Thesis for the Degree of Doctor of Philosophy

A computer simulated feeding program proposed by the optimum feeding rate trials in olive flounder, *Paralichthys olivaceus*, at the optimum temperature

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최적 수온에서 넙치의 적정사료 공급량 실험을 통한 컴퓨터 시뮬레이션 사료공급 프로그램 제안

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A computer simulated feeding program proposed by the optimum feeding rate trials in olive flounder, *Paralichthys olivaceus*, at the optimum temperature

A dissertation

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A computer simulated feeding program proposed by the optimum feeding rate trials in olive flounder, *Paralichthys olivaceus*, at the optimum temperature

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Abstract

A computer simulated feeding program was proposed by eleven feeding trials to determine the optimum feeding rates for olive flounder, *Paralichthys olivaceus*, fed extruded pellets at the optimum temperature. The entire trials were grouped into four growth stages, namely the first stage of juvenile (5 and 9 g), the second stage of juvenile (20, 30, 40 and 50 g), growing (97, 240 and 317 g) and sub-adult (384 and 525 g) stages with 2, 4, 3 and 2 feeding trials, respectively. In the first set of experiments, the optimum feeding rates for the first stage of juvenile olive flounder, *Paralichthys olivaceus*, weighing 5 g (mean±SD) and 9 g were 4.83% and 4.52% body weight (BW)/day, respectively. In the second set, the optimum feeding rates for the second stage of juvenile olive flounder, *Paralichthys olivaceus*, weighing 20 g, 30 g, 40 g and 50 g were 3.52, 3.19, 3.02 and 2.38% BW/day, respectively. The third series of experiments showed the optimum feeding rates for growing olive flounder, *Paralichthys olivaceus*, weighing 97 g, 240 g and 317 g to be 2.23, 1.09 and 0.99% BW/day, respectively. In the last experiments, the optimum feeding rates in sub-adult olive flounder, *Paralichthys olivaceus*, weighing 384 g and 525 g were shown to be 0.74 and

0.70% BW/day, respectively. The optimum feeding rates determined in these feeding trials were used to develop a computer simulated daily feeding program for olive flounder, *Paralichthys olivaceus*, by weight. This program was run using some hypothetical values for fish weight and number of fish, and selecting some preset temperatures. The output showed the daily feed allowance in terms of feeding rate and actual weight of feed to be fed.

Chapter 1. General Introduction

Feed costs account for the major portion of aquaculture production costs, making feed management one of the most important determinants of viability and productivity of any commercial aquaculture operation. Diet cost could represent 30-70% of the total operating cost of an aquaculture enterprise, the percentage increasing with increase intensification of the operation (Webster et al. 2001; Lovell 1998). Wang et al. (2007) stated that feed cost accounted for 80% of total production cost of cuneate drum. Even when at least some fertilizer is used to provide live food during the rearing of early life stages the contribution of feed to total variable costs could be up to 50% (Stickney 2005).

Feed intake is perhaps the principal factor affecting growth rate of fish (Li et al. 2004). Several studies have shown that growth correlates to food intake (Silverstein et al. 1999; Mihelakakis et al. 2002; Wang et al. 2007; Cho et al. 2007; Kim et al. 2007; Ozorio et al. 2009). Adequate supply of nutritionally balanced feed is very important to ensure optimum growth, survival, improved immunity to disease, good dress-out yield, and desirable organoleptic properties of flesh. These in turn increase profitability of an aquaculture venture. Inadequate supply of diet leads to reduced growth and survival of fish. Conversely, overfeeding increases fish production cost and reduces profitability due to increased cost of feed and deterioration of water quality, which can eventually reduce growth of fish. Food application rate does not necessarily equal food consumption rate in large scale commercial systems, since a significant and unknown portion of administered food may not be eaten (Yamada 1985).

Optimum feeding rate varies with feeding frequency, nutrient content of feed, fish species, fish size, water temperature and other water quality parameters, etc. Channel catfish fed 36% protein gained more weight than those fed 30% protein when both were fed at approximately 75% of satiation but weight gain of fish was not different when the fish were fed to satiation (Minton 1978). This shows the relationship between feeding rate and nutrient (protein) content of diet. A somewhat similar observation was made by Li and Love11 (1992a, 1992b) in the same species. Channel catfish fed twice daily consumed more feed and had faster growth rates than fish receiving a single daily meal (Collins 1971; Andrews and Page 1975), indicating the dependence of feeding rate on feeding frequency. Hung and Lutes (1987) and Hung et al. (1989, 1995) stated that the optimum feeding rates for growth of fish could be affected by fish size. Hung et al. (1993) reported the optimum feeding rate for 30 g white sturgeon, *Acipenser transmontanus*, to be 2.0-3.0% BW/day while the optimum feeding rates for the same species were reported to be 2.0% BW/day and 1.5-2.0% BW/day for 30-100 g and 0.25-0.5 kg sizes, respectively (Hung and Lutes 1987; Hung et al. 1989). The optimum feeding rate of white sturgeon, *Acipenser transmontanus*, was 2.0-2.5% BW/day at 23 °C and 2.5-3.0% BW/day at 26 °C (Hung et al. 1993).

Olive flounder, *Paralichthys olivaceus*, is one of the most commercially important marine aquaculture species in Korea, ranking first among the marine finfish aquaculture production in the country in 2007. According to FAO (2008) olive flounder, *P. olivaceus*, contributed over 42% (41,171 metric tons) of the 97,663 metric tons of marine finfish production in Korea in 2007, a huge increase from the meager 20 metric tons in 1987 and 26,274 metric tons in 1997. The actual yield is higher than the above figure due to some unreported production. This sharp increase in production is attributed to various policies by the government to encourage aquaculture production in the face of the dwindling capture production of this species. Olive flounder, *P. olivaceus*, is very popular and highly valued by Koreans for its good percent dress out and great taste. It is served in various ways; one of the most popular of these is the raw fish called hoe.

