Thesis for the Degree of Master of Science

Effects of different feeding regimes and dietary nutrient composition on compensatory growth of olive flounder *Paralichthys olivaceus*

Chung Il Kim

Department of Marine Bioscience and Environment

The Graduate School

Korea Maritime University

2009

Effects of different feeding regimes and dietary nutrient composition on compensatory growth of olive flounder *Paralichthys olivaceus*

Advisor: Prof. Sung Hwoan Cho

by

Chung Il Kim

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science

In the Department of Marine Bioscience and Environment,
the Graduate School of Korea Maritime University

February 2009

Effects of different feeding regimes and dietary nutrient composition on compensatory growth of olive flounder *Paralichthys olivaceus*

A dissertation

by

Chung Il Kim

Certified by

Sung Hwoan Cho

Thesis Advisor

Accepted by

Cheol Young Choi

Vice Chairman

In-Seok Park

Member of Committee

Sung Hwoan Cho

Member of Committee

December 2008

Contents

Page

List of tables

List of figures

Experiment 2

Effects of different feeding regimes and dietary nutrient composition on compensatory growth of olive flounder *Paralichthys olivaceus*

Chung Il Kim

Department of Marine Bioscience and Environment, Graduate School, Korea Maritime University

Korean abstract

본 연구에서는 다양한 사료공급전략 및 사료내 영양소 함량 차이에 따른 넙치(*Paralichthys olivaceus*) 유어기의 보상성장 가능성을 조사하였다.

실험 1에서는, 부상용 배합사료의 공급시 사료공급전략을 달리한 7 종류의 실험구를 설정하였으며, 각 실험구는 3반복구를 두었다[①8주간 매일 사료 공급구, 대조구(C; 8WF), ②1주 절식이후 3주간 사료 공급의 2회 반복하는 실험구(1WS+3WF)×2, ③2주 절식이후 6주간 사료 공급하는 실험구(2WS+6WF), ④5일 절식이후 9일 사료 공급의 4회 반복하는 실험구(5DS+9DF)×4, ⑤10일 절식이후 18일 사료 공급의 2회 반복하는 실험구(10DS+18DF)×2, ⑥2일 절식, 5일 사료 공급, 3일 절식 및 4일 사료 공급의 4회 반복하는 실험구(2DS+5DF+3DS+4DF)×4 ⑦4일 절식, 10일 사료 공급, 6일 절식 및 8일 사료 공급의 2회 반복하는 실험구(4DS+10DF+6DS+8DF)×2]. 8주간의 사육실험 종료시, 넙치의 체중증가량은 대조구가 다른 모든 실험구보다 유의적으로 높게 나타났으며, 2WS+6WF 실험구의 체중증가량은 (5DS+9DF)×4 및 (4DS+10DF+6DS+8DF)×2 실험구의 체중증가량보다 유의적으로 높게 나타났다. 사료소비량은 대조구가 절식을 갖

는 다른 모든 실험구보다 유의적으로 높게 나타났다. 그러나 사료전환효율(FER), 단백질전환효율(PER) 및 단백질축적율(PR)에서는 모든 실험구간에 유의적인 차이를 보이지 않았다. 간에서의 수분 함량을 제외하고는 넙치 전어체 및 간의 일반성분분석 결과 모든 실험구간에 유의적인 차이를 보이지 않았으며, T_3 (triiodothyronine) 함량에서는 대조구와 2WS+6WF 실험구가 (5DS+9DF)×4 실험구보다 유의적으로 높게 나타났다.

실험 2에서는, 실험1의 결과에 근거하여 넙치의 2주간 절식이후 6주간 사료 공급시 사료내 영양소 함량에 따른 보상성장의 가능성을 조사하였다. 5종류의 실험사료를 준비하였다[일반사료(C), 고단백질(HP), 고탄수화물(HC), 고지질(HL) 및 중간단백질탄수화물지질(IPCL) 사료]. 일반사료를 공급하는 실험구(C-8W)는 넙치 유어에게 8주간 1일2회 손으로 만복시까지 사료를 공급하였다. 다른 실험구들은 실험시작 후 처음 2주간 절식시켰으며, 그 다음 6주간 1주일에 6일간 C, HP, HC, HL 및 IPCL 사료를 1일 2회 손으로 만복시까지 사료를 공급하였으며(C-6W, HP-6W, HC-6W, HL-6W 및 IPCL-6W 실험구), 각 실험구는 3반복구를 두었다. 실험종료 이후 HP-6W 및 IPCL-6W 실험구에서 넙치 유어의 성장은 우수한 것으로 나타났다. 사육실험시작 이후 6주간, 사료섭이율은 2주 절식한 모든 실험구에서 8주간 매일 사료를 공급한 C-8W 실험구보다 유의적으로 높게 나타났다. HC-6W, HL-6W, HP-6W 및 IPCL-6W 실험구에서의 사료전환효율 및 단백질전환효율은 C-8W 및 C-6W 실험구에서의 사료전환효율 및 단백질전환효율보다 높게 나타났다.

이상의 결과를 고려하여, 넙치 유어기에 있어서 부상용 배합사료의 공급시 본 연구에서 이용된 다양한 사료공급 전략 중에서 2주 절식이후 6주간 사료를 공급하는 사료공급방법이 넙치의 우수한 보상성장 효과를 가져왔으며, 사료내 단백질, 탄수화물 및 지질원의 보충은 2주 절식 이후 6주간 사료 재공급시 넙치의 보상성장을 효율적으로 향상시켰으며, 특히 사료내 단백질 보충은 넙치의 보상성장을 극대화시킬 수 있는 것으로 판단된다.

I. General introduction

Olive flounder (*Paralichthys olivaceus*) belongs to Order Pleuronectiformes and Family Paralichthyidae. It is a carnivorous fish species inhabiting in the bottom of sea water and depth range of about 20 m and distributing into Japan, Sakhalin, Kuril island and Korean Peninsula in Western Pacific.

Since the end of the 1970's, the technique for artificial seed production of olive flounder has been developed because of its fast growth and high tolerance to environmental stress. An annual aquaculture production and value of olive flounder was estimated to be 2,437 MT and 26 billion Won, which occupied 49.4% and 58.6% of total annual marine finfish aquaculture production (4,933 MT) and value (51.4 billion Won) of Korea in 2007, respectively (MIFAFF 2008), so it is one of the most commercially important marine finfish in Korea. Therefore, many studies on the effective production of olive flounder have been conducted; dietary nutrient requirements (Lee et al. 2000a, 2000b; Kim et al. 2002; Lee et al. 2002), alternative protein sources for fishmeal in the diet (Sato and Kikuchi 1997; Kikuchi 1999), optimum feeding frequency (Lee et al. 2000b), and the feeding strategy (Cho and Lee 2002; Cho 2005b; Cho et al. 2006).

The optimum periods for growth of olive flounder were between the late spring and early autumn in Korea, however, red tide and cold-water mass frequently occurred in this period as well. Olive flounder are commonly starved to minimize mass mortality at fish farms during the occurrence of these unsuitable environment conditions and it eventually resulted to the economical loss of fish farmers due to a decrease in fish production. Since feed cost and discharged water pollution source from fish farms were directly related to use of the diet, not only the nutritional and physiological status of fish, but also the environmental conditions directly affecting fish performance must be carefully considered at fish farms before feed supply to fish.

Compensatory growth of fish, which is rapid or faster than normal growth rate of fish resulting from refeeding after undernutrition could be an applicably effective fish culture technique when fish must be starved for a certain period of time. Compensatory growth of fish was largely influenced by the several factors, such as fish size or age (Bilton and Robins 1973), feeding protocol (Jobling and Koskela 1996; Rueda et al. 1998; Gaylord and Gatlin 2000), nutrient levels in the diet (Gaylord and Gatlin 2001), feeding regimes (Wang et al. 2000; Cho and Lee 2002; Tian and Qin 2003, 2004; Cho 2005a, 2005b; Cho and Jo 2005; Cho et al. 2006; Oh et al. 2007) and so on. Also compensatory growth has been observed in the most of cold-, warm-, and tropical-water finfish species (Bilton and Robins 1973; Quinton and Blake 1990; Damsgaard and Dill 1998; Rueda et al. 1998; Gaylord and Gatlin 2000, 2001; Wang et al. 2000; Tian and Qin 2003, 2004; Cho 2005a; Cho and Jo 2005).

Olive flounder with 2-week feed deprivation achieved full compensatory growth when fish were fed by the commercially available extruded pellet in winter season and the experiment diet during summer season, respectively in the 8-week feeding trials (Cho 2005b; Cho et al. 2006).

The lipid in fish body was primarily utilized for energy source for maintenance of basal metabolism and survival while fasting (Rueda et al. 1998; Gaylord and Gatlin 2000). Gaylord and Gatlin (2001) reported that the dietary protein and energy supplementation achieved efficient compensatory growth of channel catfish (*Ictarulus puntatus*) refed after experienced in short-term (3-day) feed deprivation. Thus, manipulation of dietary nutrient composition can largely affect fish performance.

In this study, therefore, effects of different feeding regimes and dietary nutrient composition on compensatory growth of juvenile olive flounder were investigated.

