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Effect of Intermittent Feeding on Growth, Feed Utilization and Body Composition of Subadult Olive Flounder *Paralichthys olivaceus* in Suboptimal Temperature

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ABSTRACT : The effect of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder fed extruded pellet (EP) in suboptimal temperature (13.5±2.10°C) was determined. Two hundred twenty five subadult fish averaging 272 g were randomly distributed into fifteen of 300 L circular flow-through tanks (fifteen fish per tank). Five treatments with different days of feeding a week were prepared in triplicate: fish were hand-fed with EP to apparent satiation once a day, seven days a week (7DF), which was used as the control group or consecutive six, five, four and three days a week, for 12 weeks, referred to as 6DF, 5DF, 4DF and 3DF treatments, respectively. Weight gains and specific growth rates of fish in 6DF treatment were higher than those of fish in 7DF, 4DF and 3DF treatments, but not different from those of fish in 5DF treatment. Daily feed intake of fish in 6DF and 7DF treatments was higher than that of fish in 5DF, 4DF and 3DF treatments. Feed efficiency and protein efficiency ratio of fish were not different among treatments. There was no difference in the chemical composition of fish between treatments. Results of this study demonstrated that feeding five days per week could be recommended for subadult olive flounder fed extruded pellet in suboptimal temperature. (**Key Words :** Olive Flounder (*Paralichthys olivaceus*), Intermittent Feeding, Subadult, Suboptimal Temperature)

INTRODUCTION

Temperature is one of the most important environmental factors affecting not only availability of nutrient content in the diets (Lovell, 1989; Peres and Oliva-Teles, 1999; Yamamoto et al., 2007; Olsen and Ringo, 2008), but also growth of fish (Brett et al., 1969; Andrews and Stickney, 1972; Kilambi and Robinson, 1979; Koskela et al., 1997). Therefore, determining optimum temperature condition for fish growth is critical for fish farmers as it affects total production cost.

Since olive flounder (*Paralichthys olivaceus*) has been regarded as one of the most important marine fish for aquaculture in Eastern Asia for decades, many feeding trials have been undertaken to determine dietary nutrient requirements (Lee et al., 2000a, 2000b, 2002), optimum feed allowance (Lee et al., 2000b; Cho et al., 2006a, 2007), alternative protein sources for fishmeal in the diets (Sato and Kikuchi, 1997; Kikuchi, 1999) and feeding strategy (Cho et al., 2006b; Huang et al., 2008). However, these studies were all performed with juvenile olive flounder which grow quickly within moderate or optimum temperature conditions.

The optimum water temperature for growth of olive flounder was reported to be 20-25°C and its growth rate tended to decrease with lowered temperature (Iwata et al., 1994). In low temperature or suboptimal temperature conditions, oversupply of feed (excessive nutrient supply) should be avoided to lower production costs and water pollution. Under optimum temperatures (23.0±1.43°C), growth of juvenile olive flounder, averaging 38 g, fed extruded pellet (EP) for five days a week was comparable to that of fish fed EP for six days a week (Cho and Park, 2004).

Fish performance was largely affected by several factors such as fish size, water temperature and dietary nutrient composition (Lovell, 1989; Iwata et al., 1994; Yamamoto et al., 2007). Huang et al. (2008) reported that juvenile olive flounder averaging 10 g subjected to 8.5°C for 10 days followed by rearing at 22°C for the next 30 days achieved full compensatory growth and concluded that a short period of low temperature exposure might not affect the annual

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growth of fish. In addition, Kim et al. (2009, 2010) proposed that the optimum feeding frequency for both subadult olive flounder averaging 270 g and grower fish averaging 117 g was one meal a day at suboptimal temperature conditions, 12.1°C and 13°C, respectively when fish were fed with EP to satiation.

However, there is no information available of periodic feed deprivation and refeeding on performance of subadult olive flounder under suboptimal temperature conditions. In this study, therefore, the effect of intermittent feeding on growth, feed utilization and body composition of subadult olive flounder fed EP in suboptimal temperature was determined.

