatures (summer and winter seasons) to identify suitable protein and lipid (energy) levels to be used in formulating extruded pellets for olive flounder. Experiments were conducted to determine the effects of protein and lipid levels in extruded pellets on the growth and body composition of the flounder in both feeding trials. Six experimental diets were prepared containing three protein levels (46%, 51%, and 56%) and two lipid levels (10% and 17%). In the first experiment, during the summer season (22±2.2°C), a triplicate group of fish (initial weight, 114 g) were fed to satiation one of the six diets for 11 weeks. The highest weight gain was observed in fish fed the 56/17 (% protein/% lipid) diet, but this weight gain was not significantly different from that of fish fed the other diets, except for those fed the 46/10 diet. The feed efficiency and protein efficiency ratio of fish fed the 17% lipid diets were higher than those of fish fed the 10% lipid diets at each protein level. In the second experiment, during the winter season (13±1.5°C), a triplicate group of fish (initial weight, 107 g) were fed to satiation one of the six diets for 9 weeks. Weight gain was not significantly different among all groups. The feed efficiency and protein efficiency ratio tended to increase with increasing dietary lipid level at each protein level. The whole-body crude lipid content of the of fish fed the 17% lipid diets was significantly higher than that of fish fed the 10% lipid diets at each protein level in both feeding trials. Based on the data obtained in this study, the inclusion of dietary protein at a level of 46% appears to be sufficient to support optimal growth, and increasing the dietary lipid level from 10% to 17% had no beneficial effects on the growth and feed utilization of olive flounder (110-300 g), except for fish fed a 56% protein diet in the summer season.

Key words: Dietary protein and lipids, Olive flounder, Growth, Summer, Winter

Introduction

Dietary protein is the most important factor affecting the growth performance of fish and feed cost (Lovell, 1989). Therefore, improving protein utilization for tissue synthesis rather than energy use is important from a nutritional and economical point of view. Dietary lipids, as a nonprotein energy source, may also influence the growth and protein utilization of fish. Increasing the energy density of diets has been suggested as a strategy to spare protein and limit ammonia production for several fish species, including the common carp *Cyprinus carpio* (Steffens, 1996). A supplementation of lipid rather than carbohydrate as a nonprotein energy source is generally more effective for increasing the dietary energy level because lipids are an energy-dense nutrient readily metabolized by fish, especially carnivorous fish (NRC, 1993). The protein-sparing effect by dietary lipids has been reported in some fish species (Cho and Kaushik, 1990; De Silva et al., 1991; Lee et al., 2002).

The effects of temperature on nutrient utilization have been studied in some fish species (Olsen and Ringø, 1998; Peres and Oliva-Teles, 1999), and dietary composition, especially protein and lipid

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levels, may differentially influence the growth and feed efficiency at different water temperatures. The optimum temperature for the growth of flounder has been reported to be 20-25°C, and this fish reportedly maintains feeding and growth at temperatures as low as 10-20°C (Iwata et al., 1994). Most studies on the nutrient requirements for flounder have been conducted at moderate water temperatures (20-25°C) or at juvenile and adult stages (Lee et al., 2000; Lee et al., 2002a; Lee and Kim, 2005; Kim et al., 2006). Flounder are exposed to suboptimal water temperatures during certain culture periods in Korea, but the effects of dietary protein and lipid levels on the growth of olive flounder at moderate and suboptimal water temperatures are unknown. Thus, knowledge of adequate dietary composition at all relevant environmental temperatures, as well as for different fish sizes, is needed for efficient feeding of flounder throughout the year. This study was conducted to investigate the effects of protein and lipid levels in extruded feed pellets on the growth and body composition of olive flounder during the summer and winter seasons.

Materials and Methods

Feeding Trials and Experimental Diets

A factorial experimental design consisting of three protein levels (46%, 51%, and 56%) and two lipid levels (10% and 17%) with three replicates per dietary treatment was used. The energy levels of the diets were calculated based on 4 kcal, 9 kcal, and 4 kcal/g for protein, lipids, and nitrogen-free extracts, respectively (Cho et al., 1982). The ingredients and nutritional contents of the experimental diets are listed in Table 1. Anchovy meal, jack mackerel meal, soybean meal, krill meal, squid liver powder, wheat gluten, and brewer yeast were used as protein sources. To determine the effects of the two lipid levels at each protein level, the amounts of fish meals and squid liver oil in the diets were adjusted at the expense of wheat flour and cellulose. Diets were prepared with an extruder pellet mill (OEE 08 extruder; Kahl, Germany) after 35-40 g water was mixed with a 100-g mixture of ingredients and dried overnight at room temperature. The experimental diets were stored at -30°C until use.