Ⅱ. Experiment 1

Compensatory growth of olive flounder, *Paralichthys olivaceus*, fed the extruded pellet (EP) with different feeding regimes

Abstract

This study was performed to determine compensatory growth of juvenile olive flounder fed the extruded pellet (EP) with different feeding regimes. Seven treatments with triplicates of different feeding regimes were prepared; ① fish was daily fed for 6 days a week throughout 8 weeks; 8WF ② fish was starved for 1 week and then fed for 3 weeks twice; (1WS+3WF)×2 ③ fish was starved for 2 weeks and then fed for 6 weeks; 2WS+6WF ④ fish was starved for 5 days and then fed for 9 days four times; (5DS+9DF)×4 ⑤ fish was starved for 10 days and then fed for 18 days twice; (10DS+18DF)×2 ⑥ fish was starved for 2 days, fed for 5 days, starved for 3 days and then fed 4 days four times; (2DS+5DF+3DS+4DF)×4 and ⑦ fish was starved for 4 days, fed for 10 days, starved for 6 days, and then fed for 8 days twice; (4DS+10DF+6DS+8DF)×2, respectively. Total feeding day was all same, 36 days except for control group (48 days). Weight gain of flounder in the 8WF treatment was higher than that of fish in other treatments. And weight gain of flounder in the 2WS+6WF treatment was higher than that of fish in the (5DS+9DF)×4 and (4DS+10DF+6DS+8DF)×2 treatments. Feed consumption of flounder in the 8WF treatment was higher than that of fish experienced feed deprivation. Feed efficiency ration, protein efficiency ratio and protein retention were not significantly different among treatments. Chemical composition of the whole-body of fish without liver and liver, except for moisture content of liver, was not different among treatments. T_3 level of fish in the 8WF and 2WS+6WF treatments was higher than that of fish in the (5DS+9DF)×4 treatment. It can be concluded that juvenile olive flounder achieved better

compensatory growth at 6-week refeeding after 2-week feed deprivation compared to that of fish with different feeding regimes. And T_3 of fish seemed to partially play role in achieving compensatory growth.

1.1. Introduction

Olive flounder, *Paralichthys olivaceus* is a finfish species in Order Pleuronectiformes and Family Paralichthyidae. Since the end of the 1970's, the technique for artificial seed production of flounder has been developed because of its fast growth and high tolerance to environmental stress. An annual aquaculture production and value of olive flounder was estimated to be 2,437 MT and 26 billion Won, which occupied 49.4% and 58.6% of total annual marine finfish aquaculture production (4,933 MT) and value (51.4 billion Won) of Korea in 2007, respectively (MIFAFF 2008). Olive flounder is one of the most commercially important marine finfish in Eastern Asia including Korea, Japan and China. Therefore, many studies on the effective production of flounder have been conducted; dietary nutrient requirements (Lee et al. 2000a, 2000b; Kim et al. 2002; Lee et al. 2002), alternative protein sources for fishmeal in the diet (Sato and Kikuchi 1997; Kikuchi 1999), optimum feeding frequency (Lee et al. 2000b), and the feeding strategy (Cho and Lee 2002; Cho 2005b; Cho et al. 2006).

The optimum periods for growth of olive flounder were between the late spring and early autumn in Korea, however, red tide and cold-water mass frequently occurred in this period as well. Flounder are commonly starved to minimize mass mortality at fish farms during the occurrence of these unsuitable environment conditions and it eventually resulted to the economical loss of fish farmers due to a decrease in fish production. Since feed cost and discharged water pollution source from fish farms were directly related to use of the diet, not only the nutritional and physiological status of fish, but also the environmental conditions directly affecting fish performance must be carefully considered at fish farms before feed supply to fish.

Compensatory growth of fish, which is rapid or faster than normal growth rate of fish resulting from refeeding after undernutrition could be an applicably effective fish culture technique in the occurrence of red tide and coldwater mass (Cho et al. 2006).

Compensatory growth of fish was largely influenced by the several factors, such as fish size or age (Bilton and Robins 1973), feeding protocol (Jobling and Koskela 1996; Rueda et al. 1998; Gaylord and Gatlin 2000), nutrient levels in the diet (Gaylord and Gatlin 2001), feeding regimes (Wang et al. 2000; Cho and Lee 2002; Tian and Qin 2003, 2004; Cho 2005a, 2005b; Cho and Jo 2005; Cho et al. 2006; Oh et al. 2007) and so on. Also compensatory growth has been observed in the most of cold-, warm-, and tropical-water finfish species (Bilton and Robins 1973; Quinton and Blake 1990; Damsgaard and Dill 1998; Rueda et al. 1998; Gaylord and Gatlin 2000, 2001; Wang et al. 2000; Tian and Qin 2003, 2004; Cho 2005a; Cho and Jo 2005).

The use of extruded pellet (EP) is highly recommended in the most of fish farming because of easy observation of feeding activity, easy management and minimal water pollution. And a short-term feed deprivation of fish for grading, transporting and medicating frequently occurred year-around at flounder farming.

Olive flounder with 2-week feed deprivation achieved full compensatory growth when fish were fed by the commercially available extruded pellet in winter season and the experiment diet during summer season, respectively in the 8-week feeding trials (Cho 2005b; Cho et al. 2006).

In this study, therefore, effect of different feeding regimes on compensatory growth of juvenile olive flounder were investigated.

1.2. Materials and methods

1.2.1. Fish and experimental conditions

Juvenile olive flounder were purchased from a private hatchery (Kyungbook, Korea) and acclimated to the experimental conditions for 2 weeks. During the acclimation period, fish were fed the commercial EP for flounder twice a day. Forty flounder averaging 12.6 g were randomly chosen and distributed to 21 of 180 L flow-through tanks (water volume, 150 L) each. Water temperature ranged from 14.8 to 24 ℃ (Mean±SD: 18.6±2.89 ℃) and photoperiod followed natural conditions. The flow rate of water into each tank was 9.7 L/min and aeration was supplied to each tank.

1.2.2. Design of the experiment

Seven treatments with triplicate of different feeding regimes were prepared: ① fish was daily fed for 6 days a week during 8 weeks; 8WF, which was used as a control group, ② fish was starved for 1 week and then fed for 6 days a week during 3 weeks twice; (1WS+3WF)×2, ③ fish was starved for 2 weeks and then fed for 6 days a week during 6 weeks; 2WS+6WF, ④ fish was starved for 5 days and then fed for 9 days four times; (5DS+9DF)×4, ⑤ fish was starved for 10 days and then fed for 18 days twice; (10DS+18DF)×2, ⑥ fish was starved for 2 days, fed for 5 days, starved for 3 days and then fed 4 days four times; (2DS+5DF+3DS+4DF)×4, and ⑦ fish was starved for 4 days, fed for 10 days, starved for 6 days, and then fed for 8 days twice; (4DS+10DF+6DS+8DF)×2, respectively. Thus, total feeding day was all same, 36 days except for the control group (48 days). The EP (Suhyup feed; 52.0% crude protein and 11.0% crude lipid) was hand-fed to satiation twice (09:00 and 16:30) a day as feeding schedules. Remained EPs in each tank were collected in 30 minutes after feeding, and deducted from total feed consumption. The experiment lasted for 8 weeks.

1.2.3. Analysis of proximate composition and blood chemistry of fish

Five fish from each tank at the end of the experiment were sampled for the chemical analysis. Analysis of proximate composition of the whole-body of fish without liver and liver were conducted based on AOAC (1990). Crude protein content was determined using the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland), lipid content determined using ether-extraction method, moisture content determined by drying sample in a dry oven at 105 ℃ for 24 h, fiber content determined using automatic analyzer (Fibertec, Tecator, Sweden) and ash content determined using muffle furnace at 550 ℃ for 4 h.

Blood samples were obtained from the caudal vein of randomly chosen 3 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. Plasma was collected after centrifugation (3,000 rpm for 10 min), stored freezer at -70 ℃ as separate aliquots for analysis of total protein, glucose, cholesterol, triglycerides (TG), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), enzymatically analyzed by using automatic chemistry system (HITACHI 7180 and 7600-210, Hitachi, Japan) based on the manual of Daiichi Pure Chemicals Co. Ltd. (2005). In addition, total plasma T_3 (triiodothyronine) and T_4 (thyroxine) hormones of fish at the end of feeding trial and starved fish every week throughout the feeding trial were analyzed by radio-immunoassay (Gamma Counter, Cobra II, Packard, USA) using Coat-A-Count kit (DPC, Los Angeles, CA, USA).

1.2.4. Statistical analysis

One-way ANOVA and Duncan`s multiple range test (Duncan 1955) was applied to detect the differences among treatments by using SAS Version 9.1 (SAS Institute, Cary, NC, USA).

1.3. Results and Discussion

Survival (%), weight gain (g/fish) and specific growth rate (SGR) of olive flounder fed the EP with different feeding regimes are given in Table 1. Survival ranging from 98.3 to 100% was not significantly ($P > 0.05$) different among treatments. This was similar to results of studies for compensatory growth of gibel carp (Qian et al. 2000), hybrid tilapia (Wang et al. 2000) and juvenile olive flounder (Cho and Lee 2002; Cho 2005b; Cho et al. 2006) following fasting and re-feeding for 8 weeks. However, weight gain of flounder in the control group (8WF), in which fish were fed for 48 days during 8 weeks without starvation was significantly ($P < 0.05$) higher than that of fish in all other treatments, in which fish were fed for 36 days with successive starvation and feeding regimes during 8 weeks in this study. Similar trend that weight gain of Atlantic halibut, *Hippoglossus hippoglossus*, fed daily for 99 days (control) was higher than that of fish fed for 63~67 days with different starvation and feeding regimes (Heide et al. 2006).