With the rapid expansion of both aquaculture production and computer technology in the last few decades, development and use of computer tools for aquaculture has shown considerable advancements (Ernst and Nath 2000). Products are available for a wide variety of purposes, including financial analysis, development planning and project appraisal, facility design, management planning and production forecasting, facility construction, management decision support and record keeping, fish feed formulation, fish bioenergetics, disease diagnosis and treatment, environmental regulation, information systems and databases, and automated monitoring and control. The benefits of programs that control various aspects of aquaculture cannot be overstressed. Today, personal computers can be used to "least-cost" feed formulations. Linear programming, used almost exclusively in modern feed formulation in agriculture today enables feed manufacturers to calculate the combination and levels of ingredients that provide the desired nutrient content of the diet at the least cost (Halver and Hardy 2002). This not only eliminates the tediousness of manual calculation but also reduces errors and allows for easy substitution of ingredients according to availability and cost.

Aquaculture production still has a great potential for expansion if the use of computer in several other operations could be encouraged. Production is expected to expand further if computer simulation could be developed in the feeding practice of aquaculture operations, with feeding rates designed for specific species at different growth stages. Due to the importance of olive flounder, *P. olivaceus*, in Korea, various studies have been conducted in this species (Wang et al. 2002; Choi et al. 2003; Choi et al. 2004; Kim et al. 2004; Kim et al. 2005; Sun et al. 2007a,b; Yoo et al. 2007; Kim et al. 2008). Although Kim et al. (2007) determined the optimum feeding rate in juvenile olive flounder weighing 3.6 g to be 2.6% body weight/day, the optimum feeding rates over the entire growth stages have not been determined. Furthermore, no computer simulated feeding program has been developed for this species. Therefore, these feeding trials were conducted to determine the optimum feeding rates for the first and second stages of juvenile, growing and sub-adult olive flounder, *P. olivaceus*, fed extruded pellets at the optimum temperature and to propose a computer simulated feeding program for this species through results of the feeding trials.

Chapter 2. Optimum feeding rate trials in the different growth stages of olive flounder, *Paralichthys olivaceus*

I. Introduction

Feeding rate affects growth, body composition, digestibility and absorption of nutrients, weight heterogeneity, sexual growth dimorphism, and enzyme activities of fish (Fontaine et al. 1997; Shimeno et al. 1997; Halver and Hardy 2002). It is important that adequate amounts of nutritionally balanced feed be supplied, as excess or inadequate supply of feed will adversely affect the growth performance of fish and profitability of an aquaculture venture. Feeding rates have been determined in various species and growth stages of fish (De Silva et al. 1986; Hung and Lutes 1987; Hung et al. 1993; Mihelakakis et al. 2002; Fiogbe and Kestemont 2003). These have been found to be influenced by different factors among which is the fish size. Feeding rate has been found to decrease with growth of fish. Fiogbe and Kestemont (2003) found the optimum feeding levels for Eurasian perch, *Perca fluviatilis*, to decrease from 7.4%, 5.1%, 4.5% and 2.2% biomass/day for fish of 0.22, 0.73, 1.56 and 18.9g initial weights, respectively.

Olive flounder, *P. olivaceus*, is one of the most commercially important marine aquaculture species in Korea, ranking first among the marine finfish aquaculture production in the country in 2007. Olive flounder, *P. olivaceus*, contributed over 42% (41,171 metric tons) of the 97,663 metric tons of marine finfish production in Korea in 2007 (FAO 2008). This figure is a huge increase from the meager 20 metric tons in 1987 and 26,274 metric tons in 1997. Due to the importance of olive flounder, *P. olivaceus*, in Korea, various studies have been conducted in this species (Wang et al. 2002; Choi et al. 2003; Choi et al. 2004; Kim et al. 2004; Kim et al. 2005; Sun et al. 2007a,b; Yoo et al. 2007; Kim et al. 2008). Although Kim et al. (2007) determined the optimum feeding rate in juvenile olive flounder, *P. olivaceus*, weighing 3.6 g to be 2.6% body weight/day, the optimum feeding rates in all growth stages of this species have not been determined. Therefore, these feeding trials were conducted to determine the optimum feeding rates in the first and second stages of juvenile, growing and sub-adult olive flounder, *P. olivaceus*, in order to propose a computer simulated feeding program for this species.

II. Materials and methods

1. Experimental diets

Commercial feed used in all the feeding trials was supplied by Suhyup Feed Company Limited (Uiryeong, Gyeongsangnamdo, Republic of Korea). Pellet sizes with the corresponding proximate composition were selected according to the sizes of fish, as done on farm and in line with the company recommendation.

Feed of 2.0-2.2 mm diameter was used in experiment 1 (5 g) of the first stage of juvenile olive flounder and the feed contained 60.6% protein, 13.3% crude lipid, 13.4% crude ash, 8.23% moisture and 17.1 MJ/kg gross energy (Table 1). In the second experiment, 4-4.3 mm feed containing 56.8% protein, 14.4% crude lipid, 11.8% crude ash, 8.0% moisture and 17.6 MJ/kg gross energy was used (Table 4).

In the first and second experiments in the second stage of juvenile olive flounder, 4.0-4.3 mm feed containing 56.8% protein, 14.4% crude lipid, 11.8% crude ash, 8.0% moisture and 17.6 MJ/kg gross energy was used (Table 7). Experiments 3 and 4 of this stage were done with 6.0-6.3 mm feed containing 59.5% protein, 12.7% crude lipid, 11.3% crude ash, 8.51% moisture and 17.3 MJ/kg gross energy (Table 14).

Proximate composition of the diet used in experiments 1 and 2 in the growing stage of olive flounder is shown in Table 21. The 9.0-9.4 mm diameter feed contained 58.6%, 14.5%, 12.7%, 8.49% and 17.5 MJ/kg crude protein, crude lipid, crude ash, moisture and gross energy, respectively. Experiment 3 was conducted using 11.0-11.4 mm diameter feed containing 56.5% crude protein, 14.6% crude lipid, 13.2% crude ash, 8.51% moisture and 17.4 MJ/kg gross energy (Table 28).

Tables 32 and 36 contain proximate composition of feed used in the first and second experiments in the sub-adult stage of olive flounder, respectively. In the first experiment, 13.0-13.4 mm diameter feed containing 58.1% protein, 13.2%

crude lipid, 12.5% crude ash, 4.50% moisture and 17.3 MJ/kg gross energy was used while the second experiment was conducted using 15.0-15.4 mm diameter feed containing 56.0% crude protein, 14.3% crude lipid, 11.4% crude ash, 7.04% moisture and 17.6 MJ/kg gross energy.

