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Effect of Daily Feeding Ratio on Growth and Body Composition of Subadult Olive Flounder, *Paralichthys olivaceus*, Fed an Extruded Diet during the Summer Season

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Abstract

A 10-wk feeding trial to determine the effect of daily feeding ratio on growth and body composition of subadult olive flounder fed the extruded pellet (EP) was performed during the summer season. Thirteen flounder (an initial body weight of 319 g) per tank were distributed into fifteen 500-L flow-through tanks. Five treatments of feeding ratio in 5% decrement were prepared in triplicate: 100 (satiation), 95, 90, 85, and 80% of satiation. Fish in the control group (100% of satiation) were hand-fed to apparent satiation twice a day. Then, feed allowance in the rest of the four groups was determined based on average feed consumption of fish in the control group. Weight gain of fish fed to 100% of satiation was significantly ($P < 0.05$) higher than that of fish fed to 85 and 80% of satiation but not significantly ($P > 0.05$) different from that of fish fed to 95 and 90% of satiation. Serum total protein, glucose, and glutamic pyruvic transaminase were not significantly ($P > 0.05$) affected by feeding ratio but triglyceride and glutamic oxaloacetic transaminase were. In considering these results, it can be concluded that optimum daily feeding ratio for growth of subadult olive flounder seemed to be 90% of satiation when fish were fed the EP twice a day during the summer season.

Because olive flounder, *Paralichthys olivaceus*, is one of the most commercially important marine fish species for aquaculture in Eastern Asia, such as Korea and Japan, many studies to determine dietary nutrient requirements (Lee et al. 2000a; Alam et al. 2002; Kim et al. 2002a; Furuita et al. 2003), alternative protein sources for fish meal (Kikuchi et al. 1994a, 1994b; Sato and Kikuchi 1997; Kikuchi 1999; Cho et al. 2005a, 2005b) in the diet, optimum feed allowance (Lee et al. 1999, 2000b; Cho et al. 2006a), and feeding strategy (Kim et al. 2002b; Cho 2005; Cho et al. 2006b) have been performed for effective fish production.

Use of extruded pellets (EPs) is highly desirable for most of fish farms in terms of improved availability of nutrients in the diet, easy observation on fish feeding activity, reduced water pollution source in effluent discharged from fish farm and spread of disease, easy handling, and long storage time for later use. The formulation of EP to satisfy dietary nutrient requirements for olive flounder has been developed, and commercially available EP is being adapted to many olive flounder farms in Korea. Excessive amount of feed supply commonly results to lower feed availability of fish, deteriorate water quality in fish farm, and eventually increase fish production cost.

In the previous study, optimum daily feeding ratio for growth of juvenile olive flounder grown from 17 to 90 g has been reported to be 95% of

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satiation when fish were fed by the EP with various feeding ratios (100, 95, 90, 85, 80, 75, and 70% of satiation) during summer season (Cho et al. 2006a). However, optimum feed allowance for growth of fish could be largely affected by fish size as well. In this study, therefore, optimum daily feeding ratio for subadult olive flounder fed the EP was determined during the summer season.

Materials and Methods

Fish and the Experimental Conditions

Subadult olive flounder of similar size were purchased from a private hatchery and transferred to the lab (Finfish Research Center, Uljin, Kyoungsangbukdo, Korea). Before the initiation of the feeding trial, fish were acclimated to experimental conditions for 3 wk. Thirteen fish (an average body weight of 319 g) per tank were randomly chosen and distributed into fifteen 500-L flow-through tanks (water volume, 300 L). During the acclimation period, fish were fed a commercial EP for flounder twice a day. The flow rate of water into each tank was 16 L/min/tank. The water source was sand-filtered natural seawater, and aeration was supplied to each tank. Water temperature ranged from 17.5 to 23.0 C (mean $\pm$ SD = 21.1 $\pm$ 1.3 C) because the feeding trial was performed during the summer season. Natural photoperiod was used, and fish were fed for 7 d a week throughout the 10-wk feeding trial.

Design of the Feeding Trial

Five treatments of feeding ratio in 5% decrement with triplicates were prepared for this study: 100 (satiation), 95, 90, 85, and 80% of satiation. Fish in the control group were hand-fed to apparent satiation, 100% of satiation, twice a day at 0900 and 1700 h. Uneaten diet was removed 30 min after feeding and deducted from diet consumption calculations. Diet allowance of fish in the rest of the four experimental groups was determined based on the average diet consumption of fish in the control group.

