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Effects of Dietary Protein and Lipid Levels on Growth and Body Composition of Sub-adult Flounder *Paralichthys olivaceus* During the Summer Season

Kyoung-Duck Kim*, Yong Jin Kang, Jong Yun Lee, Kang-Woong Kim and Se-Min Choi
Aquafeed Research Center, National Fisheries Research & Development Institute, Pohang 791-923, Korea

A 3×2 factorial experiment was conducted to investigate the proper dietary protein and lipid levels for the growth of sub-adult flounder *Paralichthys olivaceus* reared during the summer season. Six experimental diets were formulated to contain three levels of protein (45%, 50% and 55%) and two levels of lipid (9% and 14%). Duplicate groups of fish (initial body weight of 298 g) were hand-fed to apparent satiation during the summer season (21.8±1.7°C) for 60 days. Survival of each group was over 83% and there was not significant difference among all groups. Weight gain of fish fed the 45% protein diet with 14% lipid was not significantly different from that of fish fed the 50% and 55% protein diets with 9% and 14% lipids, but weight gain of fish fed the 45% protein diet with 9% lipid was significantly lower than that of fish fed the 55% protein diets with 14% lipid. Feed efficiency tended to increase with increasing dietary lipid level at each protein levels, although no significant differences were observed at 50% and 55% protein levels. Protein efficiency ratio, daily feed intake, condition factor and hepatosomatic index were not significantly affected by dietary protein and lipid levels. Crude lipid content of the liver tended to increase with increasing dietary lipid level at the same protein levels, but the opposite appearance was found for moisture content. The contents of moisture, crude protein and crude lipid of the dorsal muscle were not significantly affected by dietary protein and lipid levels. Based on data obtained form this study, inclusion of dietary protein at level of 45% appears sufficient to support optimal growth, and an increase of dietary lipid level from 9% to 14% has beneficial effects on feed utilization of sub-adult flounder during the summer season.

Keywords: *Paralichthys olivaceus*, Sub-adult flounder, Dietary protein and lipid, Growth, Summer season

Introduction

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

Lee and Kim (2005) suggested that the diet containing 46-51% protein with 6% lipid is optimal for growth and efficient feed utilization of juvenile flounder. Although the proper levels of protein and lipid in diet for flounder had been studied, most studies of nutrient requirements for this fish have been determined in the juvenile fish (Lee et al. 2000; Lee et al. 2002; Lee and Kim 2005; Kim et al. 2006). However, nutrient utilization for growth of fish could be largely affected by fish size. Thus, knowledge of adequate dietary composition in the different fish size is considered to efficient feeding conditions for culture of the different size of fish. However, limited information about effects of dietary protein and lipid levels on growth is available for sub-adult flounder. Therefore, this study was conducted to investigate the effects of dietary protein and lipid levels on growth and body composition of sub-adult flounder during the summer season.

*Corresponding author: kdkim@nfrdi.go.kr

Materials and methods

Design of the feeding trial and experimental diets

A 3×2 factorial experimental design with two replicates was used in this study. Six experimental diets were formulated to contain three levels of protein (45%, 50% and 55%) and two levels of lipid (9% and 14%). Energy levels of the diets were calculated based on 4 kcal, 9 kcal and 4 kcal/g for protein, lipid and nitrogen-free extract, respectively (Cho et al. 1982). Ingredient and nutrient contents of the experimental diets are presented in Table 1. Brown fish meal and soybean meal, squid liver oil and wheat flour were used as the primary protein, lipid and carbohydrate sources, respectively. All ingredients were mechanically mixed with water at the ratio of 3:2 and pressure-pelleted and dried at room temperature over night. The experimental diets were stored in freezer at -30°C until use.

Fish and rearing conditions

Sub-adult flounder *Paralichythys olivaceus* were obtained from a local fish farm (Pohang, Korea). Fish were fed a commercial flounder feed for 2 weeks while being acclimated to the experimental conditions. Fish (average body weight of 298±4.7 g) were randomly distributed into each of twelve 2000 L cylindrical plastic tanks (1400 L water volume) with 20 fish to each tank in a flow-through aquarium system. Fish were hand-fed to apparent satiation twice a day (0900 and 1700 h) for 6 days a week during 60 days. Filtered seawater was supplied at a flow rate of 15 L/min in each tank, and mean water temperature was 21.8±1.7°C Photoperiod was left at natural condition during the feeding trial. All fish in each tank were collectively weighed at the beginning and end of feeding trial after being fasted for 48 h. Records were kept for daily feed consumption, mortalities and feeding behavior.