2. Experimental fish and feeding trials

Fish for the feeding trials in the first stage of olive flounder were collected from Hampyeong, Cheonnam while those for the rest of the trials were obtained from Jeju-do, both in the Republic of Korea. Prior to start of the experiment, fish were fed the commercial diet twice daily to apparent satiation for a week to acclimate them to the experimental diet and conditions.

Twenty and fifteen fish with initial body weights of 4.97±0.11 g (mean±SD) and 9.18±0.13 g in experiments 1 (5 g) and 2 (9 g) of the first stage of juvenile olive flounder, respectively were randomly distributed into each of 28 aquaria. Each aquarium was then randomly assigned to one of the four replicates of the 7 respective feeding rates: 0, 3.0, 4.0, 4.25, 4.5 and 4.75% body weight (BW)/day and satiation (5.52% BW/day) in experiment 1 and 0, 2.0, 3.0, 3.5, 4.0 and 4.5% BW/day and satiation (4.64% BW/day) in experiment 2. Fish were fed three times a day (7:00, 13:00 and 19:00 h). The feeding trials were conducted by using a semi-recirculating system with twenty-eight 40-L rectangular aquaria receiving filtered seawater at the rates of 0.8 and 1.0 L/min in experiments 1 and 2, respectively from the center tank.

In the second stage of juvenile olive flounder fifteen fish with initial body weights of 20.2±0.54 g (mean±SD), 29.8±0.52 g, 40.4±0.69 g and 50.2±0.46 g in experiments 1 (20 g), 2 (30 g), 3 (40 g) and 4 (50 g), respectively were randomly distributed into each of 18 aquaria. Each aquarium was then randomly assigned to one of the three replicates of the 6 respective feeding rates: 0, 1.0, 2.0, 3.0 and 3.5% body weight (BW)/day and satiation (4.12% BW/day) in experiment 1; 0, 1.0, 2.0, 2.6 and 3.2% BW/day and satiation (3.53% BW/day) in experiment 2; 0, 1.0, 2.0, 2.4 and 2.8% BW/day and satiation (3.04% BW/day) in experiment 3; and 0, 1.0, 1.5, 2.0 and 2.5% BW/day and satiation (2.73% BW/day) in experiment 4. Fish were fed three times a day (8:00, 12:00 and 18:00 h). The feeding trials were conducted by using a flow-through system with eighteen 50, 100, 150 and 150 L aquaria in experiments 1, 2, 3 and 4, respectively receiving filtered seawater from the center tank.

For the growing olive flounder, twenty fish with initial body weights of 96.9±3.03 g (mean±SD), 240.4±11.01 g and 316.7±6.18 g in experiments 1 (97 g), 2 (240 g), and 3 (317 g), respectively were randomly distributed into each of 10 aquaria. Each aquarium was then randomly assigned to one of the duplicates of the 5 respective feeding rates: 0, 1.0, 1.65 and 2.3% BW/day and satiation (2.52% BW/day) in experiment 1; 0, 0.5, 0.75 and 1.0% BW/day and satiation (1.25% BW/day) in experiment 2; and 0, 0.4, 0.6, 0.8% BW/day and satiation (1.0% BW/day) in experiment 3. Fish were fed twice a day (9:00 and 16:00). Each feeding trial was conducted by using a flow-through system with ten 1 metric ton aquaria receiving filtered seawater from the center tank.

The feeding trials in the sub-adult stage were conducted with twenty-seven and thirty fish with initial body weights of 384.2±5.91 g and 525.37±7.12 g in experiments 1 (384 g) and 2 (525 g), respectively. In each experiment fish were randomly distributed into each of 10 aquaria. Each aquarium was then randomly assigned to one of the duplicates of the 5 respective feeding rates: 0, 0.3, 0.5 and 0.7% body weight (BW)/day and satiation (0.9% BW/day) in experiment 1; and 0, 0.2, 0.4 and 0.6% BW/day and satiation (0.8% BW/day) in experiment 2. Fish were fed twice a day (9:00 and 16:00). Each feeding trial was conducted by using a flow-through system with ten 1 metric ton aquaria receiving filtered seawater from the center tank.

Total body weight in each aquarium was determined every week and the amount of diet fed to the fish was adjusted accordingly in each trial. All the experimental aquaria were maintained at 14:10 (light:dark) and 12:12

(light:dark) in the first and second juvenile stages, respectively while the aquaria in the growing and sub-adult stages were maintained at natural light. The seawater was maintained at 20±1 °C by heaters in the center tank in the juvenile stages while in the growing and sub-adult stages the seawater temperature was 20.5-24 °C during the whole experimental period. Supplemental aerations were provided to maintain dissolved oxygen levels near 6.5±0.5 ppm and the salinity was 33±1ppt in all experiments. The duration of the experiments in the two stages of juvenile olive flounder was 2 weeks while the trials in the growing and sub-adult stages lasted for 3 weeks.

3. Sample collection and analysis

Weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), survival and whole-body proximate composition were calculated and measured at the end of the feeding trials. Blood analyses were also carried out on fish in the second juvenile, growing and sub-adult stages, as fish in the first juvenile stage were too small for blood to be obtained easily. Hematocrit (packed cell volume; PCV), hemoglobin (Hb), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood glucose and serum total protein were determined. Blood samples were obtained from the caudal vessels by using a heparinized syringe. Hematocrit was determined from three fish per aquarium using the microhematocrit method (Brown 1980) and hemoglobin (Hb) was measured in the same three fish by the cyanmethemoglobin procedure using Drabkin's reagent. An Hb standard prepared from human blood (Sigma Chemical, St Louis, MO, USA) was used. Three fish from each aquarium were used to analyze whole-body proximate composition. Proximate composition analyses of experimental diets and fish body were performed by the standard methods of AOAC (1995). Samples of diets and fish were dried to a constant weight at 105 C to determine moisture content. Ash was determined by incineration at 550 °C; crude lipid by soxhlet extraction using Soxtec system 1046 (Foss, Hoganas, Sweden) and crude protein by Kjeldahl method (N x 6.25) after acid digestion.