Preparation of the Experimental Diet

Ingredients and chemical composition of the experimental diet are given in Table 1. Mackerel meal, dehulled soybean meal, and corn gluten meal were used as the protein sources. Wheat flour and gluten and fish oil were used as the carbohydrate and lipid sources, respectively. The ingredients of the experimental diet were well mixed and extruded by a pellet extruder from Suhyup Feed Co. Ltd. (Uiryeong, Gyeongsangnamdo, Korea). The experimental diet (diameter, 9.0–9.4 mm) contained 49.5% crude protein and 9.2% crude lipid with a gross energy level of 6.0 kcal/g diet, based on the previous studies (Lee et al. 2000a, 2002; Kim et al. 2002a; Cho et al. 2006a).

TABLE 1. *Ingredient and nutrient composition of the experimental diet.*

	Composition (%)
Ingredient	
Mackerel meal	57.0
Soybean meal	4.0
Corn gluten meal	3.0
Wheat flour	14.0
Wheat gluten	4.0
Krill meal	2.5
Fish oil	5.0
Vitamin premix[a]	1.0
Mineral premix[a]	1.7
Others	7.8
Nutrients (% DM)	
Crude protein	49.5
Crude lipid	9.2
Ash	11.2
Gross energy (kcal/g diet)	6.0
n-3 HUFA[b]	2.8

DM = dry-matter basis.

[a] Vitamin and mineral premix were provided by Suhyup Feed Co. Ltd.

[b] Highly unsaturated fatty acid (C $\geq$ 20).

Analytical Procedures of Fish

Five fish at the initiation and termination of the feeding trial were sampled and sacrificed for proximate analysis. Crude protein was determined by the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412; Flwail, Switzerland), crude lipid by using an ether extraction method, moisture by oven drying at 105 C for 24 h, fiber by using an automatic analyzer (Fibertec; Tecator, Hoganas, Sweden), and ash by using a muffle furnace at 550 C for 4 h, according to standard AOAC (1990).

Blood Analysis of Fish

Blood samples were obtained from the caudal vein of randomly chosen five fish from each tank by using a heparinized syringe after they were starved for 24 h and anesthetized with MS-222 at the concentration of 100 mg/L at the end of the feeding trial. Plasma was collected after centrifugation (3000 rpm for 10 min), stored in a freezer at −70 C as separate aliquots for analysis of total protein, glucose, cholesterol, triglyceride (TG), glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT), and analyzed by using automatic chemistry system (HITACHI 7180 and 7600-210; Hitachi, Tokyo, Japan).

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 treatments through SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results and Discussion

Survival (%), weight gain (g/fish), and specific growth rate (SGR) of olive flounder fed the EP with various feeding ratios for 10 wk are given in Table 2. Survival was not significantly ($P > 0.05$) affected by feeding ratio. However, weight gain of flounder fed to 100% of satiation was significantly ($P < 0.05$) higher than that of fish fed to 85 and 80% of satiation but not significantly ($P > 0.05$) different from that of fish fed to 95 and 90% of satiation. Weight gain of flounder fed to 95% of satiation was significantly ($P < 0.05$) higher than that of fish fed to 80% of satiation. Weight gain of flounder tended to decrease with a decrease in feeding ratio in this study. SGR of fish fed to 100 and 95% of satiation was significantly ($P < 0.05$) higher than that of fish fed to 80% of satiation but not significantly ($P > 0.05$) different from that of fish fed to 90 and 85% of satiation. When the feeding ratio was lower than optimal level, higher weight gain of fish was achieved at a higher feeding ratio (Arzel et al. 1998; Ballestrazzi et al. 1998; Van Ham et al. 2003; Eroldogan et al. 2004).