Sample collections and chemical analysis

At the end of the feeding trial, 5 fish in each tank were sampled and stored in freezer at -40°C for chemical analysis. Crude protein was determined by Kjeldahl method using Auto Kjeldahl System (Gerhardt VAP50OT/TT125, KG, Germany). Crude lipid was determined by ether extraction using Soxhlet extractor (Velp SER 148, Usmate, Italy). Moisture was determined by oven drying at 105°C for 6 h. Ash was determined by muffle furnace at 550°C for 6 h. Crude fiber was determined by an automatic analyzer (Fibertec, Tecator, Hoganas, Sweden). Nitrogen free extract was calculated by difference.

Statistical analysis

The data were subjected to one-way and two-way ANOVA to test the effects of dietary protein and lipid levels on fish

Table 1. Ingredients and nutrient contents of the experimental diets

	Diets					
	$P_{45}L_9$	$P_{45}L_{14}$	$P_{50}L_9$	$P_{50}L_{14}$	$P_{55}L_9$	$P_{55}L_{14}$
Ingredients (%)						
Brown fish meal	52.0	52.0	60.0	60.0	68.0	68.0
Soybean meal	2.0	4.0	2.0	4.0	2.0	4.0
Wheat flour	33.0	26.0	27.0	20.0	21.0	14.0
Squid liver oil	3.7	8.7	3.1	8.1	2.5	7.5
α-Cellulose	7.3	7.3	5.9	5.9	4.5	4.5
Vitamin premix [1]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix [1]	1.0	1.0	1.0	1.0	1.0	1.0
Nutrient contents (dry matter basis)						
Crude protein (%)	45.0	45.5	50.9	50.1	55.6	54.5
Crude lipid (%)	8.6	13.3	9.2	14.1	9.3	13.9
Ash (%)	7.6	8.2	8.9	9.0	10.0	9.6
Crude fiber (%)	8.7	8.4	7.3	7.2	5.8	5.8
NFE (%) [2]	30.1	24.6	23.7	19.6	19.3	16.2
Estimated energy (kcal/g) [3]	3.78	4.00	3.81	4.06	3.83	4.08
P/E ratio (mg/kcal)	119	114	134	123	145	134

[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).

performance. If significant ($P<0.05$) difference was found in one-way ANOVA test, Duncan's multiple range test (Duncan 1955) was used to rank the groups. All statistical analyses were carried out by using the SPSS program Version 11.5 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

Growth performance of sub-adult flounder fed the diets containing various protein and lipid levels during the summer season for 60 days are presented in Table 2. Survival of each group was over 83% and there was not significant difference among all groups. Weight gain was significantly affected by dietary lipid level ($P<0.05$), but not by dietary protein level. Weight gain of fish fed the 45% protein diet with 14% lipid was not significantly different from that of fish fed the 50% and 55% protein diets with 9% and 14% lipids, but weight gain of fish fed the 45% protein diet with 9% lipid was significantly lower than that of fish fed the 55% protein diets with 14% lipid. Based on weight gain of fish in this study, inclusion of dietary protein at level of 45% appears sufficient to support optimal growth of sub-adult flounder in summer season. This result was comparable with protein requirements of 45% for 23-110 g young flounder (Lee et al., 2002). However, dietary protein requirement for juvenile flounder (3-13 g) was reported to be 50% for the maximum growth when fed the diets containing various protein and lipid levels (Lee et al., 2000). The difference in dietary protein requirement for growth of flounder among these studies probably resulted from the difference in fish size, dietary protein quality or rearing conditions.

Feed efficiency was significantly affected by both dietary protein level ($P<0.05$) and lipid level ($P<0.05$), but protein efficiency ratio was not affected by either dietary protein level or lipid level. The highest feed efficiency was observed in fish fed the 55% protein diet with 14% lipid, but feed efficiency of fish fed the 55% protein diet with 14% lipid were not significantly different from that of fish fed the 50% protein diets with 9% and 14% lipids. Feed efficiency tended to increase with increasing dietary lipid level at all protein levels, although no significant differencies were observed at 50% and 55% protein levels. These results in this study showed that increasing the dietary lipid level will provide a

Table 2. Growth performance of sub-adult flounder fed the diets containing various protein and lipid levels during the summer season for 60 days

