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Effect of Fasting and Refeeding on Growth and Blood Chemistry in Juvenile Olive Flounder *Paralichthys olivaceus* L.

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Effect of fasting and refeeding on growth and blood chemistry of juvenile olive flounder *Paralichthys olivaceus* L. was investigated when fish achieved compensatory growth. Fish were fed the experimental diet for 6 days a week. Five treatments in triplicate were prepared: C, S1, S2, S3 and S4. Fish in the control group (C) were hand-fed to apparent satiation twice a day. Fish in treatments of S1, S2, S3 and S4 experienced 1, 2, 3 and 4 weeks of starvation and were then hand-fed to satiation twice daily during the remaining 7, 6, 5 and 4 weeks of the experiment, respectively. Weight gain of fish in C, S1 and S2 were higher than those of fish in S3 and S4. A significant difference in plasma total protein, glucose, triglyceride, T_3 and T_4 was observed in between starved and refed fish for the rest periods of the feeding trial. Plasma total protein and T_3 of flounder decreased with week of fasting and following correlationships were obtained; Y (Total protein) = -0.13X (week of fasting) + 1.54, R^2=0.9792 and Y (T_3) = -11.48X (week of fasting) +79.57, R^2=0.8822, respectively.

Keywords: Olive flounder, *Paralichthys olivaceus*, Fasting, Refeeding, T_3, Compensatory growth.

Introduction

The phenomenon of rapid or fast growth of fish resulted from refeeding after fasting or undernutrition, called "compensatory growth", frequently occurs in the wild due to the limited food availability. Compensatory growth has been observed in several coldwater fish (Jobling and Koskela, 1996; Damsgaard and Dill, 1998) and warmwater fish (Rueda et al., 1998; Gaylord and Gatlin, 2000; Zhu et al., 2004; Cho, 2005; Cho et al., 2006) as well as tropical fish (Wang et al., 2000; Tia and Qin, 2003, 2004). An ability of fish to achieve compensatory growth varies depending on species, size, feeding strategy, water temperature, dietary nutrient content, severity of fasting, duration of feeding trial, etc.

During fasting, physiological changes of fish, such as hormonal and/or body nutrient composition changes for basic metabolism and maintenance for survival are known to occur (Sheridan and Mommsen, 1991; Mackenzie et al., 1998; Rueda et al., 1998; Gaylord and Gatlin, 2000; Qian et al., 2000; Zhu et al., 2001). Rueda et al. (1998) demonstrated that the early phase (up to 2 weeks) of starvation of red porgy *Pagrus pagrus* seemed to result in a mobilization of protein more than lipids and lipid was mobilized in greater quantity later when fish were refed after 1-, 2-, 3-, and 4-week fasting. And the increased plasma thyroid hormones played a major role in accelerating growth rate in several fish species to achieve compensatory growth (Mackenzie et al., 1998; Gaylord et al., 2001).

Olive flounder *Paralichthys olivaceus* L. has been one of the most commercially important marine fish species for aquaculture in Eastern Asia. In the earlier studies of Cho (2005) and Cho et al. (2006), juvenile olive flounder had an ability to fully recover from 2-week feed deprivation in winter and summer season, respectively. However, few were known in changes in blood chemistry of olive flounder when fish achieved compensatory. In this study, therefore, effect of fasting and refeeding on growth and blood chemistry including thyroid hormone in olive flounder were investigated when fish achieved compensatory growth. This will be useful for farmer to scientifically understand compensatory growth of olive flounder.

Materials and Methods

Experimental conditions

Juvenile olive flounder were purchased from a private hatchery and transferred into the Lab. Fish were acclimated to the experimental conditions for 2 weeks. During the acclima-

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tion period, fish were fed by the commercial feed twice a day for 6 days a week. Twenty-five juvenile (an initial body weight of fish: 16.0 g) fish per tank were randomly distributed into 15 of 180 L flow-through tanks indoor. The flow rate of water into each tank was 6.5 L/min/tank. The water source was sand-filtered natural seawater and aeration was supplied to each tank. Water temperature ranged from 16.0 to 25.5°C (mean±SD: 23.6±0.26°C). Fish were fed for 6 days a week as designated.

Design of feeding trial

Five treatments in triplicate were prepared for this study: C, S1, S2, S3 and S4. Fish in the control group (C) were hand-fed the experimental diet to apparent satiation twice a day throughout the feeding trial (09:30 and 17:00). The experimental diet was formulated to contain 46.9% crude protein and 8.0% crude lipid, respectively. Fishmeal, wheat flour and a-starch, and pollack liver oil were used as protein, carbohydrate, and lipid sources, respectively in the experimental diet. Dietary nutrient requirements were satisfied for growth of olive flounder (Lee et al., 2000, 2002). Fish in treatments of S1, S2, S3 and S4 experienced 1, 2, 3 and 4 weeks of starvation and were then hand-fed to apparent satiation twice a day during the remaining 7, 6, 5 and 4 weeks of the experiment, respectively. The feeding trial lasted for 8 weeks.

