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Effects of Different Feeding Regimes on the Compensatory Growth of Olive Flounder *Paralichthys olivaceus*

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The effects of different feeding regimes on the compensatory growth of olive flounder *Paralichthys olivaceus* were investigated. Seven treatments with triplicates of different feeding regimes were conducted, as follows: (1) fed twice a day 6 days a week for 8 weeks; (2) starved for 1 week, then fed twice a day 6 days a week for 3 weeks; (3) starved for 2 weeks, then fed twice a day 6 days a week for 6 weeks; (4) starved for 5 days, then fed twice a day for 9 days; (5) starved for 10 days, then fed twice a day for 18 days; (6) starved for 2 days, then fed twice a day for 5 days, starved for another 3 days, and fed twice a day 4 days; and (7) starved for 4 days, then fed twice a day for 10 days, starved for another 6 days, and fed twice a day for 8 days. Forty fish averaging 12.5 g, were hand-fed to satiation according to the designated feeding schedule. Fish from the control group gained more weight than those from any other group. Feeding efficiency did not vary among treatments. In summary, olive flounder subjected to fasting with different feeding regimes did not fully compensate growth compared to fish fed for 8 weeks without fasting. In addition, the less that fasted groups were subsequently fed, the lower their compensatory growth.

Key words: Olive flounder, Compensatory growth, Feeding regimes

Introduction

When feed is oversupplied to fish without considering their nutritional and/or physiological status of fish, it leads to an increase in the cost of fish production and the deterioration of water quality. The optimum growth period of the olive flounder *Paralichthys olivaceus*, which is one of the most commercially important marine fish species for aquaculture in Eastern Asia between late spring and early autumn in Korea. However, red tide and cold-water masses frequently occurs during these periods. During such events, aquaculturists commonly starve fish to minimize mass mortality until the natural phenomena diminish, eventually resulting in suppressed fish growth and production. In addition, fish farmers are more likely to oversupply feed to fish to achieve fast growth although fish would not consume all feed well at low temperature condition. This also can lead to an increase in fish production costs and the deterioration of water quality.

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This culture method to achieve compensatory growth is a common practice aimed at improving fish production after feed deprivation. It is largely affected by fish size and age (Bilton and Robins, 1973), dietary nutrient composition (Gaylord and Gatlin, 2001), and feeding regimes (Rueda et al., 1998; Gaylord and Gatlin 2000; Wang et al. 2000; Tian and Qin, 2003, 2004; Cho, 2005a, 2005b; Cho et al., 2006; Oh et al., 2007; Cho and Cho, 2009). Juvenile olive flounder can achieve full compensatory growth after up to 2 weeks of feed deprivation, but only partial compensation after 3 or 4 weeks of fasting in 8-week feeding trials (Cho, 2005b; Cho et al., 2006). However compensatory growth at 6 week refeeding after 2 week feed deprivation when fish were fed an extruded pellet with different feeding regimes (Cho and Cho, 2009). Nonetheless fish may respond differently depending on the feed type and/or nutrient composition. In the present study, the effects of different feeding regimes, with alternate short- or long-term fasting and feeding periods, on compensatory growth of olive flounder were analyzed.

Materials and Methods

Fish and experimental conditions

Juvenile olive flounder were purchased from a private farm, transferred to the laboratory and acclimated to the experimental conditions for 2 weeks. Forty flounders, averaging 12.5 g were randomly chosen and distributed in 21 180 L fiber-reinforced plastic flow-through tanks (water volume 150 L) each. The water temperature ranged from 14.8°C to 24°C (mean±SD=18.6±2.89°C) and the fish were kept in natural photoperiod conditions. Each tank was aerated and had a water flow rate of 8.6 L/min.

Diet preparation

The ingredients and proximate composition of the experimental diet are shown in Table 1. Fish meal, dehulled soybean meal and corn gluten meal were used as a protein source. Wheat flour, and pollack liver and soybean oils were used as the carbohydrate and lipid sources, respectively. The ingredients were mixed with water at a ratio of 7:3 and then pelletized by an extruder. The experimental diet was dried at room temperature overnight and stored at -20°C until use. Dietary nutrient requirements were satisfactory for growth of olive flounder (Lee et al. 2000; Kim et al. 2002).