Feeding fish to less than satiation without growth retardation is highly recommendable for fish production in fish farms because of less cost in diet and production of water pollution source, easier management, and higher diet utilization of fish. No significant difference in weight gain among fish fed to 100, 95, and 90% of satiation in this study indicated that daily feeding ratio could be lowered to 90% of satiation for subadult olive flounder without growth retardation during summer season. However, optimum daily feeding ratio for growth of juvenile olive flounder grown from 17 to 90 g was reported to be 95% of satiation during summer season when fish were fed to satiation twice a day for 7 wk (Cho et al. 2006a). According to the previous and the present studies, optimum daily feeding ratio for growth of olive flounder seemed to lower from 95 to 90% satiation as fish grew from juvenile to subadult. Similar findings that optimum diet allowance and/or dietary nutrient requirements decreased as fish grew were reported in other fish (Page and Andrews 1973; Skalli et al. 2004; Hatlen et al. 2005; Sweilum et al. 2005).

Diet consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER),

TABLE 2. *Survival (%), weight gain (g/fish), and specific growth rate (SGR) of subadult olive flounder fed the extruded pellet for 10 wk with various feeding ratios.*[1]

Feeding ratio (%)	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[2]
100	318.6 ± 0.1[a]	487.5 ± 24.8[a]	100 ± 0.0[a]	168.8 ± 24.9[a]	0.73 ± 0.09[a]
95	319.3 ± 0.4[a]	479.1 ± 0.7[a]	100 ± 0.0[a]	159.8 ± 0.3[ab]	0.70 ± 0.00[a]
90	319.5 ± 1.0[a]	461.7 ± 5.4[a]	100 ± 0.0[a]	142.2 ± 6.0[abc]	0.63 ± 0.03[ab]
85	319.0 ± 0.1[a]	459.0 ± 0.2[a]	100 ± 0.0[a]	139.9 ± 0.2[bc]	0.63 ± 0.00[ab]
80	318.8 ± 0.1[a]	448.8 ± 7.0[a]	100 ± 0.0[a]	130.0 ± 7.0[c]	0.59 ± 0.03[b]

[1] Values (mean ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

[2] SGR = (ln final weight of fish − ln initial weight of fish) × 100/days of feeding trial.

TABLE 3. *Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER), and protein retention (PR) of subadult olive flounder fed the extruded pellet for 10 wk with various feeding ratios.*[1]

Feeding ratio (%)	Feed consumption	FER[2]	PER[3]	PR[4]	HSI[5]	CF[6]
100	170.8 ± 25.4[a]	0.99 ± 0.00[a]	2.00 ± 0.00[a]	45.3 ± 8.7[a]	1.5 ± 0.2[a]	0.99 ± 0.01[a]
95	160.2 ± 0.0[ab]	1.00 ± 0.00[a]	2.02 ± 0.00[a]	45.2 ± 2.4[a]	1.7 ± 0.1[a]	1.06 ± 0.03[a]
90	151.7 ± 0.1[abc]	0.94 ± 0.04[a]	1.89 ± 0.08[a]	41.3 ± 3.0[a]	1.7 ± 0.1[a]	1.08 ± 0.02[a]
85	143.2 ± 0.0[bc]	0.98 ± 0.00[a]	1.97 ± 0.00[a]	45.3 ± 4.8[a]	1.8 ± 0.1[a]	1.07 ± 0.02[a]
80	134.9 ± 0.2[c]	0.96 ± 0.05[a]	1.95 ± 0.10[a]	42.6 ± 3.6[a]	1.7 ± 0.3[a]	1.08 ± 0.01[a]

HSI = hepatosomatic index; CF = condition factor.

[1] Values (mean ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

[2] FER = weight gain of fish/feed consumed.

[3] PER = weight gain of fish/protein consumed.

[4] PR = protein gain of fish/protein consumed.

[5] HSI = liver weight × 100/fish weight.

[6] CF = fish weight × 100/total length^3.

protein retention (PR), hepatosomatic index (HSI), and condition factor (CF) of subadult olive flounder fed the EP with various feeding ratios for 10 wk are presented in Table 3. Because diet allowance of flounder was determined based on diet consumption of fish in the control group, consumption linearly decreased with the decrease in feeding ratio. However, efficiency of diet (FER, PER, and PR) of flounder was not significantly ($P > 0.05$) affected by feeding ratio in this study. This is probably because weight gain of fish decreased in proportion to the reduction in feeding ratio. A similar trend that no difference in feed efficiency of fish was obtained at various diet allowances was observed in other studies (Shimeno et al. 1997; Ballestrazzi et al. 1998; Eroldogan et al. 2004; Cho et al. 2006a). Wide variation in diet consumption of subadult flounder at satiation feeding in the control group as a result of easy disturbance of feeding activity of fish during the feeding trial resulted in wide variation in weight gain of fish in the control group in this study. However, fish were more likely to consume all diets in other groups (95, 90, 85, and 80% of satiation) and achieved less variation in weight gain. Body condition indexes (HSI and CF) of flounder were not significantly ($P > 0.05$) affected by feeding ratio in this study. A similar result was obtained in the previous study (Cho et al. 2006a). However, Eroldogan et al. (2004) showed that feeding ratio significantly affected CF of European sea bass, *Dicentrarchus labrax*, but not HSI.