4. Histology

Clinical signs of fish were carefully monitored daily throughout this experiment. At the end of the feeding trial fish were autopsied and processed for histological examination. Autopsies were carried out on three randomly selected fish from each tank. All tissues and organs obtained were fixed in Bouins solution and processed for a routine histological examination.

5. Statistical analysis

All data were analyzed by one-way ANOVA (Statistix 3.1, Analytical Software, St. Paul, MN, USA) to test for the dietary treatments. When a significant treatment effect was observed, a Least Significant Difference (LSD) test was used to compare means. Treatment effects were considered significant at the $P < 0.05$ level. Broken-line model was used to analyze the weight gain of olive flounder in response to feeding rate (Robbins et al. 1979).

III. Results

1. Optimum feeding rates for the first stage (5 and 9 g) of juvenile olive flounder

At the end of two weeks of feeding trial in experiment 1, weight gain (WG) and specific growth rate (SGR) of fish fed to satiation (5.52% BW/day) were significantly higher than those of fish fed at other feeding rates and the unfed fish ($P < 0.05$) as shown in Table 2. There were no significant differences in these parameters among fish fed at 4, 4.25, 4.5 and 4.75% BW/day. Weight gain and SGR of fish fed at 3% BW/day were significantly higher than those of the starved fish but significantly lower than those of the other treatments. The unfed fish (0% BW/day) had negative growth, which was significantly lower than that of fish in other treatments. Feed efficiency (FE) and protein efficiency ratio (PER) of fish fed at 4.25% BW/day were significantly higher than those of fish fed at 3% BW/day and those fed to satiation. However, there were no significant differences in these parameters among the other treatments, except for the unfed fish, which had no values for these parameters, as they received no feed. Survival of the unfed fish was significantly lower than that of other treatments except for fish fed at 4% BW/day. However, there were no significant differences among fish in the other treatments. Broken line analysis of weight gain indicated that the optimum feeding rate in 5 g juvenile olive flounder could be 4.83% BW/day (Fig. 1).

Table 3 shows the whole-body proximate composition of 5 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates. There was a trend in decreased whole-body moisture and increased whole-body crude lipid with feeding rate, although there was a drop in crude lipid content of fish fed to satiation. Moisture and crude ash of the unfed fish were significantly higher than those in other treatments ($P < 0.05$). Moisture content was significantly lower in fish fed at 4.75% BW/day and those fed to satiation than in fish fed at 0, 3, 4, and 4.25% BW/day. There were no clear trends in whole-body crude protein of fish in all treatments.

Fig. 2 shows the histological changes of the hepatopancreas, kidney and anterior intestine of 5 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. The nucleus of the interstitial cells of fasting group appeared to be highly contracted and the hepatopancreas appeared to be in critical condition due to the acidolphil of the capillary, and the reduction in pancreatic zymogen granules. It was observed that the hepatocyte of fish appeared to have contracted, the capillary expanded and zymogen granules of the pancreas reduced, although these were not as serious in fish fed at 4% BW/day and those fed to satiation as in the unfed fish. Expansion of blood cells in the glomerulus and decrease in macrophage were observed in the pancreas of unfed fish. The basement membrane of the nephridium epithelial layer appeared to have thickened in these fish.

In the second experiment, WG and SGR of fish fed to satiation (4.64% BW/day) were significantly higher ($P < 0.05$) than those of fish in the other treatments (Table 5). These parameters progressively increased, being significantly higher for each treatment than for the preceding one. Feed efficiency and PER of fish fed at 2.0% BW/day were significantly lower than values for all the other fed fish. However, there were no significant differences in FE and PER among fish fed at 3.0, 3.5, 4.0, 4.5 and 4.64% BW/day. Survival was significantly higher in the unfed fish and in fish fed at 3% BW/day than in fish fed at 2.0% BW/day. However, there were no significant differences in survival among fish fed at 2.0, 3.5, 4.0, 4.5 and 4.64% BW/day or among the unfed and those fed at 3.0, 3.5, 4.0, 4.5 and 4.64% BW/day. Broken line analysis of weight gain showed that the optimum feeding rate in 9 g olive flounder could be 4.52% BW/day (Fig. 3).

Whole-body proximate composition of 9 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates is shown in Table 6. Whole-body moisture and crude ash were significantly higher while crude protein and crude lipid were significantly lower in the unfed fish than in the other treatments ($P < 0.05$). There were no significant differences in crude protein among all the fed fish. Crude lipid increased with feeding rates; crude lipid content was significantly higher for fish fed at 4.5 and 4.64% BW/day than for those in the other treatments.

Histological changes of the hepatopancreas, kidney and anterior intestine of 9 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks are shown in fig. 4. The liver cells of the unfed fish appeared much contracted, the capillary expanded and the zymogen granules of pancreas reduced. These observations were not as serious in fish fed at 3.5% BW/day as in the unfed fish and in those fed to satiation. The blood cells in the glomerulus appeared to have expanded, more macrophage observed, and the pronephric epithelial cell of epithelial layer expanded in the unfed fish than in fish fed at 3.5% BW/day and those fed to satiation. The nucleus of the middle intestine of unfed fish appeared to have contracted, and the length of the plica circularis shortened and gradually disappeared.

2. Optimum feeding rates for the second stage (20, 30, 40 and 50 g) of juvenile olive flounder

Weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and survival of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at varying feeding rates for two weeks are shown in Table 8. Weight gain and SGR for fish fed at 3.5% body weight (BW)/day and those fed to satiation (4.12% BW/day) were significantly higher than values for fish in the other treatments ($P < 0.05$). These parameters were significantly higher at each feeding rate than the preceding one for the other treatments. Feed efficiency was significantly higher in fish fed at 3.0, 3.5% and 4.12% BW/day than in those fed at 1.0 and 2.0% BW/day. Protein efficiency ratio for fish fed to satiation was significantly higher than values obtained in other treatments except for fish fed at 3.5% BW/day. Protein efficiency ratio was significantly higher in fish fed at 3.0 and 3.5% BW/day than in fish fed at 1.0 and 2.0% BW/day. But there were no significant differences in this parameter among fish fed at 3.0 and 3.5% BW/day. Protein efficiency ratio for fish fed at 1.0% BW/day was significantly lower than that for other treatments except for the unfed fish, which had no values. There were no significant differences in survival of fish fed at all feeding rates. Broken line analysis of weight gain showed the optimum feeding rate in 20 g juvenile olive flounder to be about 3.52% BW/day (Fig. 5).