Difference in partial compensatory growth of flounder in the 2WS+6WF treatment in this study and full compensatory growth of fish in the 2WS+6WF treatment in the previous studies (Cho 2005b; Cho et al. 2006) could be resulted from difference in fish size in those studies; small fish (12.5 g) in this study vs large fish 16 g (Cho et al. 2006) and 54 g (Cho 2005b). Smaller size fish is more susceptible to feed deprivation than larger fish, so smaller flounder did not compensate fully in the same feeding regime in this study. The possibility of full compensatory growth of fish varies depending on fish species, fish size, feeding protocol, water temperature, feed nutrients and duration of feeding trial, etc. (Bilton and Robins 1973; Jobling and Koskela 1996; Rueda et al. 1998; Gaylord and Gatlin 2000, 2001; Wang et al. 2000; Tian and Qin 2004; Cho 2005b; Cho and Jo 2005; Cho et al. 2006). However, weight gain of flounder in the 2WS+6WF treatment was significantly ($P < 0.05$) higher than that of fish in the (5DS+9DF)×4 and (4DS+10DF+6DS+8DF)×2 treatments, but not significantly ($P > 0.05$) different from that of fish in the (1WS+3WF)×2, (10DS+18DF)×2 and (2DS+5DF+3DS+4DF)×4 treatments.

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

Treatments	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[2]
C (8WF)	12.5±0.02	40.0±1.70	98.3±1.67	27.5±1.71[a]	2.4±0.09[abc]
(1WS+3WF)×2	12.5±0.07	32.2±0.74	99.2±0.83	19.7±0.79[bc]	2.6±0.08[ab]
(2WS+6WF)	12.7±0.10	34.1±0.44	99.2±0.83	21.4±0.53[b]	2.7±0.06[a]
(5DS+9DF)×4	12.6±0.01	29.1±1.87	100±0.00	16.5±1.87[cd]	2.3±0.18[bc]
(10DS+18DF)×2	12.6±0.05	31.2±1.16	99.2±0.83	18.7±1.14[bcd]	2.5±0.10[abc]
(2DS+5DF+3DS+4DF)×4	12.5±0.01	32.1±2.15	99.2±0.83	19.6±2.15[bc]	2.6±0.18[ab]
(4DS+10DF+6DS+8DF)×2	12.5±0.05	27.6±0.39	100±0.00	15.1±0.40[d]	2.2±0.04[c]

[1]Values (Mean±SE, n=3) in the same column sharing a same letter are not significantly different ($P < 0.05$).

[2]SGR = (Ln final weight of fish-Ln initial weight of fish) × 100/days of feeding.

Similar result that one initial longer period of starvation achieved clear compensatory growth over 2-3 shorter intermediary periods was reported in Atlantic halibut (Heide et al. 2006). Also, SGR of flounder in the 2WS+6WF treatment was significantly ($P < 0.05$) higher than that of fish in the (5DS+9DF)×4 and (4DS+10DF+6DS+8DF)×2 treatments, but not significantly ($P > 0.05$) different from that of fish in other treatments. These results probably indicated that one initial long period of feed deprivation (2-week) is preferred to achieve better compensatory growth of flounder over the successive short period of feed deprivation for aquaculture purposes.

Although flounder accept EP well, its application is very limited in founder farming in reality due to poor growth of fish fed EP long probably resulted from its swollen digestive tract and mistrust of flounder farmers on EP, which is still controversial. Besides, compensatory growth of flounder fed the dry pellet instead of EP with same feeding strategy in this study came out with different result from this study and will be reported in another study.

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 fed the EP with different feeding regimes are shown in Table 2. Feed consumption of flounder in the 8WF treatment was significantly ($P < 0.05$) higher than that of any fish experienced feed deprivation in other treatments, probably resulting to poorer growth of fish in the latter than in the former. Weight gain of flounder seemed to be proportionally affected by feed consumption of fish in this study. Hyperphagia is one of the primary mechanisms leading to compensatory growth of fish after feed deprivation and was commonly accompanied especially when fish achieved full compensatory growth (Rueda et al. 1998; Gaylord and Gatlin 2000; Wang et al. 2000; Xie et al. 2001; Tian and Qin 2003; Cho 2005b; Cho et al. 2006; Oh et al. 2007). Also, feed consumption of flounder in the 2WS+6WF treatment was significantly ($P < 0.05$) higher than that of fish in the (4DS+10DF+6DS+8DF)×2 treatment, but not significantly ($P > 0.05$) different from that of fish in other treatments. The lowest feed consumption of flounder in the (4DS+10DF+6DS+8DF)×2 treatment resulted to the poorest weight gain of fish in this study. FER ranging from 1.11 to 1.26, PER ranging from 1.98 to 2.25 and PR ranging from 34.2 to 39.8 of flounder were not significantly ($P > 0.05$) different among treatments. Similarly, FER and PER of Atlantic halibut experienced feed deprivation was not affected by feeding regimes (Heide et al. 2006). Although fish achieved compensatory growth, improvement in feed efficiency was not observed (Wang et al. 2000; Tian and Qin 2003). HSI of flounder in the (5DS+9DF)×4 and (4DS+10DF+6DS+8DF)×2 treatments, in which fish achieved poor weight gain was significantly ($P < 0.05$) higher than that of fish in the 8WF, (1WS+3WF)×2, 2WS+6WF, and (2DS+5DF+3DS+4DF)×4 treatments, but not significantly ($P > 0.05$) different from that of fish in the (10DS+18DF)×2 treatment. Similarly, HSI of flounder decreased in proportion to weight gain of fish when fish were refed after feed deprivation (Cho 2005b). However, CF of flounder in the (10DS+18DF)×2 treatment was significantly ($P < 0.05$) higher than that of fish in the (1WS+3WF)×2 and (2DS+5DF+3DS+4DF)×4 treatments, but not significantly ($P > 0.05$) different from that of fish in other treatments in this study. However, unlike this study, HSI and CF of fish were the good indices to indicate the possibility of compensatory growth of fish and decreased rapidly with feed deprivation period and increased rapidly with subsequent refeeding (Gaylord and Gatlin 2000; Cho et al. 2006).

Table 2. 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 in the experiment 1[1]

Treatments	Feed consumption	FER[2]	PER[3]	PR[4]	CF[5]	HSI[6]
C (8WF)	21.8±1.59[a]	1.26±0.031	2.25±0.056	39.8±0.90	1.03±0.014[abc]	1.82±0.111[bc]
(1WS+3WF)×2	16.6±0.37[bc]	1.19±0.041	2.12±0.073	37.1±1.61	1.01±0.009[bc]	1.68±0.063[cd]
(2WS+6WF)	17.5±0.96[b]	1.23±0.037	2.19±0.065	38.1±1.56	1.05±0.017[ab]	1.60±0.028[d]
(5DS+9DF)×4	14.7±1.45[bc]	1.12±0.016	1.99±0.028	34.6±0.89	1.04±0.015[ab]	2.15±0.041[a]
(10DS+18DF)×2	15.6±0.40[bc]	1.20±0.048	2.13±0.085	37.3±0.92	1.06±0.001[a]	1.99±0.042[ab]
(2DS+5DF+3DS+4DF)×4	15.8±0.81[bc]	1.24±0.075	2.21±0.133	38.6±3.20	0.99±0.024[c]	1.71±0.051[cd]
(4DS+10DF+6DS+8DF)×2	13.6±0.52[c]	1.11±0.033	1.98±0.059	34.2±0.96	1.02±0.012[abc]	2.04±0.079[a]

[1]Values (Mean±SE, n=3) in the same column sharing a same 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.

Proximate composition of the whole-body of fish without liver and liver, except for moisture content of liver, was not significantly (P > 0.05) different among treatments (Table 3). Moisture content of liver in fish in the 2WS+6WF and (2DS+5DF+3DS+4DF)×4 treatments was significantly (P < 0.05) higher than that of fish in the 8WF, (5DS+9DF)×4, (10DS+18DF)×2 and (4DS+10DF+6DS+8DF)×2 treatments. Similarly, chemical composition of fish was not affected by feeding regimes with short- and long-term starvation and refeeding (Cho and Lee 2002; Cho 2005b; Cho et al. 2006; Heide et al. 2006).

Table 3. Proximate composition (% of wet weight) of olive flounder at the end of the feeding trial in the experiment 1[1]

Treatments	Whole-body of fish without liver			
	Moisture	Crude protein	Crude lipid	Ash
C (8WF)	74.0±0.32	17.1±0.13	3.3±0.28	3.6±0.20
(1WS+3WF)×2	74.9±0.51	16.8±0.18	3.1±0.21	3.4±0.18
(2WS+6WF)	74.7±0.41	16.8±0.33	3.1±0.09	3.5±0.22
(5DS+9DF)×4	75.1±0.43	16.6±0.19	2.9±0.17	3.7±0.14
(10DS+18DF)×2	74.3±0.29	16.8±0.27	3.2±0.10	3.6±0.20
(2DS+5DF+3DS+4DF)×4	74.9±0.28	16.8±0.45	2.8±0.31	3.6±0.17
(4DS+10DF+6DS+8DF)×2	75.6±0.48	16.6±0.26	2.5±0.27	3.4±0.35

	Liver		
	Moisture	Crude protein	Crude lipid
C (8WF)	68.9±0.51^{b}	10.0±0.95	10.4±1.38
(1WS+3WF)×2	70.3±0.58^{ab}	10.0±0.89	10.5±1.24
(2WS+6WF)	71.5±0.55^{a}	10.0±1.13	10.9±1.99
(5DS+9DF)×4	67.9±0.55^{b}	9.5±0.15	12.4±0.99
(10DS+18DF)×2	68.7±1.02^{b}	9.9±0.31	11.9±1.58
(2DS+5DF+3DS+4DF)×4	72.3±1.39^{a}	10.2±0.39	12.0±0.53
(4DS+10DF+6DS+8DF)×2	68.7±0.10^{b}	9.0±0.85	11.9±1.53

[1]Values (Mean±SE) for either whole-body or liver are not significantly different for any treatment ($P < 0.05$).