Experimental design

Seven treatments with triplicates of different feeding regimes were conducted as follows: (1) fed twice a day 6 days a week for 8 weeks (8WF); (2) starved for 1 week, then fed twice a day 6 days a week for 3 weeks [(1WS+3WF)×2]; (3) starved for 2 weeks, then fed twice a day 6 days a week for 6 weeks (2WS+6WF); (4) starved for 5 days, then fed twice a day for 9 days [(5DS+9DF)×4]; (5) starved for 10 days, then fed twice a day for 18 days [(10DS+18DF)×2]; (6) starved for 2 days, then fed twice a day for 5 days, starved for another 3 days, and fed twice a day 4 days [(2DS+5DF+3DS+4DF)×4]; and (7) starved for 4 days, then fed twice a day for 10 days, starved for another 6 days, and fed twice a day for 8 days [(4DS+10DF+6DS+8DF)×2]. Fish were hand-fed to satiation on the experimental diet containing 46.0% crude protein and 8.1% crude lipid twice (09:00, 16:30) a day according to the designated feeding schedule. The feeding trial lasted for 8 weeks.

Analysis of proximate composition and blood chemistry

Five olive flounders from each tank at the end of the feeding trial were randomly sampled for analysis

Table 1. Ingredients and nutrient composition of the experimental diet

	Composition (%)
Ingredients	
Fishmeal[1]	53
Dehulled soybean meal	11
Corn gluten	7
Wheat flour	24
Pollack liver oil	1.5
Soybean oil	1.4
Vitamin premix[2]	1.0
Mineral premix[3]	1.0
Choline	0.1
Nutrient (DM basis, %)	
Dry matter	81.4
Crude protein	46.0
Crude lipid	8.1
Ash	7.5
Fiber	1.6
NFE[4]	36.8
Estimated energy (kcal/g)[5]	4.0

[1]Imported from Chile.
[2]Vitamin premix is the same as Cho et al. (2007)'s study.
[3]Mineral premix is the same as Cho et al. (2007)'s study.
[4]NFE calculated by the difference.
[5]Estimated energy calculated based on the values of 4 kcal/g for protein and NFE, and 9 kcal/g for lipid, respectively (Garling and Wilson 1976).

of the proximate composition of the whole-body with and without the liver and liver according to the method described by the AOAC (1990). Crude protein content was determined using the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland). The lipid content was determined using the ether-extraction method. The moisture content was obtained by drying the samples in an oven at 105°C for 24 h. The fiber content was determined using an automatic analyzer (Fibertec, Tecator, Sweden) and ash content was obtained using a muffle furnace at 550°C for 4 h. The condition factor (CF) and hepatosomatic index (HSI) of fish at the end of the feeding trial were also measured.

Blood samples were obtained from the caudal vein of three randomly chosen fish from each tank at the end of the feeding trial using a heparinized syringe after starvation for 24 h and after the fish were anesthetized with 100 mg/L MS-222. The plasma was collected after centrifugation (3,000 rpm for 10 min), and stored at -70°C as separate aliquots for enzymatic analysis of total protein, glucose, cholesterol, triglycerides (TG), glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) using an automatic chemistry system (HITACHI 7180 and 7600-210, Hitachi, Japan) based on the manual provided by Daiichi Pure Chemicals Co. Ltd (2005). In addition, the total plasma hormones triiodothyronine (T_3) and thyroxine (T_4) of fish starved weekly and those obtained at the end of the weekly feeding trial were analyzed by radioimmunoassay (Gamma Counter, Cobra II, Packard, USA) using the Coat-A-Count kit (DPC, Los Angeles, CA, USA).

Statistical analysis

The significance in days among treatments was tested using a one-way analysis of variance (ANOVA). Duncan's multiple range test (Duncan 1955) was applied to detect significant differences among treatments using SAS Version 9.1 (SAS Institute, Cary, NC, USA). Percentage data were as log- transformed prior to the analysis.

Results and Discussion

The survival rate of olive flounder ranged from 96.7 to 100%, which was not significantly (P>0.05) different among treatments (Table 2). Given that dietary protein and energy requirements were considered satisfactory for the present study (Lee et al., 2000; Kim et al., 2002), fish growth was well achieved in the 8WF treatment. However, weight gain in the 8WF treatment (48 day feeding) was significantly (P<0.05) higher than those from other treatments (36 day feeding) with fasting. Similarly, olive flounders with different feeding regimes did not fully compensate growth compared to fish in an earlier study (Cho and Cho, 2009) that were fed an extruded pellet for 8 weeks without fasting.