Proximate composition of olive flounder fed the EP for 10 wk is presented in Table 4. Chemical composition (moisture, crude protein and lipid, and ash content) of the whole body of fish without liver or liver was not significantly ($P > 0.05$) affected by feeding ratio. Similarly, proximate composition of flounder, except for crude protein, was not affected by feeding ratio (Cho et al. 2006a) but conflicted with other studies showing that body lipid content of fish increased with the increase in diet allowance or feeding frequency (Grayton and Beamish

TABLE 4. *Proximate composition (% of wet weight) of subadult olive flounder fed the extruded pellet for 10 wk with various feeding ratios.*[a]

	Whole body of fish without liver				Liver		
Feeding ratio (%)	Moisture	Crude protein	Crude lipid	Ash	Moisture	Crude protein	Crude lipid
100	71.5 ± 1.1	20.7 ± 1.6	2.5 ± 0.8	3.9 ± 0.3	66.8 ± 1.6	12.5 ± 0.2	14.0 ± 1.3
95	71.8 ± 0.9	20.4 ± 0.4	4.5 ± 0.4	3.7 ± 0.2	63.4 ± 2.0	11.3 ± 0.5	17.6 ± 2.0
90	71.9 ± 0.9	20.2 ± 0.7	3.5 ± 1.0	3.3 ± 0.2	63.9 ± 2.7	11.6 ± 0.4	15.8 ± 3.8
85	72.7 ± 0.6	20.5 ± 0.7	3.7 ± 0.3	3.4 ± 0.2	62.6 ± 2.5	11.3 ± 0.5	20.0 ± 4.1
80	72.7 ± 0.7	20.2 ± 0.4	3.1 ± 0.7	3.6 ± 0.3	63.7 ± 2.1	11.3 ± 0.5	19.2 ± 3.4

[a] Values (mean ± SE) for either whole body or liver are not significantly different for any treatment ($P > 0.05$).

TABLE 5. *Blood analysis of subadult olive flounder fed the extruded pellet with various feeding ratios for 10 wk.*[1]

Feeding ratio (%)	Total protein (g/dL)	Glucose (mg/dL)	TG (mg/dL)	GOT (IU/L)	GPT (IU/L)
100	3.5 ± 0.0[a]	15.0 ± 1.2[a]	113.5 ± 2.5[ab]	13.7 ± 0.9[b]	2.0 ± 0.6[a]
95	3.8 ± 0.1[a]	18.0 ± 1.2[a]	146.7 ± 17.0[a]	25.5 ± 2.5[a]	3.3 ± 1.9[a]
90	3.6 ± 0.2[a]	16.0 ± 1.0[a]	166.5 ± 9.5[a]	7.0 ± 1.0[c]	0.3 ± 0.3[a]
85	3.6 ± 0.1[a]	16.7 ± 0.9[a]	158.5 ± 20.5[a]	10.3 ± 2.2[bc]	2.7 ± 0.9[a]
80	3.6 ± 0.3[a]	16.7 ± 1.3[a]	90.0 ± 10.0[b]	10.0 ± 2.5[bc]	0.3 ± 0.3[a]

TG = triglyceride; GOT = glutamic oxaloacetic transaminase; GPT = glutamic pyruvic transaminase.

[1] Values (mean ± SE, each replicate consists of measurements of five fish) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

1977; Kayano et al. 1993; Shimeno et al. 1997; Lee et al. 2000b; Mihelakakis et al. 2002; Van Ham et al. 2003; Eroldogan et al. 2004).