Effects of feeding rates on serological characteristics of 20 g juvenile olive flounder, *P. olivaceus*, are depicted in Table 9. Hematocrit (HCT) was significantly higher in the unfed fish (0% BW/day) than in fish fed at 1.0, 2.0 and 3.5% BW/day ($P < 0.05$). There were no significant differences in HCT among the unfed fish, fish fed at 3.0% BW/day and those fed to satiation (4.12% BW/day) or among all the fed fish. Glutamic oxaloacetic transaminase (GOT) was significantly higher in the unfed fish than in all the fed ones. Although there were significant differences in GOT of the fed fish, there were no clear trends in this parameter. Glutamic pyruvic transaminase (GPT) increased and decreased after a peak value which was attained in fish fed at 2.0% BW/day. This parameter was significantly higher in fish fed at 2.0% BW/day than in the unfed fish and in those fed to satiation. Total protein was significantly lower in the unfed fish than in all the fed fish except for those fed at 2.0% BW/day. There were no significant differences in hemoglobin (Hb) and blood glucose of fish in all treatments.

Whole-body proximate composition of fish fed the experimental diet at different feeding rates is shown in Table 10. Moisture, crude protein and crude ash were significantly higher while crude lipid content was significantly lower in the unfed fish than in fish fed the experimental diet at all feeding rates. These parameters fluctuated for the fed fish.

Histological changes of the hepatopancreas, kidney, and anterior intestine of 20 g juvenile olive flounder fed the experimental diet at different feeding rates are shown in Fig. 6. The liver cell appeared contracted, the capillary expanded and the zymogen granules of the pancreas reduced in the unfed fish than in fish fed at 3.0% BW/day and in those fed to satiation. The same trend was observed in the expansion of the blood cell of glomerulus, the number of macrophages and the expansion of the cells of nephridium epithelial layer. Furthermore, the nucleus of the middle intestine appeared contracted and the length of the plica circularis shortened and gradually disappeared in the unfed fish than in other treatments.

Table 11 shows growth parameters of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet in experiment 2. After two weeks of feeding trial, WG and SGR for fish fed to satiation (3.53% BW/day) were significantly higher than those for fish in other treatments ($P < 0.05$). Weight gain and SGR for fish fed at 2.0, 2.6 and 3.2% BW/day were significantly higher than those for fish fed at 1.0% BW/day and for the unfed fish. The unfed fish had negative WG and SGR, which were significantly lower than those for the other treatments. Feed efficiency and PER of fish fed at 2.6, 3.2 and 3.53% BW/day were significantly higher than values obtained for fish fed at 1.0 and 2.0% BW/day. These parameters were significantly higher in fish fed at 2.0% BW/day than in those fed at 1.0% BW/day. The unfed fish had lower survival than in other treatments except for fish fed at 2.0% BW/day. There were no significant differences in survival among fish fed at 1.0, 2.0, 2.6, 3.2 and 3.53% BW/day or between the unfed and those fed at 2.0% BW/day. Broken line analysis of weight gain indicated the optimum feeding rate in 30 g juvenile olive flounder, *P. olivaceus*, to be 3.19% BW/day (Fig. 7).

Serological characteristics of fish fed the experimental diet at different feeding rates are shown in Table 12. Hemoglobin was significantly higher in the unfed fish than in those fed at 2.0% BW/day ($P < 0.05$). However, there were no significant differences in Hb among the unfed fish and those fed at 1.0, 2.6, 3.2 and 3.53% BW/day or among all the fed fish. Blood glucose was significantly higher in the unfed fish than in fish fed the experimental diet at all feeding rates except in those fed at 2.0% BW/day. There were no significant differences in this parameter among all the fed fish. Total protein was significantly lower in the unfed fish than in fish fed at all feeding rates except for those fed at 1.0% BW/day. Total protein was significantly higher in fish fed at 3.2 and 3.53%

BW/day than in fish fed at 0, 1.0 and 2.0% BW/day. There were no significant differences in HCT, GOT and GPT of fish in all treatments.

Apart from the whole-body crude protein of fish fed to satiation, whole-body moisture, crude protein and crude ash of the unfed fish were significantly higher ($P < 0.05$) while the crude lipid was significantly lower than values for fish fed the experimental diet at all feeding rates (Table 13). No consistent trend in these parameters was observed in the fed fish except for ash, which decreased and subsequently increased again with feeding rates. Whole-body ash content was significantly higher in fish fed to satiation than in those fed at 1.0, 2.0, 2.6 and 3.2% BW/day. This parameter was significantly higher in fish fed at 2.6 and 3.2% BW/day than in those fed at 1.0 and 2.0% BW/day.

Effects of feeding rate on histological changes of the hepatopancreas, kidney and anterior intestine of fish are depicted in Fig. 8. Fish fed at 2.6 and 3.53% BW/day showed better responses than did the unfed fish. Best condition was observed, however, in hepatopancreas, kidney and anterior intestine of fish fed at 2.6% BW/day.

Growth parameters of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates in experiment 3 are shown in Table 15. Weight gain and SGR of fish fed to satiation (3.04% BW/day) were significantly higher than values obtained for the unfed fish and for those fed at 1.0 and 2.0% BW/day ($P < 0.05$). However, there were no significant differences in WG and SGR among fish fed at 2.4, 2.8 and 3.04% BW/day. Also, there were no significant differences in WG and SGR of fish fed at 2.0, 2.4 and 2.8. Weight gain for the unfed fish was negative and significantly lower than values obtained for all the fed fish. Feed efficiency and PER were significantly lower in fish fed at 1.0% BW/day than in the rest of the fish fed the experimental diet; there were no significant differences in these parameters among the other fed fish. There were no significant differences in survival of fish in all treatments. Optimum feeding rate in 40 g olive flounder was determined to be about 3.02% BW/day based on broken line analysis of WG (Fig. 9).