Analysis of blood chemistry of fish

A group of starved fish in the similar size was stocked and fasted throughout the 8-week feeding trial and 5 fish were sampled for analysis of blood chemistry each week. Blood samples were obtained from the caudal vein of 5 randomly chosen fish from each tank by using a heparinized syringe after they were starved for 24 h and anesthetized with 100 ppm MS-222 at the end of the feeding trial. Plasma was collected after centrifugation (3,000 rpm for 10 min), stored freezer at -70°C as separate aliquots for analysis of total protein, glucose, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and triglyceride (TG), and analyzed by using automatic chemistry system (Vitros DT60 II, Vitros DTE II, DTSC II Chemistry System, Johnson & Johnson Clinical Diagnostics Inc., New York, USA). In addition, plasma T_3 (triiodothyronine) and total T_4 (thyroxine) hormone levels of fish at the end of feeding trial were analyzed and compared with starved fish by radio-immunoassay method by using Gamma Counter (Cobra II, Packard, USA).

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. Student's t-test was applied to compare the difference in blood chemistry of fish between before refed and after refed for rest of feeding trial in each treatment at the end of the feeding trial (Cody and Smith, 1991). In addition, regression analysis for criteria measured for blood chemistry and weeks of starvation was conducted by using regression analysis through SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Weight gain of fish in C, S1 and S2 were significantly ($P<0.05$) higher than those of fish in S3 and S4 (Table 1). Feed consumption (g/fish) of fish in C, S1 and S2 was significantly ($P<0.05$) higher than that of fish in S3 and S4. Feed efficiency ratio (FER) of olive flounder in C and S1 were not significantly ($P>0.05$) different from that of fish in

Table 1. Weight gain (%), feed consumption (g/fish), feed efficiency ratio (FER), hepatosomatic index (HSI) and condition factor (CF) of olive flounder fed the experimental diet with different feeding strategy

Treatments	Final weight (g/fish)	Weight gain (%)	Feed consumption (g/fish)	FER[1]	HSI[2]	CF[3]
C	48.9±4.7	207.2±24.0^{a}	42.3±4.18^{a}	0.79±0.04^{a}	1.58±0.07	0.93±0.01
S1	49.4±1.7	209.0± 9.9^{a}	43.6±1.07^{a}	0.79±0.03^{a}	1.46±0.12	0.92±0.01
S2	41.6±2.3	159.7±11.7^{a}	36.0±2.06^{a}	0.71±0.02^{ab}	1.63±0.02	0.93±0.01
S3	30.8±1.6	91.6± 8.3^{b}	25.8±0.52^{b}	0.65±0.01^{b}	1.79±0.13	0.91±0.02
S4	23.8±0.0	48.6± 1.8^{b}	17.4±0.29^{c}	0.44±0.01^{c}	2.02±0.19	0.86±0.02

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

Table 2. Blood and hormone analysis of olive flounder at the end of the feeding trial or with weeks of starvation

Treatments	Plasma chemistry						
	Protein (g/dL)	Glucose (mg/dL)	TG (mg/dL)	GOT (IU/L)	GPT (IU/L)	T_3 (ng/dL)	T_4 (μg/dL)
C	2.5±0.06	293.7±41.26	57.0± 9.54	15.7±5.03	4.3±3.21	202.0±78.54	2.8±1.34
8-week fasting	0.6±0.12***	578.7±8.02**	1.0±0.00**	11.0±6.56	2.0±1.00	tr (<7.0) ***[1]	tr (<0.25) ***[1]
S1	2.6±0.21	318.7±9.45	62.0±21.17	13.3±6.81	2.7±0.58	232.9±50.73	2.9±0.78
1-week fasting	1.4±0.23**	532.0±34.04**	8.0±5.57*	22.7±21.13	1.3±0.58	63.8±7.28***	1.8±2.04***
S2	2.6±0.07	289.3±20.55	59.3± 4.04	12.0±1.73	2.7±0.58	206.5±86.53	2.8±0.63
2-week fasting	1.3±0.06***	508.0±30.51**	4.0±2.65***	28.3±14.50	1.7±0.58	61.9±9.69***	tr (<0.25) ***[1]
S3	2.5±0.32	336.0±74.65	54.0± 4.36	19.7±6.03	3.0±1.00	214.0±66.49	3.2±1.41
3-week fasting	1.1±0.06**	526.7±46.00*	1.0±0.00**	17.3±16.17	1.7±0.58	30.9±4.31***	tr (<0.25) ***[1]
S4	2.4±0.10	346.3±14.011	62.7±10.41	19.7±6.66	2.3±0.58	212.0±29.56	1.7±0.10
4-week fasting	1.0±0.12***	518.7±78.12	1.0±0.00**	19.7±19.40	1.7±1.15	38.2±1.70***	tr (<0.25) ***[1]