Blood analysis of olive flounder fed the EP with various feeding ratios for 10 wk is shown in Table 5. Serum total protein, glucose, and GPT of flounder were not significantly ($P >$ 0.05) affected by feeding ratio. However, serum TG of flounder fed to 95, 90, and 85% of satiation was significantly ($P < 0.05$) higher than that of fish fed to 100 or 80% of satiation, although a wide variation was observed within the same treatment. Serum GOT of flounder fed to 95% of satiation was significantly ($P < 0.05$) higher than that of fish fed at other feeding ratios. GOT values of fish were ranked 100, 85, 80, and 90% of satiation in order. Similarly, serum glucose, total protein, TG, and GPT were not affected by feeding ratio (Cho et al. 2006a). These results probably indicate that blood analysis of fish was not likely affected by the feeding regimes used in the present study. However, Shimeno et al. (1997) showed that serum total protein, TG, cholesterol, and ammonia in common carp, *Cyprinus carpio*, increased as feeding rate increased, while serum glucose scarcely changed when fish were fed by the commercial diet at various feeding rates (100, 90, 80, 70, 50, 30, and 0% of satiation) for 30 d.

Diet allowance largely affects not only availability of nutrients in diet, cost of fish production, and fish performance but also effluent discharged from fish farms. Also, other factors, such as fish species, fish size, dietary nutrient composition, water temperature, rearing conditions, etc., could affect optimum diet allowance for fish in the feeding trial. Therefore, the least amount of diet without growth reduction could be regarded as the optimum level for fish, and it must be carefully determined.

In considering these results, it can be concluded that optimum daily feeding ratio for growth of subadult olive flounder seemed to be 90% of satiation when fish were fed the EP to satiation twice a day during the summer season.

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Literature Cited

Alam, S., S. Teshima, D. Yaniharto, S. Koshio, and M. Ishikawa. 2002. Influence of different dietary amino acid patterns on growth and body composition of juvenile Japanese flounder, *Paralichthys olivaceus*. Aquaculture 210:359–369.

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis, 15th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Arzel, J., R. Metailler, P. Legall, and J. Guillaume. 1998. Relationship between ration size and dietary protein level varying at the expense of carbohydrate and lipid in triploid brown trout fry, *Salmo trutta*. Aquaculture 162:259–268.

Ballestrazzi, R., D. Lanari, and E. D'Agaro. 1998. Performance, nutrient retention efficiency, total ammonia and reactive phosphorous excretion of growing European sea bass (*Dicentrarchus labrax* L.) as affected by diet processing and feeding level. Aquaculture 161:55–65.

Cho, S. H. 2005. Compensatory growth of juvenile flounder *Paralichthys olivaceus* L. and changes in biochemical composition and body condition indices during starvation and after refeeding in winter season. Journal of the World Aquaculture Society 36:508–514.

Cho, S. H., S. Lee, S. Lee, B. Park, I. Park, C. Y. Choi, B. H. Min, S. Hur, and J. Jo. 2005a. Effect of partial replacement of fish meal with squid liver meal™ in the diet on growth and body composition of juvenile flounder *Paralichthys olivaceus* during winter season. Journal of Fisheries Science and Technology 8:65–69.

Cho, S. H., S. Lee, B. Park, I. Park, C. Y. Choi, S. Lee, B. H. Min, S. Hur, and Y. S. Lim. 2005b. Effect of partial dietary substitution of meat meal for fish meal on the growth and body composition of the juvenile olive flounder *Paralichthys olivaceus*. Journal of Fisheries Science and Technology 8:138–141.

Cho, S. H., S. Lee, B. H. Park, and S. Lee. 2006a. Effect of feeding ratio on growth and body composition of juvenile olive flounder *Paralichthys olivaceus* fed extruded pellets during the summer season. Aquaculture 251:78–84.

Cho, S. H., S. Lee, B. H. Park, S. Ji, J. Lee, J. Bae, and S. Oh. 2006b. Compensatory growth of juvenile olive flounder *Paralichthys olivaceus* L. and changes in proximate composition and body condition indices during fasting and after refeeding in summer season. Journal of the World Aquaculture Society 37:168–174.

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

Eroldogan, O. T., M. Kumlu, and M. Aktas. 2004. Optimum feeding rates for European sea bass *Dicentrarchus labrax* L. reared in seawater and freshwater. Aquaculture 231:501–515.

Furuita, H., H. Tanaka, T. Yamamoto, N. Suzuki, and T. Takeuchi. 2003. Supplemental effect of vitamin A in diet on the reproductive performance and egg quality of the Japanese flounder *Paralichthys olivaceus* (T & S). Aquaculture Research 34:461–468.