Blood chemistry of olive flounder at the end of the 8-week feeding trial is presented in Table 4. Plasma protein, glucose, TG, GOT, GPT and T_4 levels of flounder were not significantly ($P > 0.05$) different among treatments. However, cholesterol level of flounder in the 8WF and (10DS+18DF)×2 treatments was significantly ($P < 0.05$) higher than that of fish in the (5DS+9DF)×4, (2DS+5DF+3DS+4DF)×4 and (4DS+10DF+6DS+8DF)×2 treatments, but not significantly ($P > 0.05$) different from that of fish in the (1WS+3WF)×2 and 2WS+6WF treatments. T_3 level of flounder in the 8WF and 2WS+6WF treatments was significantly ($P < 0.05$) higher than that of fish in the (5DS+9DF)×4 treatment, which was the lowest. A trend toward an increased T_3 level of fish in the proportion to weight gain of fish which was the highest T_3 level in the 8WF treatment, followed by fish in the 2WF+6WF treatment and relatively low for fish in the (4DS+10DF+6DS+8DF)×2 treatment in this study probably indicated that T_3 of fish played role in achieving compensatory growth of fish after feed deprivation and partially agreed with Van der Geyten et al. (1998)'s study. Eales (1988) explained that inhibition of thyroid function appeared to be one of the most consistent endocrine responses to feed deprivation, and feed deprivation resulted to the decrease in growth and circulating levels of the T_3 and T_4. Similarly, feed restriction affected on thyroid function. Circulating T_3 level correlated with weight gain of red drum, *Sciaenops ocellatus*, 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 (Mackenzie et al. 1993). However, in this study, it was difficult to explain why the lowest T_3 level was observed in flounder in the (5DS+9DF)×4 treatment rather than the (4DS+10DF+6DS+8DF)×2 treatment, in which fish achieved the lowest weight gain with

Table 4. Plasma chemistry of olive flounder at the end of the 8-week feeding trial in the experiment 1[1]

Treatments	Plasma chemistry							
	Protein (g/dL)	Glucose (mg/dL)	TG[2] (mg/dL)	GOT[3] (IU/L)	GPT[4] (IU/L)	Cholesterol (mg/dL)	T_3 (ng/ml)	T_4 (ng/ml)
C (8WF)	3.4±0.08	17.0±4.32	97.2±11.94	11.0±1.85	5.6±0.58	192.2±13.09[a]	4.9±0.30[a]	69.1±19.23
(1WS+3WF)×2	3.5±0.08	13.6±0.67	114.9±7.38	13.0±1.59	4.0±0.17	183.2±9.37[ab]	4.5±0.26[ab]	65.0±3.24
(2WS+6WF)	3.6±0.11	16.3±1.45	127.2±21.07	18.8±0.94	6.5±0.33	181.5±8.19[ab]	4.7±0.07[a]	62.9±9.24
(5DS+9DF)×4	3.5±0.09	17.0±5.82	108.1±12.96	16.5±3.89	4.5±1.39	154.3±4.39[c]	3.7±0.12[b]	70.8±17.50
(10DS+18DF)×2	3.5±0.20	18.0±3.44	119.8±39.58	17.7±3.20	6.0±0.91	207.9±2.26[a]	4.1±0.17[ab]	58.3±8.36
(2DS+5DF+3DS+4DF)×4	3.2±0.01	16.2±3.69	96.7±16.00	16.7±3.10	3.6±0.15	152.2±8.38[c]	4.1±0.46[ab]	98.4±21.01
(4DS+10DF+6DS+8DF)×2	3.4±0.10	15.5±5.75	106.8±29.18	13.7±2.74	4.6±0.69	161.9±7.78[bc]	4.0±0.38[ab]	93.0±18.23

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

[2]TG: Triglycerides.

[3]GOT: Glutamic oxaloacetic transaminase.

[4]GPT: Glutamic pyruvic transaminase.

the least feed consumption. Gaylord et al. (2001) reported that plasma thyroid hormone following realimentation minimized the effects of feed deprivation on growth and feed efficiency of channel catfish subjected to the 3-day feed deprivation treatment when compared to longer periods (5- and 7-day feed deprivation).

In considering these results, it can be concluded that juvenile olive flounder achieved better compensatory growth at 6-week refeeding after 2-week feed deprivation compared to that of fish with different feeding regimes. And T_3 of fish seemed to partially play role in achieving compensatory growth when fish were refed after feed deprivation.

Ⅲ. Experiment 2

Effect of dietary nutrient composition on compensatory growth of juvenile olive flounder *Paralichthys olivaceus* during summer season

Abstract

Effect of dietary nutrient composition on compensatory growth of olive flounder was investigated. Twenty-five fish per tank were randomly distributed into 18 flow-through tanks. Five experimental diets were prepared: control (C), high protein (HP), high carbohydrate (HC), high lipid (HL), and intermediate protein, carbohydrate and lipid (IPCL) diets. Fish were hand-fed to satiation twice daily by the C diet for 6 days a week during 8 week (C-8W). Other five groups of fish were starved for 2 weeks and then fed to satiation twice a day by the C, HP, HC, HL and IPCL diets during 6 weeks, referred to as C-6W, HP-6W, HC-6W, HL-6W, and IPCL-6W treatments, respectively. An accelerated growth rate of fish in HP-6W and IPCL-6W treatments was achieved. Feeding rate of fish in all treatments with 2-week fasting was higher than that of fish in C-8W treatment in 6 week after the initiation of the feeding trial. Feed and protein efficiency ratios of fish in HC-, HL-, HP- and IPCL-6W treatments were higher than those of fish in C-8W and C-6W treatments. Dietary supplementation of protein, carbohydrate and lipid sources could improve compensatory growth of fish refed for 6 weeks after 2-week fasting especially, dietary protein supplementation can maximize it.

2.1. Introduction

When red tide or coldwater mass frequently occurs during summer season, in which most of marine fish for aquaculture grow relatively fast, in Eastern Asia, such as Korea, Japan and China, economical loss due to not only high mortality of fish, but also reduction in fish growth during feed deprivation to minimize mortality highly loads many fish farms. Since occurrence of red tide or coldwater mass is hardly controllable environmental condition at fish farm to date, development of a new fish culture technique to improve growth of fish after the environmental condition becomes normal or better is highly needed.

Application of feeding strategy leading to compensatory growth of fish could be one. It is an effective fish culture technique in fish farming to improve feeding activity of fish after refeeding, elevate growth of fish and efficiency of feed, and eventually increase fish production. Since a naturally available food for fish is limited, most of fish are highly endurable against short period of fasting and compensatory growth of fish commonly occurs in nature. Many feeding trials on compensatory growth of fish have been performed and reported that possible duration of feed deprivation to achieve full compensatory growth of fish ranged 1-3 weeks although it varied depending on fish species, fish size (age), water temperature, feed allowance, feeding ratio, dietary nutrient composition, duration of feeding trial, etc (Bilton and Robins 1973; Jobling and Koskela 1996; Rueda et al. 1998; Saether and Jobling 1999; Gaylord and Gatlin 2000; Wang et al. 2000; Zhu et al. 2001; Cho 2005a, 2005b; Cho et al. 2006).

The lipid in fish body was primarily utilized for energy source for maintenance of basal metabolism and survival while fasting (Rueda et al. 1998; Gaylord and Gatlin 2000). Gaylord and Gatlin (2001) reported that the dietary protein and energy supplementation achieved efficient compensatory growth of channel catfish (*Ictarulus puntatus*) refed after experienced in short-term (3-day) feed deprivation. Thus manipulation of dietary nutrient composition can largely affect fish performance.

Olive flounder *Paralichthys olivaceus* is one of the most commercially important marine fish species for aquaculture in Korea. Juvenile olive flounder with 2-week feed deprivation achieved full compensatory growth when fish were fed by the commercially available extruded pellet in winter season and the experiment diet during summer season, respectively in the 8-week feeding trials (Cho 2005b; Cho et al. 2006).

In this study, therefore, effect of dietary nutrient composition on compensatory growth of juvenile olive flounder was investigated when fish were refed for 6 weeks after 2-week feed deprivation during summer season.

2.2. Materials and methods

2.2.1. Fish and the experimental conditions

Juvenile olive flounder were purchased from a private hatchery (Tongyoung City, Korea) and transferred into the Lab. Before the initiation of the feeding trial, fish were acclimated to the experimental conditions for 2 weeks. During the acclimation period, fish were fed by the commercial extruded pellet (Ewha Oil and Fat Industry Co. Ltd., Korea) containing 50.0% crude protein and 7.0% crude lipid twice a day. Twenty-five fish (an initial body weight of fish: 16.0±0.01 g) per tank were randomly chosen and distributed into 18, 180 L flow-through tanks (water volume; 150 L). The flow rate of water into each tank was 6.5 L/min. The water source was the sand-filtered natural seawater and aeration supplied to each tank. Water temperature ranged from 16.0 to 25.5℃ (Mean±SD: 23.6±0.26℃) and photoperiod followed natural condition. After an initiation of the feeding trial, fish in each tank were collectively weighed bi-weekly to minimize retardation of growth of fish resulted from the stress during the measurement.

2.2.2. Preparation of the experimental diets

Five experimental diets were prepared: control (C), high protein (HP), high carbohydrate (HC), high lipid (HL), and intermediate protein, carbohydrate and lipid (IPCL) diets. Ingredient and nutrient composition of the experimental diets are given in Table 1.