Experiment 1 (Summer Season)

Olive flounder (*Paralichythys olivaceus*) were obtained from a local fish farm (Pohang, Korea). Fish having an average body weight of 114±2.7 g (mean ± SD) were distributed randomly into 18 350-L cylindrical plastic tanks with 20 fish per tank in a flow-through aquarium system. Fish were hand-fed to

Table 1. Ingredients and nutrient contents of the experimental diets

	Diets (% protein/% lipid)					
	46/10	46/17	51/10	51/17	56/10	56/17
Ingredients (%)						
Anchovy meal	22	22	26	26	30	30
Jack mackerel meal	22	22	26	26	30	30
Soybean meal	3	5	3	5	3	5
Krill meal	2.5	2.5	2.5	2.5	2.5	2.5
Squid liver powder	4	4	4	4	4	4
Wheat gluten	4	4	4	4	4	4
Brewer yeast	1	1	1	1	1	1
Squid liver oil	2.6	7.6	1.9	6.9	1.2	6.2
Wheat flour	31	24	26	19	21	14
α-Cellulose	4.6	4.6	2.3	2.3		
Vitamin premix[1]	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix[1]	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.3	0.3	0.3	0.3	0.3	0.3
Nutrient contents (DM basis)						
Crude protein (%)	46.4	46.3	51.9	50.9	56.0	55.2
Crude lipid (%)	10.2	17.0	9.9	16.0	11.0	16.5
Ash (%)	10.4	10.3	11.6	11.6	12.9	11.8
Crude fiber (%)	4.4	4.5	2.9	2.7	1.1	1.2
Nitrogen-free extract (%)[2]	28.7	21.9	23.7	18.9	18.9	14.3
Estimated energy (kcal/g)[3]	3.92	4.26	3.92	4.23	3.99	4.27
P/E ratio (mg/kcal)	118	109	133	120	140	129

[1]Same as Lee et al. (2003).
[2]Calculated by the difference (=100-crude protein-crude lipid-ash-crude fiber).
[3]Based on 4 kcal/g protein, 9 kcal/g lipid and 4 kcal/g carbohydrate (Cho et al., 1982).

apparent satiation twice a day (09:00 and 17:00 h, 6 days a week) for 11 weeks (August-October 2006). Seawater was supplied at a flow rate of 10 L/min in each tank and the mean water temperature was maintained at 22.0±2.2°C. The photoperiod was left under natural conditions during the feeding trial. The fish in each tank were weighed collectively at the beginning and end of the feeding trial after fasting for 48 h. Records were kept for daily feed consumption, mortalities, and feeding behavior.

Experiment 2 (Winter Season)

Flounder having an average body weight of 107±2.9 g (mean ± SD) were distributed randomly into 18 350-L cylindrical plastic tanks with 20 fish per tank in a flow-through aquarium system. Fish were hand-fed to apparent satiation twice a day (0900 and 1700 h, 6 days a week) for 9 weeks (March-May 2006). Seawater was supplied at a flow rate of 10 L/min in each tank and the mean water temperature was maintained at 13.4±1.54°C.

Sample Collection and Chemical Analysis

At the end of each feeding trial, three fish were selected randomly from each tank for body composition analysis. All samples were stored at -40°C in sealed plastic bags prior to chemical analysis. The crude protein content was determined by the Kjeldahl method using an Auto Kjeldahl System (VAP50OT/TT125; Gerhardt, Germany), and crude lipid was determined by ether extraction using a Soxhlet extractor (Velp SER 148; Usmate, Italy). Moisture was determined by oven-drying at 105°C for 6 h, ash was determined using a muffle furnace at 550°C for 6 h, and crude fiber was determined using an automatic analyzer (Fibertec; Tecator AB, Sweden). Nitrogen-free extract was calculated based on the difference.

Statistical Analysis

The data were subjected to factorial ANOVA to test the effects of the three dietary protein and two lipid levels on fish performance. Duncan's multiple range test (Duncan, 1955) was used for comparisons between means at a significance level of α=0.05. All statistical analyses were carried out using the SPSS program version 11.5 for Windows (SPSS Inc., USA).