MATERIALS AND METHODS

Fish and the experimental conditions

Subadult olive flounder were purchased from a private farm and transferred into the laboratory. Fish were acclimated to the experimental conditions for 3 weeks before the initiation of the feeding trial. Two hundred twenty five subadult fish averaging 272 g were randomly distributed into fifteen of 300 L circular flow-through tanks (fifteen fish per tank) and the water source was sand-filtered natural seawater. During the acclimation period, fish were fed with EP once a day (09:00). The flow rate of water into each tank was 8.9 L/min and photoperiod followed natural conditions. Water temperature ranged from 9.8°C to 17.1°C (mean±SD:13.5±2.10°C). Fish in each tank were collectively weighed at the end of the 12-week feeding trial after they had been starved for a day.

Experimental design

Five treatments with different feeding regimes were prepared in triplicate: Fish in the control group were hand-fed with EP (Suhyup Feed Co. Ltd, Korea; 9.0-9.4 mm) containing 54.4% crude protein, 9.2% crude lipid and 11.8% ash (18.9 kJ/g diet gross energy) to apparent satiation once a day (09:00), seven days a week (7DF). The experimental groups were fed to apparent satiation for consecutive six, five, four and three days a week, for 12 weeks during winter season, referred to as 6DF, 5DF, 4DF and 3DF treatments, respectively. Uneaten feed was removed 30 min after feeding and deducted from the feed consumption calculation.

Chemical analysis of fish

Three fish at the initiation and termination of the feeding trial were sacrificed for proximate analysis. Crude protein was determined by the Kjeldahl method (Kjeltec 2100 Distillation Unit, Foss Tecator, Hoganas, Sweden), crude lipid was determined using an ether-extraction method, moisture was determined by oven drying at 105°C for 24 h, fiber was determined using an automatic analyzer (Fibertec, Tecator, Sweden) and ash was determined using a muffle furnace at 550°C for 4 h, all methods were according to standard AOAC (1990).

Calculation and statistical analysis

Calculations were made as follows: specific growth rate (SGR, %/d) = (Ln final weight of fish-Ln initial weight of fish)×100/days of feeding trial, daily feeding rate (DFR, %/body weight) = feed intake×100/((initial fish weight+final fish weight+dead fish weight)/2×days of the experiment), feed efficiency (FE) = weight gain of fish/dry feed fed, protein efficiency ratio (PER) = weight gain of fish/protein fed, protein retention (PR) = fish protein gained ×100/protein fed, condition factor (CF) = body weight (g)× 100/total body length (cm)^3 and hepatosomatic index (HSI) = liver weight×100/body weight.

One-way ANOVA and least significant difference (Fisher's LSD) test was applied to determine significant differences between the means of treatments using the SAS program version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA).

RESULTS

Survival ranged from 93.3% to 100% was not significantly (p>0.4) different among treatments (Table 1). However, the weight gain (p<0.03) and SGR (p<0.04) of

Table 1. Survival (%), weight gain (g/fish) and specific growth rate (SGR) of subadult olive flounder *Paralichthys olivaceus* fed with extruded pellet under different feeding regimes for 12 weeks in suboptimal temperature

Treatments	Initial weight (g/fish)	Final weight (g/fish)	Survival (%)	Weight gain (g/fish)	SGR[1] (%/d)
7DF	271.44±0.37	326.81±12.15^b	93.33±6.67	55.37±11.91^b	0.21±0.09^b
6DF	271.78±0.22	355.00±5.47^a	88.89±8.01	83.22±5.26^a	0.30±0.01^a
5DF	271.67±0.54	336.51±4.69^{ab}	100.00±0.00	64.84±4.16^{ab}	0.24±0.01^{ab}
4DF	271.87±0.44	318.67±4.72^b	97.78±2.22	46.80±5.14^b	0.18±0.02^b
3DF	271.60±0.07	318.89±7.71^b	100.00±0.00	47.29±7.77^b	0.18±0.03^b

Values (mean of triplicate±SE) in the same column sharing a common superscript are not significantly different (p>0.05).