Hematocrit (HCT) was significantly higher in the unfed fish than in fish fed at 1.0 and 2.8% BW/day ($P < 0.05$), as shown in Table 16. There were no significant differences in HCT among the unfed fish and those fed at 2.0, 2.4 and 3.04% BW/day or among those fed at 1.0 and 2.8% BW/day. There were no significant differences in Hb of fish in all treatments except that of the unfed fish, which was significantly higher than that in fish fed at 2.8% BW/day. Glutamic oxaloacetic transaminase was significantly higher in the unfed fish than in fish fed the experimental diet at all feeding rates, except for fish fed at 2.0% BW/day. There were no significant differences in GPT, blood glucose and total protein in all treatments except for the blood glucose in fish fed to satiation, which was significantly lower than values for all other treatments and the total protein of fish fed at 1.0% BW/day, which was significantly lower than values obtained for fish fed at 2.4, 2.8 and 3.04% BW/day.

Table 17 shows the effects of feeding rates on whole-body proximate composition of 40 g olive flounder fed the experimental diet at different feeding rates. Whole-body moisture content was significantly higher in the unfed fish than in fish fed the experimental diet at all feeding rates except for the whole-body moisture of fish fed to satiation. Crude lipid increased to a peak point for fish fed at 2.8% BW/day, which was significantly higher than values in all other treatments, and then decreased. Although there were significant differences in whole-body moisture, crude protein and crude ash, there were no clear trends in these parameters.

Fig. 10 shows the histological observation of tissues of 40 g juvenile olive flounder, *P. olivaceus*. Hepatopancreas, kidney and anterior intestine of fish fed at 2.4 and 3.04% BW/day were in better condition than those of the unfed fish.

In experiment 4, WG, SGR and PER for fish fed at 2.5% BW/day and those fed to satiation (2.73% BW/day) were significantly higher (*P* < 0.05) than those for fish in the other treatments (Table 18). These parameters increased with feeding rate for other treatments, being significantly higher at each feeding rate than the preceding one. Values of FE for the fed fish were not significantly different except for fish fed at 1.0% BW/day, which was significantly lower than values for other treatments. There were no significant differences in survival of fish in all treatments. The optimum feeding rate in 50 g olive flounder was estimated to be about 2.38% BW/day based on broken line analysis of WG (Fig. 11).

Hemoglobin was significantly higher (*P* < 0.05) in fish fed to satiation than in the unfed fish and in those fed at 2.0% BW/day (Table 19). There were no significant differences in HCT among fish fed at 1.0, 1.5, 2.0 and 2.5% BW/day or among those fed at 1.0, 1.5, 2.5 and 2.73% BW/day. There were no clear trends in GOT and no significant differences in GPT, blood glucose and total protein except for the total protein of the starved fish which was significantly lower than values in all fed fish, and for the blood glucose of fish fed to satiation, which was significantly higher than values in fish fed at 0, 1.0, 1.5, and 2.0% BW/day.

Moisture and crude ash were significantly higher while crude lipid was significantly lower in the whole-body of unfed fish than in fish fed the diet at all feeding rates (*P* < 0.05), as indicated in Table 20. Whole-body crude protein decreased to the minimum value at the feeding rate of 2.0% BW/day and subsequently increased. Whole-body crude lipid increased with feeding rate, except for fish fed to satiation, which was significantly lower than in the preceding treatment. Whole-body crude lipid was significantly higher in fish fed at 2.5% BW/day than in other treatments. There were no clear trends in crude ash of fish in all treatments.

Histological observation of tissues of 50 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates is shown in Fig. 12. Much difference was not found in the hepatopancreas, kidney and anterior intestine of fish fed at 0, 2 and 2.73% BW/day.

3. Optimum feeding rates for growing (97, 240 and 317 g) olive flounder

Weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER) and survival of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates in experiment 1 are shown in Table 22. Weight gain and specific growth rate for fish fed to satiation (2.52% BW/day) were significantly higher than those for the unfed fish and for fish fed at 1.0 and 1.65% BW/day ($P < 0.05$). There were no significant differences in values of these parameters between fish fed at 1.65% and those fed at 2.3% BW/day and between fish fed at 2.3% and those fed to satiation. Weight gain and SGR for the unfed fish were negative and significantly lower than those for fish in the other treatments. Feed efficiency and PER were significantly higher in fish fed to satiation than in the rest of the treatments. Also, FE was significantly higher in fish fed at 1.65 and 2.3% BW/day than in fish fed at 1.0% BW/day. Protein efficiency ratio increased with feeding rate, being higher at each feeding rate than the preceding one. Broken line analysis of WG indicated the optimum feeding rate in 97 g growing olive flounder, *P. olivaceus*, to be 2.23% BW/day as shown in Fig. 13.

Table 23 shows the effects of feeding rates on serological characteristics of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates. Blood glucose of fish fed at 1.65% BW/day was significantly lower than that of fish fed at 2.3% BW/day ($P < 0.05$). There were no significant differences in this parameter among fish fed at 0, 1.0, 1.65 and 2.52% BW/day or among those fed at 0, 1.0, 2.3 and 2.52% BW/day. There were no significant differences in hematocrit (HCT) and Glutamic pyruvic transaminase (GPT), and there were no clear trends in hemoglobin (Hb), Glutamic oxaloacetic transaminase (GOT) and total protein in all treatments.

Whole-body proximate composition of fish is shown in Table 24. Whole-body moisture and crude lipid were significantly higher while crude ash content was significantly lower in the unfed fish than in fish fed the experimental diet at all feeding rates ($P < 0.05$). Although there were significant differences in proximate composition of fish, there were no clear trends in any of the analyzed parameters.

Histological changes caused by the varying feeding rates in fish are displayed in Fig. 14. There was not much difference in the response of the hepatopancreas, kidney and anterior intestine of fish to the feeding rate for fish fed at 0, 1 and 2.52% BW/day.