However, weight gain was not significantly (P> 0.05) different among fish groups subjected to fasting with different feeding regimes contradicting the results of Cho and Cho (2009). Their study showed that enhanced compensatory growth in olive flounder fed for 6-week after a 2 week fasting period compared to fish with alternate feeding regimes with the same number of feeding days (36 days) using an extruded pellet (crude protein 52.0% and crude lipid 11.0%). Heide et al. (2006) reported that higher levels of compensatory growth in Atlantic halibut *Hippoglossus hippoglossus* subjected to one long initial period of fasting compared to two or three short fasting periods. Given that similar results were obtained from the present study and previous reports, the difference in fish performance suggests that the compensatory growth of olive flounder varies depending on feed type and/or dietary nutrient composition. However, the specific growth rate was not significantly (P>0.05) different in this study.

Feed consumption (g/fish) of olive flounder in the 8WF treatment without fasting was significantly (P>0.05) higher than those from treatments with fasting (Table 3). Hyperphagia is common in may fish that achieve full compensatory growth after feed deprivation (Rueda et al., 1998; Gaylord and Gatlin, 2000; Wang et al., 2000; Xie et al., 2001; Tian and Qin, 2003; Cho, 2005b). Therefore, less feed consumption of allfish groups subjected to fasting with different feeding regimes resulted to poor compensatory growth compared to from the 8WF treatment. Feed consumption in the (10DS+18DF)×2 and (4DS+10DF+6DS+8DF)×2 groups was significantly (P<0.05) less than that in the (2DS+5DF+3DS+4DF)×4 group, but not significantly (P>0.05) different from the (1WS+3WF)×2, 2WS+6WF or (5DS+9DF)×4 groups. However, the feed efficiency ratio (0.65-0.73), the protein efficiency ratio (1.41-1.59), protein retention (25.6-28.0), CF (0.95-1.02), and HSI (1.55-1.71) were not significantly (P>0.05) different among treatments. In contrast, improvements in feed utilization have been observed in

Table 2. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of juvenile olive flounder fed the experimental diet with different feeding regime for 8 weeks[1]

Treatments	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[2]
8WF	12.5 ± 0.03	38.7 ± 1.59	98.3 ± 1.67[a]	26.2 ± 1.61[a]	2.3 ± 0.09[a]
(1WS+3WF)×2	12.6 ± 0.01	31.4 ± 0.53	99.2 ± 0.83[a]	18.8 ± 0.51[b]	2.5 ± 0.04[a]
(2WS+6WF)	12.6 ± 0.03	31.6 ± 1.15	96.7 ± 2.20[a]	19.1 ± 1.17[b]	2.6 ± 0.10[a]
(5DS+9DF)×4	12.6 ± 0.03	31.1 ± 0.45	99.2 ± 0.83[a]	18.5 ± 0.42[b]	2.5 ± 0.03[a]
(10DS+18DF)×2	12.5 ± 0.02	30.0 ± 0.96	100 ± 0.00[a]	17.6 ± 0.97[b]	2.4 ± 0.09[a]
(2DS+5DF+3DS+4DF)×4	12.5 ± 0.05	32.3 ± 1.68	99.2 ± 0.83[a]	19.8 ± 1.65[b]	2.6 ± 0.14[a]
(4DS+10DF+6DS+8DF)×2	12.7 ± 0.14	29.4 ± 0.81	100 ± 0.00[a]	16.7 ± 0.70[b]	2.3 ± 0.06[a]

[1]Values (mean ± SE) in the same column sharing a same superscript letter are not significantly different (P<0.05).
[2]SGR = (Ln final weight of fish-Ln initial weight of fish) × 100/days of feeding.

Table 3. Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER), protein retention (PR), condition factor (CF) and hepatosomatic index (HSI) of olive flounder with different feeding regime[1]