Table 1. Ingredients and nutrient composition of the experimental diets used in the experiment 2

	Diets				
	C	HP	HC	HL	IPCL
Ingredients (%)					
Fishmeal[1]	60	74	60	60	68
Cellulose	10		2	6.3	
α-starch	5	5	13	5	9
Wheat flour	19.3	16.6	19.3	19.3	15.3
Pollack liver oil	2	0.7	2	2	2
Soybean oil				3.7	2
Vitamin premix[2]	1.5	1.5	1.5	1.5	1.5
Mineral premix[3]	2	2	2	2	2
Choline	0.2	0.2	0.2	0.2	0.2
Nutrient (DM basis, %)					
Moisture	9.6	11.7	10.0	10.3	10.0
Crude protein	48.6	56.3	48.1	48.1	53.1
Crude lipid	7.3	7.3	7.4	12.4	9.2
Ash	10.0	12.1	9.8	9.9	11.0
Fiber	11.6	0.9	3.0	7.6	0.9
NFE[4]	22.5	23.4	31.6	22.0	25.8
n-3 HUFA[5]	1.4	1.5	1.4	1.4	1.5
Estimated energy (kJ/g)[6]	14.6	16.1	16.1	16.4	16.6

[1]Imported from Chile.

[2]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

[3]Mineral premix contained the following ingredients (g/kg mix): $MgSO_47H_2O$, 80.0; $NaH_2PO_42H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_47H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_36H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4H_2O$, 2.0; $CoCl_26H_2O$, 1.0.

[4]NFE calculated by differences.

[5]n-3 HUFA: Sum of n-3 highly unsaturated fatty acids ($C \geq 20$).

[6]Estimated energy calculated based on 16.7 kJ/g for protein and NFE, and 37.7 kJ/g for lipid (Garling and Wilson 1976).

Fishmeal, wheat flour and -starch, and pollack liver oil were used as the primary protein, carbohydrate and lipid sources, respectively. Dietary nutrient composition in the C diet satisfied its requirement for growth of olive flounder (Lee et al. 2000a, Lee et al. 2002; Kim et al. 2002). Protein level was constant at 48.1% in the C, HC and HL diets, but carbohydrate and lipid levels were supplemented into the HC and HL diets, respectively at the expense of cellulose to increase energy level to 16.1 and 16.4 kJ/g diet each, calculated by the value of 16.7 kJ/g for protein and NFE, and 37.7 kJ/g for lipid, respectively (Garling and Wilson 1976). However, protein level increased to 56.3%, but maintained constant energy level of 16.1 kJ/g diet in the HP diet, and protein and energy levels of the IPCL diet were 53.1% and 16.6 kJ/g diet, respectively. Ingredients of the experimental diets were well mixed with water at the ratio of 7:3 and pelletized by pellet-extruder. The experimental diets dried overnight at room temperature and stored at -20℃ until use.

2.2.3. Design of the feeding trial

Fish were hand-fed to apparent satiation twice daily (09:30 and 17:00) by the C diet for 6 days a week during 8 weeks, referred to as C-8W, which was used as a control group. Other five groups of fish were starved for the first 2 weeks and then fed to satiation twice daily by the C, HP, HC, HL and IPCL diets for 6 days a week during the next 6 weeks referred to as C-6W, HP-6W, HC-6W, HL-6W, and IPCL-6W treatments, respectively. Each treatment was triplicated.

2.2.4. Analysis of chemical composition and blood analysis of fish

Fifteen fish at the initiation and five fish from each tank at the termination of the feeding trial were sampled and sacrificed for proximate analysis. Crude protein content determined using Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland), lipid content determined using ether-extraction method, moisture content determined by drying sample in a dry oven at 105℃ for 24 h, fiber content determined using automatic analyzer (Fibertec, Tecator, Sweden) and ash content determined using muffle furnace at 550℃ for 4 h, all methods were according to standard AOAC (1990). Blood samples were obtained from the caudal vein of randomly chosen 5 fish from each tank by using a heparinized syringe after they were starved for 24 h and anesthetized with 100 ppm MS-222. Also, biological index of fish such as condition factor (CF) and hepatosomatic index (HSI) of fish was measured as follows: CF = (body weight, g)/(total length, cm)^3 and HSI = (liver weight, g)/(body weight, g).

2.2.5. Calculation and statistical analysis

Calculation was made as follows: feeding rate (% body weight/day) = 100C/[(Wi+Wf)/2]/t feed efficiency ratio (FER) = (Wf-Wi)/C; protein efficiency ratio (PER) = (Wf-Wi)/Cp, where, Wi and Wf are initial and final weights, t is the feeding duration (day), C is feed consumption (g) and Cp is protein consumption (g).

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 by using SAS Program Version 9.1 (SAS Institute, Cary, NC, USA). Repeated measures ANOCOVA were used to assess the effect of dietary nutrient composition on fish weight from week 2 to week 8 in the bi-week interval, and the treatment effect on FR and FER. The initial size of fish was used as a covariate to test the effect of dietary nutrient composition on fish weight. The initial fish weight of the bi-week interval was used as a covariate to assess the treatment effect on FR, FER and PER.

2.3. Results

Relatively high survival (96%) was obtained in all olive flounder at the end of the feeding trial and no significant difference was observed among treatments. No significant difference in initial body weight of fish was found among all treatments (P > 0.05). However, significant (P < 0.05) difference in body weight of fish was observed in between C-8W treatment, in which fish were fed on the C diet for the initial 2-week and all treatments with 2-week feed deprivation. After refeeding, an accelerated growth rate was observed in all starved fish in the rest periods of the feeding trial (4, 6 and 8 weeks) and fish in HP-6W and IPCL-6W treatments overcompensated compared to growth of fish in C-8W treatment at the end of the feeding trial (Fig. 1, Table 2).

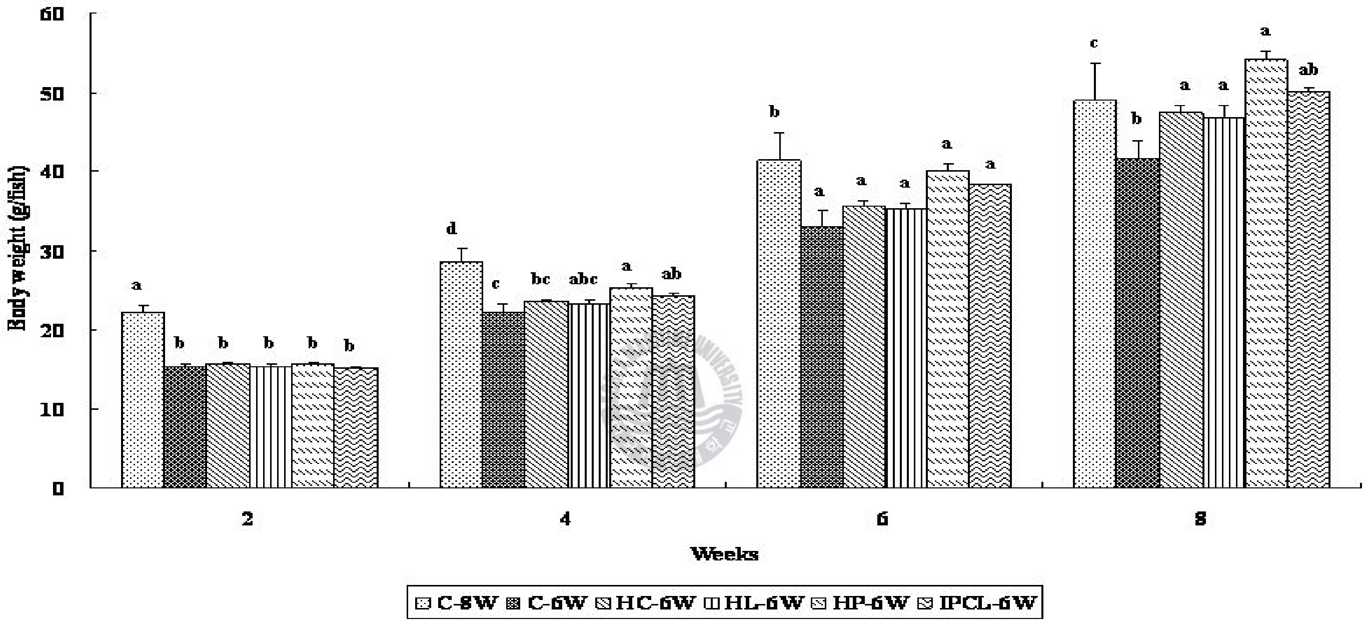

Figure 1. Changes in body weight (g/fish) of flounder fed the experimental diets with different feeding strategies (Mean±SE). Different letters indicate significant differences among treatments in the experiment 2 ($P < 0.05$).

Table 2. Survival (%), weight gain (g/fish), feed consumption (g/fish) and feed efficiency ratio (FER) of olive flounder fed the experimental diets containing various levels of nutrients for 6 weeks after 2-week starvation in the experiment 2[1]

Treatments	Final weight (g/fish)	Survival (%)	Weight gain (g)	SGR	Feed consumption (g/fish)	FER
C-8W	48.9±4.67	96.0±0.00	33.0±46.7[ab]	2.6±0.24[ab]	42.3±4.18[a]	0.79±0.040[b]
C-6W	41.6±2.27	96.0±2.31	25.6±2.29[b]	2.3±0.13[b]	36.0±2.06[a]	0.71±0.025[c]
HP-6W	54.1±1.17	100.0±0.00	38.1±1.19[a]	2.9±0.06[a]	39.1± 0.94[a]	0.97±0.008[a]
HC-6W	47.5±0.86	100.0±0.00	31.5±0.87[ab]	2.6±0.05[ab]	37.1±0.54[a]	0.85±0.011[b]
HL-6W	46.9±1.57	97.3±1.33	30.8±1.56[ab]	2.6±0.08[ab]	36.5±1.18[a]	0.84±0.016[b]
IPCL-6W	50.1±0.33	100.0±0.00	34.1±0.33[a]	2.7±0.02[a]	36.1±0.34[a]	0.95±0.004[a]

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

Feeding rate (%) of fish in all treatments with 2-week feed deprivation was significantly (*P*< 0.05) higher than that of fish in C-8W treatment in 6 week after the initiation of the feeding trial (Fig. 2). However, feeding rate of fish in C-6W treatment, in which fish were fed by the C diet for 6 weeks after 2-week feed deprivation was significantly (*P* < 0.05) higher than that of fish in other treatments, in which fish were fed on the C diet for 8 weeks or fed on the HC, HL, HP and IPCL diets for 6 weeks after 2-week feed deprivation.