In experiment 2, WG for fish fed to satiation (1.25% BW/day) was significantly higher ($P < 0.05$) than values for the unfed fish and for fish fed at 0.5 and 0.75% BW/day (Table 25). Weight gain of fish fed at 1.0% BW/day was significantly higher than values for the unfed fish and for those fed at 0.5% BW/day. However, there were no significant differences in WG between fish fed at 0.5% BW/day and those fed at 0.75% BW/day, between fish fed at 0.75% BW/day and those fed at 1.0% BW/day and between fish fed at 1.0% BW/day and those fed to satiation. Values of SGR for fish fed at 1.0% BW/day and those fed to satiation were significantly higher than values for the unfed fish and for those fed at 0.5% BW/day. There were no significant differences in SGR between fish fed at 0.5% BW/day and those fed at 0.75% BW/day or among fish fed at 0.75% BW/day, 1.0% BW/day and satiation. The optimum feeding rate in 240 g growing olive flounder, *P. olivaceus*, was indicated to be 1.09% BW/day based on broken line analysis of WG (Fig. 15).

Serological characteristics of growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates are shown in Table 26. Glutamic oxaloacetic transaminase of fish fed at 1.0% BW/day was significantly higher than values obtained in other treatments. There were no significant differences in this parameter among fish in other treatments. Total protein was significantly higher in unfed fish than in fish fed at 0.5% BW/day. There were no significant differences in total protein among the unfed fish and those fed at 0.75, 1.0 and 1.25% BW/day or among those fed at 0.5, 0.75, 1.0 and 1.25% BW/day. There were no significant differences in HCT, Hb, GPT and blood glucose of fish in all treatments.

Whole-body moisture content in fish fed to satiation was significantly lower ($P < 0.05$) than values for fish fed at 0.5 and 0.75% BW/day (Table 27). There were no significant differences in whole-body moisture among the unfed fish and those fed at 0.5, 0.75, and 1.0% BW/day or among the unfed and those fed at 1.0 and 1.25% BW/day. Crude protein was significantly higher in fish fed at 0.5 and 0.75% BW/day than in the unfed fish and in those fed at 1.0 and 1.25% BW/day. Crude protein was significantly lower in fish fed to satiation than in the other treatments. Whole-body crude lipid was significantly higher in fish fed to satiation than in the other treatments. Crude ash was significantly higher in fish fed at 0.75% BW/day than in the starved fish and in those fed at 1.0 and 1.25% BW/day.

Histological changes in tissues of olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates are shown Fig. 16. All the tissues were in good condition in fish fed at 0, 0.75 and 1.25% BW/day.

Growth performance of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates is shown in Table 29. Weight gain and SGR were significantly higher in fish fed to satiation (1.0% BW/day) than in other treatments ($P < 0.05$). These parameters were negative and significantly lower in the starved fish than in fish fed the experimental diet at all feeding rates. There were no significant differences in WG and SGR among fish fed at 0.4, 0.6

and 0.8% BW/day. There were no significant differences in FE, PER and survival of fish in all treatments. Based on broken line analysis of WG, the optimum feeding rate in 317 g growing olive flounder, *P. olivaceus*, could be 0.99% BW/day (Fig. 17).

Hematocrit (HCT) of fish fed to satiation (1.0% BW/day) was significantly lower ($P < 0.05$) than values obtained in other treatments (Table 30). There were no significant differences in HCT among the unfed fish and those fed at 0.4, 0.6 and 0.8% BW/day. Total protein was significantly higher in fish fed at 0.8% BW/day than in the unfed fish and in those fed at 1.0% BW/day. There were no significant differences in total protein among the starved fish and those fed at 0.4, 0.6 and 1.0% BW/day or among those fed at 0.6, 0.8 and 1.0% BW/day. There were no significant differences in Hb, GOT, GPT and blood glucose among all treatments, except for the GPT of fish fed at 0.8% BW/day, which was significantly lower than that of the starved fish.

Whole-body crude protein was significantly higher ($P < 0.05$) in fish fed at 0.6% BW/day than in other treatments (Table 31). Whole-body crude protein was significantly higher in the unfed fish and in those fed at 0.4% BW/day than in fish fed at 0.8 and 1.0% BW/day. There were no significant differences in whole-body crude protein of the starved fish and those fed at 0.4% BW/day or among those fed at 0.8 and 1.0% BW/day. Whole-body crude lipid of fish fed at 0.8% BW/day was significantly higher while that in fish fed at 0.6% BW/day was significantly lower than values in other treatments. Crude ash was significantly higher in fish fed at 0.6 and 1.0% BW/day than in other treatments.

Histological observation of tissues of fish in this feeding trial is shown in Fig. 18. Although hepatopancreas, kidney and anterior intestine of fish fed at 0.6% BW/day was in the best condition much difference was not found in tissues of fish fed at this feeding rate and those of fish fed at 0 and 1.0% BW/day.

4. Optimum feeding rates for sub-adult (384 and 525 g) olive flounder

At the end of the 3 weeks of feeding trial, weight gain (WG) and specific growth rate (SGR) were significantly higher ($P < 0.05$) in fish fed at 0.7% body weight (BW)/day and those fed to satiation (0.9% BW/day) than in fish fed at other feeding rates and in the unfed fish in experiment 1 (Table 33). The above parameters were negative and significantly lower in the unfed fish than in those fed the experimental diet. There were no significant differences in WG and SGR among fish fed at 0.3 and 0.5% BW/day and among those fed at 0.7% BW/day and satiation. There were no significant differences in FE, PER and survival of fish in all treatments. The optimum feeding rate in 384 g sub-adult olive flounder, *P. olivaceus*, was found to be 0.74% BW/day based on broken line analysis of WG as indicated in Fig. 19.

Table 34 shows the serological characteristics of 384 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for three weeks. Glutamic oxaloacetic transaminase (GOT) was significantly higher in fish fed at 0.5% BW/day than in other treatments except for fish fed to satiation (0.9% BW/day). There were no significant differences in this parameter among the unfed fish and those fed at 0.3, 0.7 and 0.9% BW/day or among those fed at 0.5 and 0.9% BW/day. There were no significant differences in Glutamic pyruvic transaminase (GPT) and total protein of fish in all treatments except in the value of GPT for fish fed at 0.5% BW/day, which was significantly lower than in those fed to satiation and also in total protein of fish fed at 0.5% BW/day, which was significantly higher than values for the unfed fish and those fed at 0.3 and 0.7% BW/day. There were no significant differences in hemoglobin (Hb) or blood glucose of fish in all treatments.