Results

Experiment 1 (Summer Season)

The survival of olive flounder in response to diets containing 46-56% protein and 10-17% lipid for 11 weeks in the summer season was 80-95%, and no significant differences were observed among the main effects (Table 2). A significant effect of dietary protein and lipid levels on weight gain was detected. The highest weight gain was observed in fish fed the 56/17 (% protein/% lipid) diet, although the difference was only significant when compared to that of fish fed the 46/10 diet. Feed efficiency was significantly affected by the dietary protein level (P<0.01) and lipid level (P<0.05), and the feed efficiency of fish fed the 56/17 diet was the highest (P<0.05). The feed efficiency of fish fed a 17% lipid diet was significantly (P<0.05) higher than that of the 10%

Table 2. Growth performance of grower flounder fed extruded pellets containing three protein and two lipid levels for 11 weeks in summer season

Protein	Lipid	P/E ratio (mg/kcal)	Initial weight (g/fish)	Survival (%)	Weight gain (%)[1]	Feed efficiency (%)[2]	Protein efficiency ratio[3]	Daily feed intake[4]
46	10	118	113 ± 0.7	80 ± 7.6	145 ± 13.3^{b}	101 ± 1.3^{c}	2.17 ± 0.03^{ab}	1.41 ± 0.07
46	17	109	114 ± 1.5	93 ± 1.7	161 ± 11.0^{ab}	108 ± 1.8^{bc}	2.33 ± 0.04^{a}	1.27 ± 0.07
51	10	133	112 ± 2.8	85 ± 5.0	176 ± 15.4^{ab}	116 ± 4.9^{b}	2.24 ± 0.09^{a}	1.32 ± 0.04
51	17	120	114 ± 1.3	90 ± 2.9	151 ± 13.0^{ab}	115 ± 2.8^{b}	2.26 ± 0.05^{a}	1.25 ± 0.05
56	10	140	115 ± 1.2	95 ± 0.0	152 ± 6.9^{ab}	114 ± 1.7^{b}	2.04 ± 0.03^{b}	1.22 ± 0.02
56	17	129	115 ± 1.5	95 ± 0.0	187 ± 6.2^{a}	128 ± 3.9^{a}	2.32 ± 0.07^{a}	1.25 ± 0.05
Two-way ANOVA								
Protein			P<0.3	P<0.2	P<0.2	P<0.01	P<0.5	P<0.3
Lipid			P<0.5	P<0.1	P<0.2	P<0.05	P<0.01	P<0.2
Protein × lipid			P<0.9	P<0.3	P<0.05	P<0.1	P<0.2	P<0.3

Values (mean ± SEM of three replications) in each column with different superscripts are significantly different (P<0.05).
[1](Final body weight - initial body weight) × 100/initial body weight.
[2]Fish wet weight gain × 100/feed intake (dry matter).
[3]Fish wet weight gain/protein intake.
[4]Feed intake (dry matter) × 100/[(initial fish wt.+ final fish wt.+ dead fish wt.) × days fed/2].

lipid diet at 56% protein, but no significant differences were observed between diets containing 10% and 17% lipid at 46% or 51% protein. The protein efficiency ratio was significantly ($P<0.05$) affected by the dietary lipid level but not by dietary protein. The protein efficiency ratio tended to increase with increasing dietary lipid levels at each protein level. Daily feed intake was not significantly affected by dietary protein or lipid levels.

The whole-body crude lipid content was significantly ($P<0.01$) affected by the dietary lipid level (Table 3). The crude lipid content of fish fed a 17% lipid diet was significantly ($P<0.01$) higher than that of fish fed a 10% lipid diet at each protein level. The whole-body contents of moisture, crude protein, and ash were not significantly different among all groups.

Experiment 2 (Winter Season)

The survival and weight gain of flounder in response to diets containing 46-56% protein and 10-17% lipid for 9 weeks in the winter season were not significantly affected by dietary protein and lipid levels (Table 4). Feed efficiency and daily feed intake of fish were significantly ($P<0.05$) affected by the dietary protein level but not by the dietary lipid level, and feed efficiency tended to increase with an increasing dietary protein level at each lipid level. The highest feed efficiency of fish fed the 56/17 diet was significantly ($P<0.05$) higher than that of fish fed the 46/10, 46/17, and 51/10 diets, but was not significantly different from that of fish fed the 51/17 and 51/10 diets. The daily feed intake of fish fed the 46/10 diet was the highest, but not significantly different from that of fish fed the 46/17, 51/10, and 51/17 diets. The protein efficiency ratio was not significantly different among all groups.

The whole-body contents of moisture and crude lipids were significantly ($P<0.05$) affected by the dietary lipid level. The crude lipid content of fish fed a 17% lipid diet was significantly ($P<0.05$) higher than that of fish fed a 10% lipid diet at each protein level (Table 5).