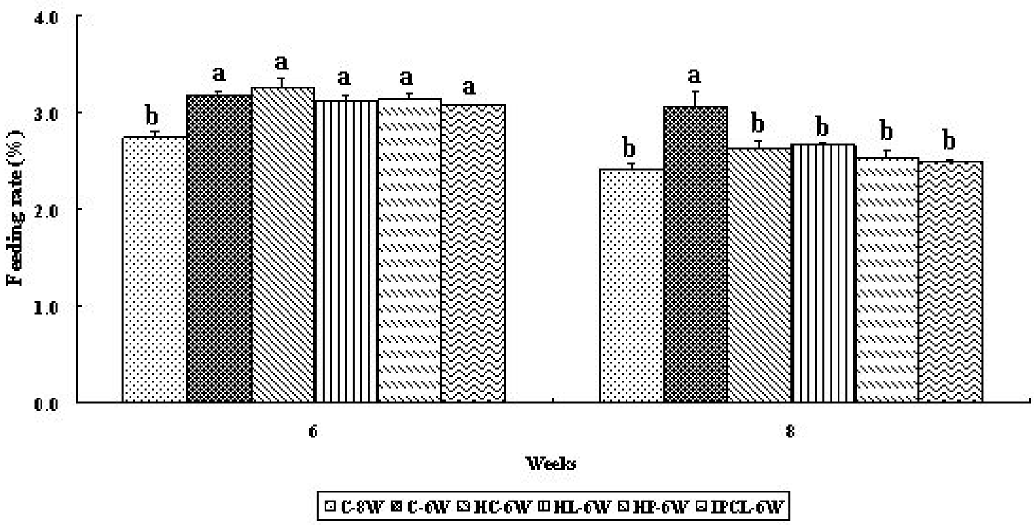

Figure 2. Changes in feeding rate (%, DM basis) of flounder fed the experimental diets with different feeding strategies from week 6 to week 8. Different letters indicate significant differences among treatments in the experiment 2 ($P < 0.05$).

Feed efficiency ratio (FER) of fish in C-8W treatment was significantly ($P < 0.05$) higher than that of fish in C-6W and HC-6W treatments, but not significantly ($P > 0.05$) different from that of fish in HL-6W, HP-6W and IPCL-6W treatments in 6 week after the initiation of the feeding trial (Fig. 3, Table 2). However, FER of fish in HC-6W, HL-6W, HP-6W and IPCL-6W treatments was significantly ($P < 0.05$) higher than that of fish in C-8W and C-6W treatments at the end of the feeding trial.

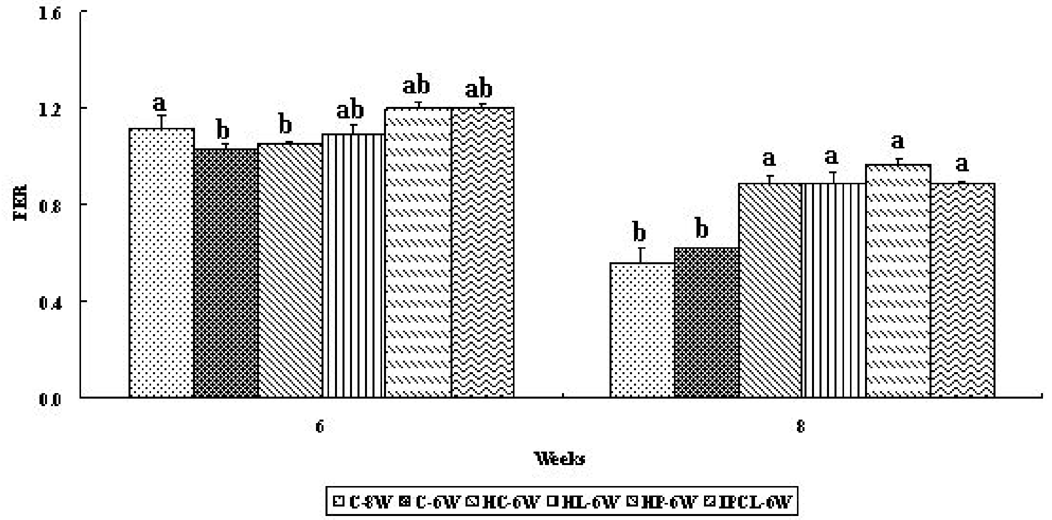

Figure 3. Changes in feed efficiency ratio (FER) of flounder fed the experimental diets with different feeding strategies from week 6 to week 8. Different letters indicate significant differences among treatments in the experiment 2 ($P < 0.05$).

Protein efficiency ratio (PER) of fish in C-8W treatment was significantly ($P < 0.05$) higher than that of fish in C-6W and HP-6W treatments, but not significantly ($P > 0.05$) different from that of fish in HC-6W HL-6W and IPCL-6W treatments in 6 week after the initiation of the feeding trial (Fig. 4). However, PER of fish in HC-6W, HL-6W, HP-6W and IPCL-6W treatments was significantly ($P < 0.05$) higher than that of fish in C-8W and C-6W treatments at the end of the feeding trial.

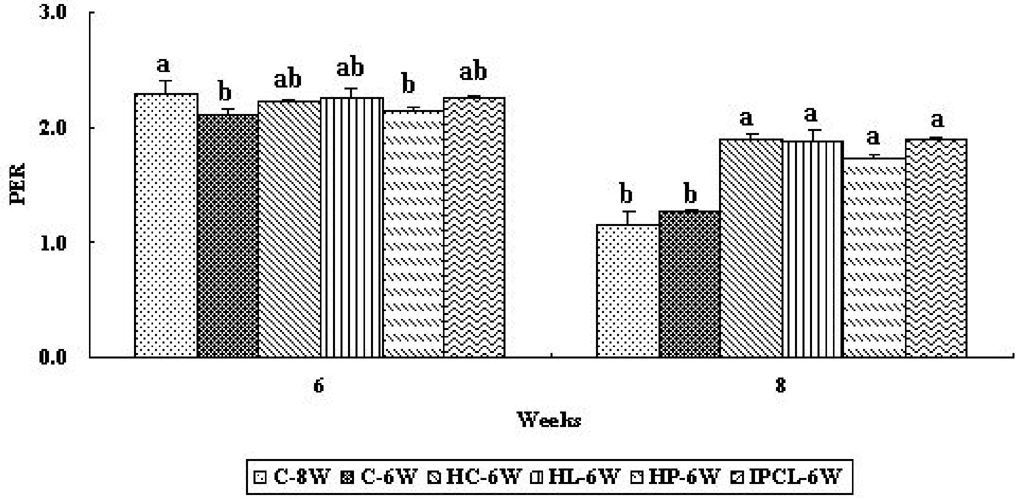

Figure 4. Changes in protein feed efficiency ratio (PER) of flounder fed the experimental diets with different feeding strategies from week 6 to week 8. Different letters indicate significant differences among treatments in the experiment 2 ($P < 0.05$).

Hematocrit was not significantly ($P > 0.05$) different among treatments (Table 3). However, condition factor (CF) of fish in HP-6W, HC-6W and IPCL-6W treatments was significantly ($P < 0.05$) higher than that of fish in C-8W and C-6W treatments, but not significantly ($P > 0.05$) different from that of fish in HL-6W treatment. Hepatosomatic index (HSI) of fish in HC-6W, HL-6W and IPCL-6W treatments was significantly ($P < 0.05$) higher than that of fish in C-8W, C-6W or HP-6W treatment. The lowest HSI was obtained in fish in C-6W treatment.

Table 3. Hematocrit (%), condition factor (CF) and hepatosomatic index (HSI) of olive flounder at the end of the feeding trial in the experiment 2[1]

Treatments	Hematocrit	CF[2]	HSI[3]
C-8W	26.6±1.67	0.93±0.012^{b}	1.58±0.069^{c}
C-6W	26.2±2.01	0.93±0.011^{b}	1.63±0.021^{c}
HP-6W	28.1±2.01	0.99±0.023^{a}	1.96±0.020^{b}
HC-6W	28.2±1.55	0.98±0.009^{a}	2.50±0.076^{a}
HL-6W	27.2±1.10	0.96±0.010^{ab}	2.55±0.022^{a}
IPCL-6W	27.5±1.22	0.99±0.003^{a}	2.64±0.225^{a}

[1]Values (Mean±SE) in the same row sharing a common superscript are not significantly different ($P < 0.05$).

Hematocrit, condition factor and hepatosomatic index of the initial fish were 15.8, 0.69 and 0.96, respectively.

[2]CF = (body weight) / (total length)^3.

[3]HSI = (liver weight) / (body weight).