Plasma total protein, glucose, TG, GOT, GPT, T_3 and T_4 of the initial fish were 1.6 g/dL, 454.7 mg/dL, 20.7 mg/dL, 2.0 IU/L, 18.3 IU/L, 84.6 ng/dL and 2.5 μg/dL, respectively.
Values (mean±SD) in the same column sharing a common superscript are not significantly different ($P<0.05$).
tr (<7.0)[1] and tr (<0.25)[1] indicate that trace amount of T_3 and T_4 hormones was detected, but not measurable by the instrument used in this study.
*, **, ***indicate significance levels of t-test between two groups were $P<0.05$, $0.01<P<0.001$ and $P<0.0001$, respectively.

S2, but significantly ($P<0.05$) higher than that of fish in S3 and S4. Condition factor (CF) and hepatosomatic index (HSI) of olive flounder was not significantly ($P>0.05$) affected by feeding strategy at the end of the feeding trial.

Blood chemistry of olive flounder, such as total protein, glucose, TG, GOT, GPT, T_3 and T_4 was not significantly ($P>0.05$) affected by feeding regime at the end of the feeding trial, probably due to high variation within the same treatment (Table 2). Comparison in blood chemistry of fish between before and after refed for the rest of the feeding trail in each treatment was given in Table 2. A significant difference in plasma total protein was observed in between fish fed for 8 weeks in the control group and fish starved for 8 weeks ($P<0.0002$), fish in S1 and fish starved for 1 week ($P<0.002$), fish in S2 and fish starved for 2 weeks ($P<0.0001$), fish in S3 and fish starved for 3 weeks ($P<0.01$) or fish in S4 at the end of the 8-week feeding trial and fish starved for 4 weeks ($P<0.0001$), respectively. A significant difference in plasma glucose was observed in between fish in the control group and fish starved for 8 weeks ($P<0.005$), fish in S1 and fish starved for 1 week ($P<0.005$), fish in S2 and fish starved for 2 weeks ($P<0.001$) or fish in S3 and fish starved for 3 weeks ($P<0.03$), respectively. A significant difference in plasma TG was observed in between fish in the control group and fish starved for 8 weeks ($P<0.01$), fish in S1 and fish starved for 1 week ($P<0.04$), fish in S2 and fish starved for 2 weeks ($P<0.0001$), fish in S3 and fish starved for 3 weeks ($P<0.002$) or fish in S4 and fish starved for 4 weeks ($P<0.009$), respectively. However, no significant difference was observed in either plasma GOT or GPT of fish. A significant difference in T_3 and T_4 levels was observed in between fish in the control group and fish starved for 8 weeks ($P<0.0001$), fish in S1 and fish starved for 1 week ($P<0.0001$), fish in S2 and fish starved for 2 weeks ($P<0.0001$), fish in S3 and fish starved for 3 weeks ($P<0.0001$) or fish in S4 and fish starved for 4 weeks ($P<0.0001$), respectively although T_3 and T_4 values were trace in some of the starved fish.

Total protein and T_3 of olive flounder decreased with week of fasting and the following correlationships were obtained; Y (Total protein) = -0.13X (week of fasting) + 1.54, R^2=0.9792, $P<0.0001$ (Fig. 1) and Y (T_3) = -11.48X (week of fasting) +79.57, R^2=0.8822, $P<0.0001$ (Fig. 2), respectively. However, there was no correlationship between other plasma chemistry criteria measured and week of fasting.

Discussion

Full compensatory growth was achieved in olive flounder fed for 7 and 6 weeks after 1- and 2-week fasting, respectively in this study. Also, growth of olive flounder fed on the commercial feed for 6 weeks after 2-week fasting was comparable to that of fish fed for 8 weeks during the winter season (Cho, 2005). Although feeding conditions such as water temperature, feed nutrients and fish size were differed between two studies, juvenile olive flounder seemed to have a limited ability to recover full compensatory growth after up

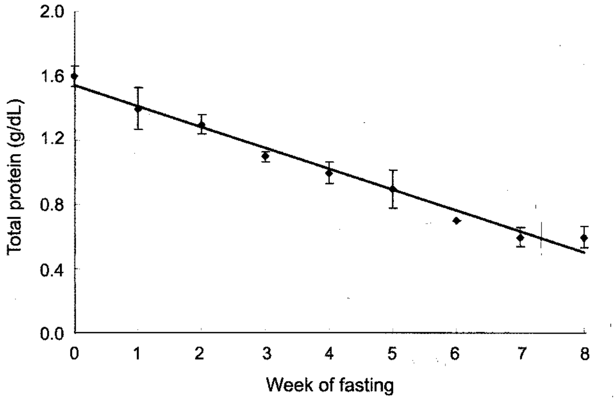

Fig. 1. Weekly change in plasma total protein (g/dL) in olive flounder with fasting (Y = -0.13X +1.54, R^2=0.9792, *P*<0.0001).