	Diets					
	$P_{45}L_9$	$P_{45}L_{14}$	$P_{50}L_9$	$P_{50}L_{14}$	$P_{55}L_9$	$P_{55}L_{14}$
Initial weight (g/fish)	298±0.0	303±1.5	298±1.7	299±2.3	300±2.1	296±1.7
Survival (%)	95±0.0	85±10.0	93±2.5	83±2.5	98±2.5	95±0.0
Weight gain (g/fish)	161±6.9^{a}	183±1.5^{ab}	170±0.4^{ab}	182±0.8^{ab}	180±21.0^{ab}	211±16.5^{b}
Feed efficiency (%)[1]	87±1.3^{a}	102±4.9^{b}	100±3.9^{ab}	105±5.5^{bc}	107±4.2^{bc}	116±0.8^{c}
Protein efficiency ratio[2]	1.93±0.03	2.23±0.11	1.96±0.08	2.09±0.11	1.92±0.08	2.13±0.02
Daily feed intake[3]	0.88±0.04	0.75±0.00	0.77±0.00	0.73±0.03	0.76±0.04	0.78±0.05
Condition factor[4]	1.21±0.03	1.19±0.02	1.15±0.03	1.22±0.02	1.21±0.03	1.21±0.07
Hepatosomatic index[5]	1.61±0.13	1.77±0.02	1.72±0.17	1.67±0.10	1.74±0.21	1.83±0.15

Two-way ANOVA	Dietary protein	Dietary lipid	Interaction
Survival	$P<0.3$	$P<0.1$	$P<0.7$
Weight gain	$P<0.2$	$P<0.05$	$P<0.7$
Feed efficiency	$P<0.05$	$P<0.05$	$P<0.5$
Protein efficiency ratio	$P<0.8$	$P<0.2$	$P<0.6$
Daily feed intake	$P<0.3$	$P<0.1$	$P<0.2$
Condition factor	$P<0.9$	$P<0.5$	$P<0.6$
Hepatosomatic index	$P<0.8$	$P<0.6$	$P<0.8$

Values (mean ± SE of two replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).
[1]Fish wet weight gain × 100/feed intake (dry matter).
[2]Fish wet weight gain/protein intake.
[3]Feed intake (dry matter) × 100/[(initial fish wt.+ final fish wt.+ dead fish wt.) × days fed/2].
[4]Fish weight × 100/fish length^3.
[5]Liver weight × 100/body weight.

Table 3. Proximate compositions of the liver and dorsal muscle of sub-adult flounder fed the diets containing various protein and lipid levels during the summer season for 60 days

	Diets					
	$P_{45}L_9$	$P_{45}L_{14}$	$P_{50}L_9$	$P_{50}L_{14}$	$P_{55}L_9$	$P_{55}L_{14}$
Liver						
Moisture (%)	62.5±1.94[ab]	60.7±0.37[ab]	64.4±0.29[ab]	60.6±0.71[ab]	65.0±3.32[b]	58.2±1.41[a]
Crude protein (%)	10.6±0.47	9.6±0.29	10.1±0.64	10.1±0.08	10.8±1.55	9.6±0.06
Crude lipid (%)	10.9±0.87[a]	15.1±2.17[ab]	10.7±1.98[a]	15.6±3.51[ab]	13.2±0.12[ab]	19.0±2.56[b]
Dorsal muscle						
Moisture (%)	75.3±0.38	75.9±0.48	75.5±0.17	75.6±0.48	76.0±0.17	75.4±0.29
Crude protein (%)	22.3±0.31	22.2±0.27	22.3±0.10	22.2±0.10	21.7±0.70	22.8±0.80
Crude lipid (%)	0.27±0.05	0.33±0.04	0.30±0.07	0.42±0.05	0.25±0.02	0.29±0.03

Two-way ANOVA	Dietary protein	Dietary lipid	Interaction
Liver			
Moisture (%)	$P < 0.9$	$P < 0.05$	$P < 0.4$
Crude protein (%)	$P < 0.9$	$P < 0.3$	$P < 0.7$
Crude lipid (%)	$P < 0.4$	$P < 0.05$	$P < 0.9$
Dorsal muscle			
Moisture (%)	$P < 0.9$	$P < 0.9$	$P < 0.3$
Crude protein (%)	$P < 0.9$	$P < 0.6$	$P < 0.5$
Crude lipid (%)	$P < 0.2$	$P < 0.1$	$P < 0.8$

Values (mean ± SE of two replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).

more efficient utilization of dietary protein for the growth of sub-adult flounder. However, Lee et al. (2000; 2003) reported that an increase in dietary lipid did not appear to improve feed efficiency or show a clear protein-sparing effect in juvenile flounder (3-13 g). These different responses with respect to increased dietary lipid level could be related to the fish size. Similarly, no beneficial effects of increasing dietary lipid from 12% to 24% on growth and feed utilization of sea bass has been reported during 6-25 g juvenile (Peres and Oliva-Teles, 1999). However, Lanari et al. (1999) reported that growing sea bass (92-342 g) fed a diet of 19% lipid showed increased growth compared with diets containing 11% and 15% dietary lipid.