Treatments	Feed consumption (g/fish)	FER[2]	PER[3]	PR[4]	CF[5]	HSI[6]
8WF	35.8 ± 1.49[a]	0.73 ± 0.015[a]	1.59 ± 0.032[a]	27.8 ± 0.38[a]	1.01 ± 0.018[a]	1.61 ± 0.092[a]
(1WS+3WF)×2	26.6 ± 0.81[bc]	0.71 ± 0.008[a]	1.53 ± 0.014[a]	26.9 ± 0.70[a]	0.99 ± 0.039[a]	1.55 ± 0.027[a]
(2WS+6WF)	28.4 ± 1.61[bc]	0.67 ± 0.011[a]	1.43 ± 0.005[a]	26.2 ± 0.50[a]	0.95 ± 0.006[a]	1.55 ± 0.026[a]
(5DS+9DF)×4	28.1 ± 1.02[bc]	0.66 ± 0.011[a]	1.43 ± 0.020[a]	25.6 ± 0.79[a]	1.02 ± 0.018[a]	1.63 ± 0.080[a]
(10DS+18DF)×2	25.6 ± 0.87[c]	0.68 ± 0.028[a]	1.49 ± 0.060[a]	25.8 ± 2.62[a]	0.98 ± 0.016[a]	1.68 ± 0.106[a]
(2DS+5DF+3DS+4DF)×4	29.9 ± 0.88[b]	0.66 ± 0.043[a]	1.44 ± 0.089[a]	25.9 ± 1.52[a]	0.98 ± 0.030[a]	1.71 ± 0.068[a]
(4DS+10DF+6DS+8DF)×2	25.7 ± 0.81[c]	0.65 ± 0.010[a]	1.41 ± 0.021[a]	28.0 ± 2.40[a]	0.98 ± 0.011[a]	1.67 ± 0.075[a]

[1]Values (mean±SE) in the same column sharing a same superscript letter are not significantly different ($P<0.05$).
[2]Feed efficiency ratio (FER) = Weight gain of fish/feed consumed.
[3]Protein efficiency ratio (PER) = Weight gain of fish/protein consumed.
[4]Protein retention (PR) = Protein gain of fish × 100/protein consumed.
[5]Condition factor (CF) = Fish weight × 100/total length^3.
[6]Hepatosomatic index (HSI) = Liver weight × 100/fish weight.

Table 4. Proximate composition (% of wet weight) of olive flounder at the end of the feeding trial[1]

Treatments	Whole body of fish without liver				Liver		
	Moisture	Crude protein	Crude lipid	Ash	Moisture	Crude protein	Crude lipid
8WF	70.4 ± 1.01[a]	11.5 ± 0.77[a]	10.5 ± 0.69[a]	3.3 ± 0.09[a]	70.4 ± 1.01[a]	11.5 ± 0.77[a]	10.5 ± 0.69[a]
(1WS+3WF)×2	70.1 ± 0.26[a]	11.4 ± 0.45[a]	10.0 ± 0.92[a]	3.3 ± 0.20[a]	70.1 ± 0.26[a]	11.4 ± 0.45[a]	10.0 ± 0.92[a]
(2WS+6WF)	71.6 ± 0.98[a]	11.9 ± 0.40[a]	9.9 ± 1.61[a]	3.4 ± 0.26[a]	71.6 ± 0.98[a]	11.9 ± 0.40[a]	9.9 ± 1.61[a]
(5DS+9DF)×4	71.2 ± 0.16[a]	11.7 ± 0.17[a]	9.5 ± 0.47[a]	3.1 ± 0.32[a]	71.2 ± 0.16[a]	11.7 ± 0.17[a]	9.5 ± 0.47[a]
(10DS+18DF)×2	69.4 ± 0.53[a]	10.9 ± 0.68[a]	11.0 ± 0.48[a]	3.3 ± 0.14[a]	69.4 ± 0.53[a]	10.9 ± 0.68[a]	11.0 ± 0.48[a]
(2DS+5DF+3DS+4DF)×4	71.5 ± 0.08[a]	12.5 ± 0.54[a]	8.7 ± 0.50[a]	3.5 ± 0.19[a]	71.5 ± 0.08[a]	12.5 ± 0.54[a]	8.7 ± 0.50[a]
(4DS+10DF+6DS+8DF)×2	70.7 ± 0.54[a]	11.5 ± 0.43[a]	10.7 ± 0.80[a]	3.5 ± 0.20[a]	70.7 ± 0.54[a]	11.5 ± 0.43[a]	10.7 ± 0.80[a]

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

fish reaching compensatory growth (Qian et al., 2000; Gaylord and Gatlin, 2001; Cho, 2005b; Cho et al., 2006). Gaylord and Gatlin (2000) reported that HSI and CF of fingerling channel catfish *Ictalurus punctatus* de-creased rapidly during the fasting period and increased quickly during subsequent re-feeding, which showed that HSI and CF were useful for in-dicating possible compensatory growth. Cho (2005b) also demonstrated that HSI could be a good indicator of compensatory growth.