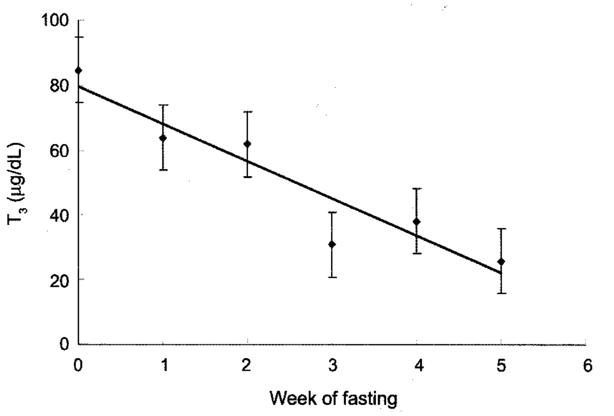

Fig. 2. Weekly change in T_3 (ß'/dL) level in olive flounder with fasting (Y = -11.48X +79.57, R^2=0.8822, *P*<0.0001).

to 2-week feed deprivation. Similarly, critical period to recover growth from fasting was reported to be up to 2-week in warmwater fish (Qian et al., 2000; Zhu et al., 2001).

Hyperphagia is one of the primary mechanisms leading to compensatory growth of fish after refeeding. Feed consumption of fish in S1 and S2 was comparable to that of fish in C due to hyperphagia of fish in S1 and S2, reported in the earlier study (Cho et al., 2006). However, improvement in weight gain of fish achieved by hyperphagia did not lead to improvement in FER because weight gain of fish improved with a proportion to feed consumption (Wang et al., 2000; Tian and Qin, 2003; Zhu et al., 2004).

Sheridan and Mommsen (1991) showed that plasma glucose level of coho salmon *Oncorhynchus kisutch* fasted for 1 week increased, but glucose level of fish fasted for 3 weeks was similar to that of fish fed for 2 weeks after 1-week fasting or that of fish without fasting, and concluded that plasma glucose level maintained in fish during fasting due to fasting-induced hyperlipidemia mediated by lipolytic enzyme activity. Similar result that glucose level of 1-week fasted olive flounder increased from 454 (an initial fish) to 532 mg/dL and then maintained similar level for 2-, 3- and 4-week fasted fish, but decreased in fish refed for the rest periods of feeding trial compared to that of fish before refed in each treatment (Table 2). Although blood chemistry of olive flounder was not affected by feeding regime at the end of the feeding trial in this study, significant difference in total protein, TG, T_3 and T_4 of fish between before and after refed for the rest periods of the feeding trial. The linear decrease in plasma total protein (Fig. 1) and T_3 (Fig. 2) with week of fasting in this study indicated that these criteria could be the good indexes of olive flounder subjected to the severity of fasting.

Inhibition of thyroid function appeared to be one of the most consistent endocrine responses to feed deprivation. Feed deprivation resulted to decrease in growth and circulating levels of the T_4 and T_3 (Eales, 1988). Significant difference in T_3 and T_4 levels of olive flounder between before and after refed for the rest periods of the feeding trial, but no difference in T_3 and T_4 levels of fish among treatment at the end of the feeding indicated that T_3 and/or T_4 could partially play the important role to achieve compensatory growth of fish in this experimental conditions. Gaylord et al. (2001) reported that more rapid recovery of thyroid hormone production following realimentation minimized the effects of feed deprivation on growth of fish subjected to short feed deprivation compared to longer periods. Feed restriction also affected on thyroid function. Circulating T_3 level correlated with weight gain of fish at lower feeding ratio levels, actually reaching maximum levels at a feeding ratio below that yielding maximal weight gain, in contrast, T_4 level did not differ among fish at different feeding ratios (Eales and Shostak, 1985).

In considering these results, it could be concluded that growth of juvenile olive flounder fed for 6 weeks after 2-week fasting was able to catch up that of fish fed for 8 weeks and plasma total protein, glucose, TG, T_3 and T_4 levels were different between fish before and after refed for the rest periods of the feeding trial. The linear relationships between plasma total protein and T_3 levels and fasting could be the good indexes of fish subjected to the severity of fasting.

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