Moisture, crude protein and ash content of the whole body of fish without liver was not significantly ($P > 0.05$) different among treatments (Table 4). However, crude lipid content of the whole body of fish without liver in HL-6W treatment was significantly ($P < 0.05$) higher than that of fish in C-8W and C-6W treatments, but not significantly ($P > 0.05$) different from that of fish in HP-6W, HC-6W or IPCL-6W treatment. Moisture and crude protein content of liver of fish in C-8W and C-6W treatments were significantly ($P < 0.05$) higher than those of fish in HP-6W, HC-6W, HL-6W and IPCL-6W treatments. And moisture and crude protein content of liver of fish in HP-W and HC-6W treatments were significantly ($P < 0.05$) higher than those of fish in HL-6W and IPCL-6W treatments. However, crude lipid content of liver of fish in HL-6W and IPCL-6W treatments was significantly ($P < 0.05$) higher than that of fish in C-8W, C-6W, HP-6W and HC-6W treatments.

Table 4. Chemical composition (% of wet weight) of the liver and whole body without liver of olive flounder at the end of the 8-week feeding trial in the experiment 2[1]

Whole body without liver				
Treatments	Moisture	Crude protein	Crude lipid	Ash
C-8W	75.4±0.11	18.7±0.28	1.3±0.65^{c}	4.1±0.12
C-6W	75.2±0.15	18.6±0.32	1.8±0.26^{bc}	4.0±0.13
HP-6W	75.0±0.04	18.5±0.20	2.4±0.27^{abc}	3.8±0.18
HC-6W	75.1±0.33	18.4±0.27	2.6±0.13^{abc}	3.6±0.23
HL-6W	74.3±0.12	18.3±0.32	3.9±0.17^{a}	3.8±0.16
IPCL-6W	74.3±0.55	18.3±0.35	3.2±0.55^{ab}	3.5±0.08
Liver				
	Moisture	Crude protein	Crude lipid	
C-8W	71.0±0.95^{a}	12.7±0.04^{a}	9.9±1.41^{b}	
C-6W	70.9±0.40^{b}	13.0±0.13^{a}	9.2±0.90^{b}	
HP-6W	67.9±0.48^{b}	10.9±0.21^{b}	10.7±0.12^{b}	
HC-6W	67.5±0.78^{b}	10.2±0.17^{b}	12.2±0.95^{b}	
HL-6W	63.8±0.35^{c}	9.2±0.18^{c}	15.6±0.64^{a}	
IPCL-6W	63.4±0.28^{c}	9.2±0.42^{c}	15.7±0.30^{a}	

[1]Values (Mean±SE) in the same row sharing a common superscript are not significantly different ($P < 0.05$).

Moisture, crude protein, crude lipid and ash content of the initial whole body of fish without liver were 76.0, 17.5, 1.7 and 4.5 (%) and 73.0, 17.3 and 4.2 (%) for moisture, crude protein and crude lipid of liver, respectively.

Blood chemistry and thyroid hormones of olive flounder fed the experimental diets for 6 weeks after 2-week fasting are presented in Table 5. Serum total protein ranged from 2.5 to 3.0 g/dL, glucose ranged from 256.3 to 298.0 mg/dL, GOT ranged from 15.7 to 19.7 IU/L, GPT ranged from 2.7 to 4.3 IU/L, TG ranged from 57.0 to 108.0 mg/dL, and T_4 ranged from 1.7 to 3.6 g/dL, respectively, and were not significantly ($P > 0.05$) affected by the experimental diets. However, T_3 of flounder fed the HL and IPCL diets was significantly ($P > 0.05$) higher than that of fish fed the C diet, but not significantly ($P > 0.05$) higher than that of fish fed the HP and HC diets.

Table 5. Blood chemistry and hormone levels of olive flounder at the end of the feeding trial in the experiment 2[1]

Treatments	Total protein (g/dL)	Glucose (mg/dL)	GOT (IU/L)	GPT (IU/L)	TG (mg/dL)	T_3 (ng/dL)	T_4 (mg/dL)
C-8W	2.5±0.03	293.7±23.82	15.7±2.91	4.3±1.86	57.0±5.51[c]	189.0±15.10[b]	2.3±0.10
C-6W	2.6±0.07	289.3±11.86	12.0±1.00	2.7±0.33	59.3±2.33[c]	198.0±15.95[b]	2.5±0.74
HP-6W	2.9±0.07	285.0±13.20	18.0±5.51	2.7±0.33	92.7±13.98[bc]	274.6±27.19[ab]	2.1±0.29
HC-6W	3.0±0.27	256.3±26.10	19.3±2.19	3.0±0.00	97.7±9.84[bc]	306.1±67.17[ab]	3.6±1.88
HL-6W	3.0±0.12	294.0±18.89	19.5±2.33	3.0±0.33	108.0±19.66[a]	405.3±37.38[a]	2.0±0.69
IPCL-6W	3.0±0.15	298.0±3.00	19.7±0.50	3.0±0.00	107.0±1.00[b]	379.6±46.05[a]	1.7±0.31

[1]Values (Mean±SE) in the same row sharing a common superscript are not significantly different (*P* < 0.05).

2.4. Discussion

Juvenile (initial body weight of 3.1 g) olive flounder grew better on the low energy diet (12.6 kJ/g) than the high energy diet (16.7 kJ/g) at 30, 40 and 50% protein levels (Lee et al. 2000a) and dietary protein requirement was estimated to be 45% for growth of juvenile olive flounder when fish were fed to satiation twice daily (Lee et al. 2002). Therefore, growth of fish fed on the C diet twice a day for 8 weeks (C-8W treatment) seemed to be well achieved in this study. The accelerated growth rate of olive flounder in all treatments with 2-week feed deprivation in 4, 6 and 8 week after the initiation of the feeding trial in this study indicated that compensatory growth was well achieved in fish experienced 2-week fasting and supplementation of dietary nutrient composition (protein and/or lipid) could effectively improve compensatory growth of fish. Especially, a better improvement in growth of olive flounder in HP-6W treatment compared to that of fish in C-8W treatment in this study indicated that dietary protein supplementation could maximize compensatory growth of fish after 2-week fasting and result into overcompensating growth of fish fed the C diet for 8 weeks without fasting (C-8W treatment). Diet nutrient composition largely affected thyroid hormone level of fish, which seemed to play a major role in achieving compensatory growth; especially, a high protein diet greatly increased T_3 level of fish (Riley et al. 1993; MacKenzie et al. 1998). This probably explained why the better compensatory growth of olive flounder fed on the HP diet than the HC or HL diet for 6 weeks after 2-week fasting was achieved in this study. Similarly, Gaylord and Gatlin (2001) showed that compensatory growth of fingerling channel catfish was primarily affected by dietary protein level rather than dietary energy level. However, an increase in dietary protein level over 45% deteriorated growth of juvenile olive flounder when fish were fed to satiation twice a day for 9 weeks without fasting (Lee et al. 2002).

An increased feeding rate of 2-week fasting all fish groups compared to that of fish in C-8W treatment in 6week after the initiation of the feeding trial in this study was probably resulted from hyperphagia, which is one of the primary mechanisms leading to compensatory growth of fish after refeeding. Similarly, hyperphagia was observed in other fish achieving compensatory growth (Rueda et al. 1998; Gaylord and Gatlin 2000; Wang et al. 2000; Cho 2005b; Cho et al. 2006). Unlike these studies, however, feed consumption of channel catfish achieving compensatory growth was affected by dietary protein level, but not by dietary energy level (Gaylord and Gatlin 2001). However, poorer weight gain would have been observed in fish in C-6W treatment compared to that of fish in all other treatments with 2-week fasting although the highest feeding rate had been observed in the former at the end of the feeding trial. This probably indicated that dietary nutrient (protein, carbohydrate and/or lipid) supplementation should be needed to effectively achieve compensatory growth of fish. An improvement in FER and PR of fish in HP-6W, HC-6W, HL-6Wand IPCL-6W treatments compared to those of fish in C-6W or C-8W treatment at the end of the feeding trial in this study indicated that supplementation of dietary nutrient composition (protein, carbohydrate and/or lipid) effectively improved feed utilization, which agreed to other studies (Jobling et al. 1994; Gaylord and Gatlin 2001).

Higher CF was observed in olive flounder in HP-6W, HC-6W and IPCL-6W treatments compared to that of fish in C-6W and C-8W treatments at the end of the feeding trial in this study, probably indicating that fish fed the diets supplemented with protein, carbohydrate, and all of protein, carbohydrate and lipid became fattier when compensatory growth of fish was achieved after refeeding. Also higher HSI of olive flounder in HP-6W, HC-6W, HL-6W and IPCL-6W treatments was observed compared to that of fish in C-6W and C-8W treatments in this study. Similarly, high HSI was observed in other fish achieving compensatory growth (Farbridge and Leatherland 1992; Gaylord and Gatlin 2000; Cho 2005b). Especially, Gaylord and Gatlin (2000) proposed that HSI could be the index for detecting when refeeding should be made to maximize compensatory growth.

High crude lipid content of fish without liver and liver in fish in HL-6W and IPCL-6W treatments in this study indicated that body lipid content of olive flounder was relatively well reflected from dietary energy level and its surplus was deposited into body lipid. Similarly, high dietary energy (lipid) level resulted into an increase in body lipid content of fish (El-Dahhar and Lovell 1995; Lee et al. 2000a Lee et al. 2000b; Gaylord and Gatlin 2001; Cho and Jo 2002).

T_3 of flounder fed the HL and IPCL diets was significantly ($P > 0.05$) higher than that of fish fed the C diet, but not significantly ($P > 0.05$) higher than that of fish fed the HP and HC diets, partially agreeing with Riley et al. (1993) and MacKenzie et al. (1998)'s studies showing that diet quality largely affected thyroid hormone levels of fish, which seemed to play a major role in achieving compensatory growth; especially, a high protein diet greatly increased T_3 level.