[1] Specific growth rate (SGR, %/d) = (Ln final weight of fish-Ln initial weight of fish)×100/days of feeding trial.

Table 2. Daily feeding rate (DFR), feed efficiency (FE), protein efficiency ratio (PER), protein retention (PR), condition factor (CF) and hepatosomatic index (HSI) of subadult olive flounder *Paralichthys olivaceus* under different feeding regimes for 12 weeks in suboptimal temperature

Treatments	DFR[1]	FE[2]	PER[3]	PR[4]	CF[5]	HSI[6]
7DF	0.24±0.00[a]	0.81±0.09	1.50±0.17	31.6±3.13[b]	1.00±0.02	1.13±0.47
6DF	0.24±0.00[a]	1.13±0.10	2.07±0.18	43.2±4.36[b]	0.98±0.03	1.66±0.24
5DF	0.21±0.00[b]	1.14±0.08	2.11±0.15	43.8±1.06[b]	0.96±0.03	1.48±0.22
4DF	0.18±0.00[c]	0.98±0.10	1.80±0.18	41.9±1.62[b]	0.94±0.03	1.70±0.13
3DF	0.14±0.00[d]	1.32±0.18	2.44±0.32	56.9±6.97[a]	0.98±.05	1.62±0.35

Values (mean of triplicate±SE) in the same column sharing a common superscript are not significantly different (p>0.05).

[1] Daily feeding rate (DFR, %/body weight) = Feed intake×100/((initial fish weight+final fish weight+dead fish weight)/2×days of the experiment).

[2] Feed efficiency (FE) = Weight gain of fish/dry feed fed. [3] Protein efficiency ratio (PER) = Weight gain of fish/protein fed.

[4] Protein retention (PR) = Fish protein gained×100/protein fed. [5] Condition factor (CF) = Body weight (g)×100/total body length (cm)^3.

[6] Hepatosomatic index (HSI) = Liver weight×100/body weight.

subadult olive flounder in 6DF treatment were significantly higher than those of fish in 7DF, 4DF and 3DF treatments, but not significantly (p>0.05) different from those of fish in 5DF treatment.

DFR of subadult olive flounder in 6DF and 7DF treatments was significantly (p<0.0001) higher than that of fish in 5DF, 4DF and 3DF treatments (Table 2). In addition, DFR of fish in 5DF treatment was significantly higher than that of fish in 4DF and 3DF treatments, which was lowest. FE (p>0.08) and PER (p>0.08) of subadult olive flounder was not significantly different among treatments. However, PR of fish in 3DF treatment was significantly (p<0.02) higher than that of fish in all other treatments. Neither CF (p>0.5) nor HSI (p>0.5) of fish was significantly affected by different days of feeding.

None of the following parameters differed significantly between treatments: moisture content, ranged from 70.3% to 72.2% (p>0.3), crude protein content, ranged from 18.6% to 18.9% (p>0.8), crude lipid content, ranged from 4.0% to 4.2% (p>0.3) and ash content, ranged from 2.7% to 3.2% (p>0.1) of the whole body of fish excluding the liver, moisture content, ranged from 69.8% to 72.9% (p>0.6), crude protein content, ranged from 11.1% to 11.8% (p>0.7) and crude lipid content of the liver, ranged from 11.1% to 12.8% (p>0.1) (Table 3).