Discussion

In this study, considerably higher feed intake, growth, and feed efficiency were observed in fish reared in the summer season (22°C) compared to those reared in the winter season (13°C). The increased feed intake at the higher water temperature may have been the primary response of fish to the increased temperature (Bureau et al., 2002), and these results agree with those of a previous study (Iwata et al., 1994). Similar results have also been reported for other fish species (Goolish and Adelman, 1984; Peres and Oliva-Teles, 1999). The improvement in feed efficiency at the higher temperature probably resulted from an increase in feed intake for growth as the temperature approached the optimum temperature for a given fish species (Peres and Oliva-Teles, 1999).

Based on the weight gain of fish in this study, the inclusion dietary protein at a level of 46% appears to be sufficient to support the growth of olive flounder (110-300 g) in the summer and winter seasons. This result is comparable to the protein requirements of 45% observed for 23-110 g (Lee et al., 2002a) and 300-500 g flounder (Kim et al., 2008). However, the dietary protein requirement for 3-13 g juvenile flounder was reported to be 50% for maximum growth (Lee et al., 2000; Lee and Kim, 2005). These differences are probably a result of the differences in

Table 3. Proximate composition of whole body of grower flounder fed extruded pellets containing three protein and two lipid levels for 11 weeks in summer season

Protein	Lipid	P/E ratio (mg/kcal)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
46	10	118	71.1 ± 1.18	18.8 ± 0.23	3.5 ± 0.26[b]	3.2 ± 0.07
46	17	109	70.9 ± 0.09	18.5 ± 0.08	4.5 ± 0.28[a]	3.1 ± 0.04
51	10	133	71.4 ± 1.43	18.7 ± 0.20	3.2 ± 0.19[b]	3.2 ± 0.10
51	17	120	71.6 ± 0.76	18.3 ± 0.24	4.7 ± 0.27[a]	3.4 ± 0.02
56	10	140	72.4 ± 0.54	18.9 ± 0.23	3.3 ± 0.53[b]	3.2 ± 0.07
56	17	129	71.5 ± 0.27	18.6 ± 0.15	4.8 ± 0.03[a]	3.3 ± 0.04
Two-way ANOVA						
Protein			P<0.6	P<0.5	P<0.2	P<0.1
Lipid			P<0.7	P<0.1	P<0.01	P<0.3
Protein × lipid			P<0.9	P<0.9	P<0.8	P<0.3

Values (mean ± SEM of three replications) in each column with different superscripts are significantly different (P<0.05).

Table 4. Growth performance of grower flounder fed extruded pellets containing three protein and two lipid levels for 9 weeks in winter season

Protein	Lipid	P/E ratio (mg/kcal)	Initial weight (g/fish)	Survival (%)	Weight gain (%)	Feed efficiency (%)[1]	Protein efficiency ratio[2]	Daily feed intake[3]
46	10	118	108 ± 2.2	97 ± 3.3	37 ± 2.3	81 ± 4.5[b]	1.74 ± 0.09	0.56 ± 0.02[a]
46	17	109	107 ± 1.5	97 ± 1.6	36 ± 5.7	89 ± 4.0[b]	1.89 ± 0.08	0.52 ± 0.06[ab]
51	10	133	107 ± 2.6	98 ± 1.6	38 ± 5.7	85 ± 9.2[b]	1.64 ± 0.17	0.55 ± 0.02[ab]
51	17	120	107 ± 1.8	100 ± 0.0	45 ± 7.2	103 ± 11.6[ab]	2.03 ± 0.22	0.54 ± 0.01[ab]
56	10	140	107 ± 2.0	98 ± 1.6	33 ± 2.6	102 ± 6.6[ab]	1.82 ± 0.11	0.42 ± 0.01[c]
56	17	129	107 ± 1.9	97 ± 1.6	43 ± 4.4	113 ± 6.8[a]	2.04 ± 0.12	0.46 ± 0.01[bc]
Two-way ANOVA								
Protein			P<0.9	P<0.5	P<0.9	P<0.05	P<0.8	P<0.01
Lipid			P<0.7	P<0.9	P<0.3	P<0.1	P<0.1	P<0.6
Protein × lipid			P<0.9	P<0.7	P<0.6	P<0.8	P<0.8	P<0.4

Values (mean ± SEM of three replications) in each column with different superscripts are significantly different (P<0.05).
[1](Final body weight - initial body weight) × 100/initial body weight.
[2]Fish wet weight gain × 100/feed intake (dry matter).
[3]Fish wet weight gain/protein intake.
[4]Feed intake (dry matter) × 100/[(initial fish wt.+ final fish wt.+ dead fish wt.) × days fed/2].