Once a red tide or coldwater mass occurs in summer season, it commonly lasts over 2 weeks or even up to a few months. Therefore, result of this study that dietary nutrient composition to maximize compensatory growth of fish after 2-week feed deprivation is applicable limitedly in reality in case of long term (over 2 weeks) fasting of fish. Therefore, the further study on dietary nutrient composition to maximize compensatory growth of olive flounder during the long term fasting will be needed. According to these results, it can be concluded that supplementation of protein, carbohydrate and lipid sources into the diet could effectively improve compensatory growth of juvenile olive flounder when fish were refed for 6 weeks after 2-week fasting during the summer season especially, dietary protein supplementation can maximize it.

IV. Conclusion

Effects of different feeding regimes and dietary nutrient composition on compensatory growth of juvenile olive flounder (*Paralichthys olivaceus*) were investigated.

In the experiment 1, compensatory growth of olive flounder fed the extruded pellet with different feeding regimes was determined. It can be concluded that juvenile olive flounder achieved better compensatory growth at 6-week refeeding after 2-week feed deprivation compared to that of fish with different feeding regimes.

In the experiment 2, effect of dietary nutrient composition on compensatory growth of olive flounder was investigated. Dietary supplementation of protein, carbohydrate and lipid sources could improve compensatory growth of fish refed for 6 weeks after 2-week fasting especially, dietary protein supplementation can maximize it.

V. Acknowledgements

I would like to express my sincere gratitude to Prof. Sung Hwoan Cho, Department of Marine Bioscience and Environment, Korea Maritime University, for his invaluable advice and continuous encouragement throughout this study.

I wish to thanks to my committees, Profs. Cheol Young Choi and In-Seok Park, for their critical advices for my thesis, and to Profs. Hyo Jin Kang, Il Noh, Youngwan Seo, Ho Jin Lee, Jong Woong Ahn, Sun Young Lim and Kyung Eun Lee for their kind advices and interests in this thesis.

I also wish to thank all staffs in East Sea Specific Research Center (East Sea Fisheries Research Institute, National Fisheries Research & Development Institute) for their suppling experimental space.

For my colleagues, Sang Mok Lee, Byum Hee Park, Sung Choon Ji, Tae Kyoung Lee, Mi Na Jang, Young Jin Cho, Jin Woo Lee, Ha Na Kim, Hae Won Yang, Kyoung Tae Kim, Hyun Jung Cho and Bom Sok Lee at the Feed Nutrition & Engineering Lab, I warmly thank for their help at practical things as well as sharing the good and bad moments of research and life.

Finally, I would like to express my hearty thanks to my family who have incessantly loved, encouraged and supported me.

VI. References

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

Bilton, H. T. and G. L. Robins. 1973. The effects of starvation and subsequent feeding on survival and growth of Fulton channel sockeye salmon fry. Journal of Fisheries Research Board of Canada 30:1-5.

Cho, S. H. and J. K. Lee. 2002. Compensatory growth in juvenile olive flounder (*Paralichthys olivaceus*) in the spring. Journal of Fisheries Science and Technology 5:122-126.

Cho, S. H. and J. Jo. 2002. Effects of dietary energy level and number of meals on growth and body composition of Nile tilapia, *Oreochromis niloticus* (L.) during summer and winter seasons. Journal of the World Aquaculture Society 33:48-56.

Cho, S. H. 2005a. Effect of alternate-week feeding strategies on growth and feed efficiency ratio of juvenile Nile tilapia, *Oreochromis niloticus* in a recirculating system. Journal of Fisheries Science and Technology 8:128-131.

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

Cho, S. H. and J. Jo. 2005. The effect of different feeding strategies on the growth of young Nile tilapia, *Oreochromis niloticus* L. in a freshwater recirculating system during summer. Journal of Fisheries Science and Technology 8:38-42.

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

Daiichi Pure Chemicals Co. Ltd. 2005. The manual of Daiichi Pure Chemicals Company Limited, 13-5. Nihombashi 3-chome Chuoku, Tokyo. Japan.

Damsgaard, B. and L. M. Dill. 1998. Risk-taking behavior in weight-compensating coho salmon, *Oncorhynchus kisutch*. Behavioral Ecology 9:26-32.

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

Eales, J. G. 1988. The influence of nutritional state on thyroid function in various vertebrates. American Zoologist 28:351-362.

El-Dahhar, A. A. and R. T. Lovell. 1995. Effect of protein to energy ratio in purified diets on growth performance, feed utilization and body composition of Mozambique tilapia, *Oreochromis mossambicus* (Peters). Aquaculture Research 26:451-457.

Farbridge, K. J. and J. F. Leatherland. 1992. Temporal changes in plasma thyroid hormone, growth hormone and free fatty acid concentrations, and hepatic 5'-monodeiodinase activity, lipid and protein content during chronic fasting and re-feeding in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry 10:245-257.

Garling, D. L. and R. P. Wilson. 1976. Optimum dietary protein to energy ratios for channel catfish fingerlings, *Ictalurus punctatus*. Journal of Nutrition 106:1368-1375.

Gaylord, T. G. and D. M. Gatlin. 2000. Assessment of compensatory growth in channel catfish *Ictalurus punctatus* R. and associated changes in body condition indices. Journal of the World Aquaculture Society 31:326-336.

Gaylord, T. G. and D. M. Gatlin. 2001. Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). Aquaculture 194:337-348.

Gaylord, T. G., D. S. Mackenzie, and D. M. Gatlin. 2001. Growth performance, body composition and plasma thyroid hormone status of channel catfish (*Ictalurus punctatus*) in response to short-term feed deprivation and refeeding. Fish Physiology and Biochemistry 24:73-79.

Heide, A., A. Foss, S. O. Stefansson, I. Mayer, B. Norberg, B. Roth, M. D. Jenssen, R. Nortvedt, and A. K. Imsland. 2006. Compensatory growth and fillet crude composition in juvenile Atlantic halibut: Effects of short term starvation periods and subsequent feeding. Aquaculture 261:109-117.

Jobling, M. and J. Koskela. 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. Journal of Fish Biology 49:658-667.

Jobling, M., O. H. Meloy, J. Dos Santos and B. Christiansen. 1994. The compensatory growth response of the Atlantic cod: effects of nutritional history. Aquaculture International 2:75-90.

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.

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

Lee, S. M., 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. M., S. H. Cho, and D. Kim. 2000b. Effects of feeding frequency and dietary energy level on growth and body composition of juvenile flounder (*Paralichthys olivaceus*). Aquaculture Research 12:917-921.

Lee, S. M., 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.

Mackenzie, D. S., H. Y. Moon, D. M. Gatlin, and L. R. Perez. 1993. Dietary effects on thyroid hormones in the red drum, *Sciaenops ocellatus*. Fish Physiology and Biochemistry 11: 329-335.

MacKenzie, D. S., C. M. Van Putte and K. A. Leiner. 1998. Nutrient regulation of endocrine function in fish. Aquaculture 161:3-25.

MIFAFF. 2008. Ministry for Food, Agriculture, Forestry and Fisheries. Seoul, Korea.

Oh, S., C. H. Noh, and S. H. Cho. 2007. Effect of restricted feeding regimes on compensatory growth and body composition of red sea bream, *Pagrus major*. Journal of the World Aquaculture Society 38:443-449.

Qian, X., Y. Cui, B. Xiong, and Y. Yang. 2000. Compensatory growth, feed utilization and activity in gibel carp, following feed deprivation. Journal of Fish Biology 56:228-232.

Quinton, J. C. and R. W. Blake. 1990. The effect of feed cycling and ration level on the compensatory growth response in rainbow trout, *Oncorhynchus mykiss*. Journal of Fish Biology 37:33-41.

Riley, W. W., D. A. Jr. Higgs, B. S. Dosanjh and J. G. Eales. 1993. Influence of dietary amino acid composition on thyroid function of juvenile rainbow trout, *Oncorhynchus mykiss*.

Aquaculture 112:253-269.

Rueda, F. M., F. J. Martinez, S. Zamora, M. Kentouri, and P. Divanach. 1998. Effect of fasting and refeeding on growth and body composition of red porgy, *Pagrus pagrus* L. Aquaculture Research 29:447-452.

Saether, B. S. and M. Jobling. 1999. The effects of ration level on feed intake and growth, and compensatory growth after restricted feeding, in turbot *Scophthalmus maximus* L. Aquaculture Research 30:647-653.

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

Tian, X. and J. G. Qin. 2003. A single phase of food deprivation provoked compensatory growth in barramundi *Lates calcarifer*. Aquaculture 224:169-179.

Tian, X. and J. G. Qin. 2004. Effects of previous ration restriction on compensatory growth in barramundi *Lates calcarifer*. Aquaculture 235:273-283.

Van der Geyten, S., K. Mol, W. Pluymers, E. R. Khn, and V. M. Darras. 1998. Changes in plasma T_3 during fasting-refeeding in tilapia (*Oreochromis niloticus*) are mainly regulated through changes in hepatic type II iodothyronine deiodinase. Fish Physiology and Biochemistry 19:135-143.

Wang, Y., Y. Cui, Y. Yang, and F. Cai. 2000. Compensatory growth in hybrid tilapia, *Oreochromis mossambicus* × *O. niloticus,* reared in seawater. Aquaculture 189:101-108.

Xie, S., X. Zhu, Y. Cui, R. J. Wootton, W. Lei, and Y. Yang. 2001. Compensatory growth of the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. Journal of Fish Biology 58:999-100.

Zhu, X., Y. Cui, M. Ali and R. J. Wootton. 2001. Comparison of compensatory growth responses of juvenile three-spined stickleback and minnow following similar food deprivation protocols. Journal of Fish Biology 58:1149-1165.