The proximate composition of the whole body of olive flounder with and without the liver was not significantly ($P>0.05$) different among treatments, except for moisture content of the whole body without liver (Table 4). In the 8WF group, the moisture content of the whole body without the liver was significantly ($P<0.05$) lower than that in the (1WS+3WF)×2, (10DS+18DF)×2, (2DS+5DF+3DS+4DF)×4, and (4DS+10DF+6DS+8DF)×2 groups, which was the highest, although not significantly ($P>0.05$) different from those in the 2WS+6WF and (5DS+9DF)×4 groups. Similarly, the chemical com-position was unaffected by feeding regimes in several previous studies (Gaylord and Gatlin, 2000; Xie et al., 2001; Cho et al., 2006, 2007).

At the end of the 8-week feeding trial, the plasma chemistry as well as T_3 and T_4 levels was measured. Plasma protein (3.0-3.7 g/dL), glucose (12.1-21.9 mg/dL), triglyceride (126.1-202.4 mg/dL), GOT (18.2-38.0 IU/L), GPT (5.5-12.5 IU/L), cholesterol (162.0-222.4 mg/dL), T_3 (4.2-5.7 ng/ml), and T_4 (38.2-135.1 ng/mL) levels were not significantly ($P>0.05$) different among treatments (Table 5). These results are not in agreement with several reports that have shown effects of enzyme and hormones on compensatory growth (Eales, 1988; Van der Geyten et al., 1998; Gaylord et al., 2001; Meton et al., 2003). Specifically, Gaylord et al. (2001) showed that T_3 and T_4 levels of channel catfish increased rapidly within 2 days of subsequent re-feeding after 3 or 5 days of fasting. Cho and Cho (2009) reported that T_3 levels of olive flounder appeared to partially play a role in achieving compensatory growth when fish were fed an extruded pellet with different feeding regimes.

In summary, juvenile olive flounder subjected to fasting with different feeding regimes did not fully

Table 5. Plasma chemistry of olive flounder at the end of the 8-week feeding trial[1]

Treatments	Plasma chemistry							
	Total protein (g/dL)	Glucose (mg/dL)	TG (mg/dL)	GOT (IU/L)	GPT (IU/L)	Cholesterol (mg/dL)	T_3 (ng/mL)	T_4 (ng/mL)
8WF	3.0 ± 0.09	17.3 ± 6.46	129.0 ± 7.07	18.2 ± 1.62	5.5 ± 1.40	162.0 ± 5.77	4.2 ± 0.33	53.1 ± 4.80
(1WS+3WF)×2	3.0 ± 0.10	16.5 ± 5.49	126.1 ± 5.21	29.9 ± 5.76	7.5 ± 0.91	187.5 ± 17.54	4.7 ± 0.39	89.8 ± 16.60
(2WS+6WF)	3.4 ± 0.34	12.1 ± 1.57	166.4 ± 26.34	23.8 ± 3.22	8.9 ± 2.27	184.1 ± 29.18	5.0 ± 0.64	181.0 ± 48.10
(5DS+9DF)×4	3.7 ± 0.19	17.1 ± 2.88	172.5 ± 9.54	38.0 ± 7.44	12.5 ± 1.46	199.4 ± 6.55	5.1 ± 0.23	79.8 ± 43.32
(10DS+18DF)×2	3.7 ± 0.13	21.9 ± 6.17	142.6 ± 5.58	29.9 ± 4.84	12.4 ± 1.88	222.4 ± 11.98	5.7 ± 0.14	58.8 ± 26.86
(2DS+5DF+3DS+4DF)×4	3.6 ± 0.39	14.3 ± 2.17	154.2 ± 33.81	25.0 ± 7.70	10.7 ± 3.25	199.3 ± 26.28	5.7 ± 1.08	54.9 ± 12.35
(4DS+10DF+6DS+8DF)×2	3.7 ± 0.56	15.4 ± 1.96	202.4 ± 60.10	25.9 ± 4.05	11.2 ± 1.67	207.9 ± 16.18	5.8 ± 0.63	38.2 ± 3.37

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

compensate growth compared to fish fed an experimental diet for 8 weeks without fasting. In addition, the less that fasted groups were subsequently fed the lower their compensatory growth.

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