Daily feed intake of fish fed the 9% lipid diets was slightly higher than that of fish fed the 14% lipid diets at 45% and 50% protein levels in this study. Similar results of reduced feed consumption of fish fed a high-energy diet compared with a low-energy diet have been reported for a previous study (Lee et al., 2000). The high-energy diet caused a reduction in feed intake, probably because fish eat to satisfy their energy requirements. Condition factor and hepatosomatic index were not significantly affected by dietary protein level and lipid level.

The contents of moisture and crude lipid of the liver were significantly affected by dietary lipid level ($P<0.05$), but crude protein content was not significantly affected by dietary protein level and lipid level (Table 3). Crude lipid contents of the liver tended to increase with increasing dietary lipid level at the same protein levels, but the opposite appearances were found for moisture content. The contents of moisture, crude protein and crude lipid of the dorsal muscle were not significantly different among all groups. The increase of dietary lipid levels should be carefully considered as it may affect carcass quality, mainly due to an increase of lipid deposition (Cowey, 1993; Hillestad and Johnsen, 1994). In this study, the lipid content of the liver was positively correlated with dietary lipid level. However, increased dietary lipid had little effect on the dorsal muscle lipid in this study. Similar results have been reported in the previous study for juvenile flounder (Lee and Kim, 2005). This phenomenon might be explained by a tendency in flatfish including flounder to deposit high content of lipid in the liver or viscera rather than in muscle tissue (Sheridan, 1988).

Based on data obtained form this study, inclusion of dietary protein at level of 45% appears sufficient to support optimal growth, and an increase of dietary lipid level from 9% to 14% has beneficial effects on feed utilization of sub-adult flounder during the summer season.

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References

Cho, C.Y. and S.J. Kaushik, 1990. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Rev. Nutr. Diet, 61, 132–172.

Cho, C.Y., S.J. Slinger and H.S. Bayley, 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol., 73B, 25–41.

Cowey, C.B., 1993. Some effects of nutrition and flesh quality of cultured fish. (in) Kaushik, S.J. and P. Luquet. (eds.), Fish Nutrition in Practice. Proc. IV Int. Symp. on Fish Nutrition and Feeding, vol. 61, Les Colloques INRA, Paris, pp. 227–236.

De Silva, S.S., R.M. Gunasekera and K.F. Shim, 1991. Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. Aquaculture, 95, 305–318.

Duncan, D. B., 1955. Multiple-range and multiple F tests. Biometrics, 11, 1–42.

Hillestad, M. and F.T. Johnsen, 1994. High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. Aquaculture, 124, 109–116.

Kim, K.-D., K.-M. Kim, K.-W. Kim, Y. J. Kang and S.-M. Lee, 2006. Influence of lipid level and supplemental lecithin in diet on growth, feed utilization and body composition of juvenile flounder (*Paralichthys olivaceus*) in suboptimal water temperatures. Aquaculture, 251, 484–490.

Lanari, D., B.M. Poli, R. Ballestrazzi, P, Lupi, E. D'Agaro and M. Mecatti, 1999. The effects of dietary fat and NFE levels on growing European sea bass (*Dicentrarchus labrax* L.). Growth rate, body and fillet composition, carcass traits and nutrient retention efficiency. Aquaculture, 179, 351–364.

Lee, S.-M. and K.-D. Kim, 2005. Effect of various levels of lipid exchanged with dextrin at different protein level in diet on growth and body composition of juvenile flounder *Paralichthys olivaceus*. Aquacult. Nutr., 11, 435–442.

Lee, S.-M., S.H. Cho and K.-D. Kim, 2000. Effects of dietary protein and energy levels on growth and body composition of juvenile flounder *Paralichthys olivaceus*. J. World Aquacult. Soc., 31, 306–315.

Lee, S.-M., C.S. Park and I.C. Bang, 2002. Dietary protein requirement of young Japanese flounder *Paralichthys olivaceus* fed isocaloric diets. Fish. Sci., 68, 158–164.

Lee, S.-M., K.-D. Kim and S.P. Lall, 2003. Utilization of glucose, maltose, dextrin and cellulose by juvenile flounder (*Paralichthys olivaceus*). Aquaculture, 221, 427–438.

Lovell, R. T., 1989. Nutrition and Feeding of Fish. Van Nostrand Reinhold, New York. 260 pp.

National Research Council (NRC), 1993. Nutrient requirements of fish. National Academy Press. Washington, DC, 114 pp.

Peres, H. and A. Oliva-Teles, 1999. Influence of temperature on protein utilization in juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture, 170, 337–348.

Sheridan, M. A., 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp. Biochem. Physiol., 90, 679–690.

Steffens, W., 1996. Protein sparing effects and nutritive significance of lipid supplementation in carp diet. Arch. Anim. Nutr., 49, 93–98.

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