Grayton, B. D. and F. W. H. Beamish. 1977. Effects of feeding frequency on food intake, growth and body composition of rainbow trout (*Salmo gairdneri*). Aquaculture 11:159–172.

Hatlen, B., B. Grisdale-Helland, and S. J. Helland. 2005. Growth, feed utilization and body composition in two size groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in protein and carbohydrate content. Aquaculture 249:401–408.

Kayano, Y., S. Yao, S. Yamamoto, and H. Nakagawa. 1993. Effects of feeding frequency on the growth and body constituents of young red-spotted grouper, *Epinephellus akaara*. Aquaculture 110:272–278.

Kikuchi, K. 1999. Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). Aquaculture 179:3–11.

Kikuchi, K., T. Furuta, and H. Honda. 1994a. Utilization of feather meal as a protein source in the diet of juvenile flounder. Fisheries Science 60:203–206.

Kikuchi, K., T. Furuta, and H. Honda. 1994b. Utilization of soybean meal as a protein source in the diet of juvenile flounder, *Paralichthys olivaceus*. Suisanzoshoku 42:601–604.

Kim, K. D., S. Lee, H. G. Park, S. Bai, and Y. H. Lee. 2002a. Essentiality of dietary n-3 highly unsaturated fatty acids in juvenile Japanese flounder (*Paralichthys olivaceus*). Journal of the World Aquaculture Society 33:432–440.

Kim, J. D., S. H. Shin, K. J. Cho, and S. Lee. 2002b. Effect of daily and alternate day feeding regimens on growth and food utilization by juvenile flounder *Paralichthys olivaceus*. Journal of Aquaculture 15:15–21 (in Korean with English abstract).

Lee, S., C. H. Seo, and Y. S. Cho. 1999. Growth of the juvenile olive flounder (*Paralichthys olivaceus*) fed the diets at different feeding frequencies. Journal of the Korean Fisheries Society 32:18–21.

Lee, S., S. H. Cho, and K. D. Kim. 2000a. Effects of dietary protein and energy levels on growth and body composition of juvenile flounder (*Paralichthys olivaceus*). Journal of the World Aquaculture Society 31:306–315.

Lee, S., S. H. Cho, and D. Kim. 2000b. Effects of feeding frequency and dietary energy level on growth and body composition of juvenile olive flounder (*Paralichthys olivaceus*). Aquaculture Research 12:917–923.

Lee, S., C. S. Park, and I. C. Bang. 2002. Dietary protein requirement of young Japanese flounder *Paralichthys olivaceus* fed isocaloric diets. Fisheries Science 68:158–164.

Mihelakakis, A., C. Tsolkas, and T. Yoshimatsu. 2002. Optimization of feeding rate for hatchery-produced juvenile gilthead sea bream *Sparus aurata*. Journal of the World Aquaculture Society 33:169–175.

Page, J. W. and J. W. Andrews. 1973. Interaction of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). Journal of Nutrition 103:1339–1346.

Sato, T. and K. Kikuchi. 1997. Meat meal as a protein source in the diet of juvenile Japanese flounder. Fisheries Science 63:877–880.

Shimeno, S., T. Shikata, H. Hosokawa, T. Masumoto, and D. Kheyyali. 1997. Metabolic responses to feeding rates in common carp, *Cyprinus carpio*. Aquaculture 151:371–377.

Skalli, A., M. C. Hidalgo, E. Abellan, M. Arizcun, and G. Cardenete. 2004. Effects of the dietary protein/lipid ratio on growth and nutrient utilization in common dentex (*Dentex dentex* L.) at different growth stages. Aquaculture 235:1–11.

Sweilum, M. A., M. M. Abdella, and A. S. El-Din. 2005. Effect of dietary protein-energy levels and fish initial sizes on growth rate, development and production of Nile tilapia, *Oreochromis niloticus* L. Aquaculture Research 36:1414–1421.

Van Ham, E. H., M. H. G. Berntssen, A. K. Imsland, A. C. Parpoura, S. E. Wendelaar Bonga, and S. O. Stefansson. 2003. The influence of temperature and ration on growth, feed conversion, body composition and nutrient retention of juvenile turbot (*Scophthalmus maximus*). Aquaculture 217:547–558.