DISCUSSION

Higher weight gains and SGR were achieved in subadult olive flounder in 6DF treatment compared to those of fish in 7DF, 4DF and 3DF treatments, but were comparable to those of fish in 5DF treatment. These results indicated that seven days of satiation feeding per week produced no additional effect on growth of fish and that six or five days of satiation feeding a week was sufficient when fish were fed with EP in suboptimal temperature. This is in agreement with other studies (Brett et al., 1969; Andrews and Stickney, 1972; Kilambi and Robinson, 1979; Lovell, 1989; Koskela et al., 1997; Peres and Oliva-Teles, 1999; Yamamoto et al., 2007; Olsen and Ringo, 2008; Zhao et al., 2009) showing that metabolic rate and digestibility of nutrients in fish at lower temperature decreased when compared to those at higher temperatures. Consequently, surplus food either deteriorated or did not improve weight gains and feed efficiency (Brett et al., 1969; Andrews and Stickney, 1972; Kim et al., 2005, 2007). The optimum feeding frequency for grower and subadult olive flounder was one meal a day in suboptimal temperature conditions (Kim et al., 2009; Kim et al., 2010).

Juvenile catfish *Ictalurus pnuctatus* averaging 14.4 and 44.3 g, whitefish *Coregonus lavaretus* averaging about 50 g and walleye *Sander vitreus* averaging 26.1 g fed the

Table 3. Chemical composition (%, wet weight basis) of the whole body excluding the liver and liver of subadult olive flounder *Paralichthys olivaceus* at the end of the 12-week feeding trial

Treatments	Whole body excluding the liver				Liver		
	Moisture	Crude protein	Crude lipid	Ash	Moisture	Crude protein	Crude lipid
7DF	72.13±1.05	18.63±0.26	3.97±0.18	2.73±0.07	72.93±1.50	11.17±0.60	12.40±0.57
6DF	71.83±0.49	18.73±0.09	4.17±0.07	3.03±0.03	71.07±1.33	11.40±0.58	11.13±0.46
5DF	71.73±0.77	18.63±0.22	4.40±0.20	2.93±0.12	71.77±1.60	11.73±0.52	12.70±0.40
4DF	72.17±0.58	18.87±0.23	4.20±0.15	3.17±0.15	69.80±1.64	11.10±0.23	12.83±0.09
3DF	70.33±0.47	18.87±0.09	4.03±0.12	3.00±0.15	70.70±1.00	11.80±0.15	11.37±0.74

Values (mean of triplicate±SE) in the same column sharing a common superscript are not significantly different (p>0.05).

commercial feeds for consecutive 5 days a week achieved comparable growth of fish fed daily for 7 days a week in optimal temperatures (Li et al., 2005; Kankanen and Pirhonen, 2009; Rosauer et al., 2009). Similarly, Chatakondi and Yant (2001) also reported that intermittent feeding of channel catfish achieved an improvement in growth rate, feed consumption and survival after artificial *Edwardsiella ictaluri* infection compared with fish with on a daily feeding regime in a 10-week feeding trial. However, unlike these studies, body weight of juvenile turbot *Scophthalmus maximus* averaging 33 g and 62 g was directly related to the number of feeding days when fish were fed with commercial diet to apparent satiation for 7, 6, 5 or 4 days a week (Blanquet and Oliva-Teles, 2010) and the authors concluded that juvenile turbot should be fed daily as even cycles of short periods of feed deprivation adversely affected growth in this species.

Higher (0.30 and 0.24%/d), and comparable (0.21%/d) SGR of subadult olive flounder in 6DF and 5DF treatments, and 7DF treatment in this study were achieved in the present study which contrasts with that of (0.20%/d) of subadult fish grown from 270 g to 350 g in Kim et al. (2009)'s study, where fish were fed with EP containing 52.3% crude protein and 12.8% crude lipid to satiation daily at 12°C for 15 weeks. One satiation feeding a day was recommended for growth of subadult olive flounder by Kim et al. (2009). However, optimum feeding frequency for juvenile olive flounder averaging 6.3 g was two meals a day when fish were fed with EP and moist pellet at either 12 or 17°C for 60 days (Kim et al., 2007). Furthermore, Kim et al. (2010) recommended that the optimum feeding frequency was one meal per day for grower olive flounder averaging 117 g in a study where fish were fed with EP to satiation once in 2 days, once a day or twice a day at 13°C. Thus, small (juvenile) olive flounder seemed to require more feeding frequency than large (grower or subadult) fish in suboptimal temperature.