Table 5. Proximate composition of whole body of grower flounder fed extruded pellets containing three protein and two lipid levels for 9 weeks in winter season

Protein	Lipid	P/E ratio (mg/kcal)	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
46	10	118	76.1 ± 0.97[ab]	17.7 ± 0.36	3.0 ± 0.40[c]	3.4 ± 0.26
46	17	109	74.4 ± 0.39[b]	17.3 ± 0.18	4.6 ± 0.27[a]	3.6 ± 0.15
51	10	133	75.7 ± 0.23[ab]	17.6 ± 0.18	3.2 ± 0.16[bc]	3.2 ± 0.23
51	17	120	74.4 ± 0.36[b]	17.4 ± 0.12	4.3 ± 0.38[a]	3.4 ± 0.28
56	10	140	76.6 ± 0.88[a]	17.6 ± 0.25	2.3 ± 0.50[c]	3.8 ± 0.12
56	17	129	75.3 ± 0.39[ab]	17.5 ± 0.21	4.1 ± 0.30[ab]	3.9 ± 0.11
Two-way ANOVA						
Protein			P<0.4	P<0.9	P<0.3	P<0.1
Lipid			P<0.05	P<0.3	P<0.01	P<0.3
Protein × lipid			P<0.9	P<0.9	P<0.7	P<0.9

Values (mean ± SEM of three replications) in each column with different superscripts are significantly different (P<0.05).

fish size, dietary protein quality, or rearing conditions in these studies.

The feed efficiency and protein efficiency ratio tended to increase with increasing dietary lipid levels at each protein level, although for the most part, no significant differences were observed in the summer or winter seasons. These results indicated that increasing the dietary lipid level could provide a more efficient utilization of dietary protein for the growth of olive flounder. Similar results of improved feed utilization in juvenile flounder fed a 50% protein diet with 14% lipid compared to 7% lipid at suboptimal water temperatures of 12 and 17°C have been reported previously (Kim et al., 2006). However, Lee et al. (2000; 2003) reported that an increase in dietary lipid did not improve the feed efficiency or show a clear protein-sparing effect in juvenile flounder (3-13 g). These different responses with respect to increased dietary lipid levels could be related to differences in fish size, nutrient digestibility, or feed utilization with respect to rearing water temperature. Similarly, no beneficial effects were observed on the growth and feed utilization of 6-25 g juvenile sea bass by increasing the dietary lipid from 12% to 24% (Peres and Oliva-Teles, 1999). However, Lanari et al. (1999) reported that sea bass (92-342 g) fed a diet of 19% lipids displayed increased growth compared to those fed diets containing 11% and 15% dietary lipids. In addition, Bendiksen et al. (2003) observed higher fat digestibility in the Atlantic salmon *Salmo salar* when the dietary fat levels were increased from 20% to 33% at 8°C. This could be also related to the effects of dietary fat and temperature on gastric evacuation, which is slowed by both increased dietary fat and lower temperatures. A slowing of gastric emptying may provide more time for lipases to hydrolyze fats, leading to improved digestion and absorption. Olsen and Ringø (1998) reported that feeding high carbohydrate diets to the Arctic charr *Salvelinus alpinus* had no significant influence on nutrient utilization at 10°C, but appeared to reduce the apparent digestibility coefficient for most macronutrients at 0.6°C.

In this study, the lipid content of the whole body was positively correlated with the dietary lipid level. Similar results have been reported for juvenile flounder (Lee et al., 2000; Lee and Kim, 2005). The practice of increasing dietary lipid levels should be carefully considered as it may affect carcass quality, flavor, and storage characteristics as a result of increased lipid deposition (Cowey, 1993; Hillestad and Johnsen, 1994). In the previous studies, the lipid levels of liver and viscera increased with increasing dietary lipid levels, whereas those of the dorsal muscle were not (Lee et al., 2000; Lee and Kim, 2005; Kim et al., 2008). This phenomenon might be explained by a tendency in flatfish, including flounder, to deposit high concentrations of lipids in the liver or viscera rather than in muscle tissue (Sheridan, 1988).

Based on the data obtained from this study, the inclusion of dietary protein at level of 46% appears to be sufficient to support optimal growth, and an increase in the dietary lipid level from 10% to 17% has no beneficial effects on the growth and feed utilization of olive flounder (110-300 g), except for fish fed the 56% protein diet in the summer season.

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