A lower DFR, with no difference in weight gain of subadult olive flounder in 5 DF treatment compared to those of fish in 7DF and 6DF treatments in this study (Table 2) indicated that five days of feeding a week could be recommended for subadult olive flounder in suboptimal temperature. However, a 12.5% lower DFR in 5DF treatment produced 5.2% less weight gain in fish compared to that of fish in 6DF treatment means that these two variables must be considered in determining the optimum number of feeding days for subadult olive flounder. Furthermore, lower DFR and slightly, but not significantly, higher weight gains of fish in 5DF treatment compared to those of fish in 7DF treatment indicated that compensatory growth of fish in the former was not resulted from hyperphagia, which has been commonly observed in many fish species achieving full compensatory growth (Chatakondi and Yant, 2001; Wu et al., 2003; Cho et al., 2006b; Kankanen and Pirhonen, 2009). However, subadult olive flounder seemed to consume more feed on the days after 1, 2, 3 and 4-day feed deprivation in this study.

Higher DFR (0.32%/body weight), but comparable SGR (0.20%/d) of subadult olive flounder averaging 279 g in Kim et al. (2009)'s study compared to values (0.24%/body weight and 0.21%/d) of fish in 7DF treatment in the present study might indicate that fish were overfed by Kim et al. (2009). In addition, DFR of fish in the present study was comparable to that of subadult olive flounder averaging 176 g grown at 10°C, but lower than fish grown at 15°C (Iwata et al., 1994). Similarly, feeding rate of 0.30%/body weight was recommendable for growth of subadult olive flounder when fish were fed with EP to satiation daily with various daily feeding rates (0.1, 0.15, 0.2, 0.3 and 0.32% body weight) at 12.1°C for 15 weeks (Kim et al., 2009). In addition, Kim et al. (2010) recommended that the optimum feeding rate was 0.3% body weight for grower olive flounder when fish were fed with EP to satiation twice a day with various at feeding rates (0.1, 0.25, 0.4, 0.55 and 0.57% body weight) at 13°C.

However, DFR of subadult olive flounder in the present study was relatively low compared to that (0.71%/body weight) of subadult fish averaging 319 g fed EP to satiation daily at optimum temperature (21.1°C) for 10 weeks (Cho et al., 2007). Another reason for lower DFR could be differences in the daily feeding frequency between studies (one satiation feeding in the former vs. twice satiation feeding in the latter). Feed consumption of fish generally decreased with lowered temperatures within an acceptable range of temperatures (Kilambi and Robinson, 1979; Iwata et al., 1994; Yamamoto et al., 2007).

That neither FE nor PER of subadult olive flounder were different among treatments in the present study, probably resulted from wide variation within the treatments. Similarly, FE and PER of olive flounder were not affected by different days of feeding (Cho and Park, 2004; Kankanen and Pirhonen, 2009) or feeding frequency (Lee et al., 2000b; Kim et al., 2007).

None of chemical composition of the whole body excluding the liver and the liver of olive flounder was affected by different days of feeding in this study. Similarly, chemical composition of olive flounder was unaffected by different days of feeding (Cho and Park, 2004), feeding frequency (Kim et al., 2005, 2007, 2009), feeding ratio (Kim et al., 2005; Cho et al., 2007) or feeding strategy (Cho et al., 2006b). However, unlike this study, visceral fat of channel catfish increased with the number of days of feeding (Li et al., 2005).

Results of this study demonstrated that five days of feeding a week could be recommended for subadult olive flounder fed extruded pellet to satiation in suboptimal temperature. However, since the optimum number of days of feeding per week can be affected by factors such as fish species, fish size, water temperature, feed allowance and dietary nutrient composition, the final recommendation must also consider these variables.

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