Whole-body proximate composition of fish is shown in Table 35. Whole-body crude protein was significantly higher in fish fed to satiation (0.9% BW/day) than in other treatments ($P < 0.05$). There were no significant differences in whole-body crude protein among the unfed fish and those fed at 0.3, 0.5 and 0.7% BW/day. Whole-body crude lipid was significantly higher in fish fed at 0.3 and 0.7% BW/day, and significantly lower in fish fed to satiation than in other treatments. There were no clear trends in whole-body crude ash content of fish in all treatments although crude ash content was significantly higher in the starved fish than in all other treatments.

Fig. 20 depicts the histological changes in tissues of olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates. Tissues of fish fed at 0, 0.5 and 0.9% BW/day were all in good condition.

In experiment 2 (Table 37), WG and SGR were significantly higher in fish fed to satiation (0.8% BW/day) than in those fed at 0.2% BW/day and in the unfed fish ($P < 0.05$). There were no significant differences in WG and SGR among fish fed at 0.2, 0.4 and 0.6% BW/day and among those fed at 0.4, 0.6 and 0.8% BW/day. Weight gain and SGR of the unfed fish were negative and significantly lower than those of fish fed the experimental diet at all feeding rates. There were no significant differences in FE and PER of fish in all treatments. Broken line analysis of WG indicated the optimum feeding rate in 525 g sub-adult olive flounder, *P. olivaceus*, to be 0.70% BW/day (Fig 21).

Serological characteristics of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for three weeks is shown in Table 38. Hematocrit was significantly higher ($P < 0.05$) in fish fed at 0.2, 0.4 and 0.6% BW/day than in the unfed and in fish fed to satiation (0.8% BW/day). There were no significant differences in HCT among the unfed fish and those fed to satiation or among those fed at 0.2, 0.4 and 0.6% BW/day. Blood glucose was significantly higher in fish fed at 0.6% BW/day than in the unfed fish and in those fed at 0.2 and 0.8% BW/day. There were no significant differences in Hb, GOT, GPT or total protein of fish in all treatments.

Table 39 shows the whole-body proximate composition of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for three weeks. Although there were significant differences in all analyzed parameters, there were no clear trends in any of them.

Histological changes in tissues of fish fed the experimental diet at different feeding rates are shown in Fig. 22. There was not much difference in the condition of hepatopancreas, kidney and anterior intestine of fish fed at 0, 0.4 and 0.8% BW/day.

IV. Discussion and summary

Growth of olive flounder, *P. olivaceus*, improved with feeding rate in all experiments. Both weight gain (WG) and specific growth rate (SGR) increased with feeding rate up to satiation. Growth has been reported to increase with feeding rate in several fish species (De Silva et al. 1986; Hung and Lutes 1987; Xiao-Jun and Ruyung 1992; Adebayo et al. 2000; Ng et al. 2000; Mihelakakis et al. 2002). Fish appear to continuously increase in weight with feeding rate up to satiation although this increase may not correspond with the incremental feed consumption. Continuous increase in weight gain with feeding rate has been recorded in blackspot seabream, *Pagellus Bogaraveo* (Ozorio et al. 2009); olive flounder, *P. olivaceus* (Cho et al. 2007); European sea bass, *Dicentrarchus labrax* L. (Eroldogan et al. 2004); white sturgeon, *Acipenser transmontanus* (Deng et al. 2003); and gilthead sea bream, *Spurus aurata* (Mihelakakis et al. 2002) among others. However, improvement in growth performance of fish below satiation varied with fish sizes in this study. Weight gain of 9, 40, 97, 240 and 525 g fish improved more remarkably below satiation than did this parameter in other sizes of fish. Although WG continued to increase up to satiation in all sizes of fish there was no marked change in WG of 5 g fish for instance, between the feeding rates of 4 and 4.75% body weight (BW)/day. A similar observation was recorded in this parameter among the last three treatments before satiation in 30 and 317 g fish while there were no marked improvements in growth of fish fed at the level immediately below satiation and those fed to satiation in 20, 50, and 384 g fish. But in all the experiments, growth continued to improve with feeding rate up to satiation.

Feed utilization on the other hand leveled out after a certain feeding rate in almost all feeding trials. This could be seen from the peaks in feed efficiency and protein efficiency ratio and the drop after the peaks in these parameters in the 5 g fish. Maximum feed efficiency has been reported to be achieved at feeding rates below that required for maximum growth (Brett and Groves, 1979). At low feeding rates, fish tend to optimize their digestion to extract nutrients more efficiently (Zoccarato et al. 1994, Van Ham et al. 2003). This implies an inverse relationship between feeding rate and feed efficiency. The peak in feed efficiency in the present study could be explained on the basis of greater weight gain than the incremental feed intake at feeding rates below the peak value. But beyond the peak fish could not efficiently utilize the additional food, leading to the drop in feed efficiency.

Comparing the optimum feeding rates in the different growth stages, it could be seen that the optimum feeding rates decreased with fish size. This observation agrees with results in previous studies in various species (Page and Andrews 1973; Skalli et al. 2004; Hatlen et al. 2005; Sweilum et al. 2005). The optimum feeding rate for 30 g white sturgeon, *Acipenser transmontanus*, was reported to be 2.0-3.0% BW/day (Hung et al. 1993) while the feeding rates for 30-100 g and 0.25-0.5 kg fish of the same species were found to be 2.0% BW/day and 1.5-2.0% BW/day, respectively (Hung and Lutes 1987; Hung et al. 1989). Similarly, Cho et al. (2007) reported the optimum feeding rate for juvenile olive flounder, *P. olivaceus*, grown from 17 to 90 g to be 95% of satiation while that for 319 g sub-adult fish was 90% of satiation. Growth rates of fish decrease with age. This implies increased demand for food to meet high metabolic rates in small fish; hence, the corresponding decrease in feeding rates with size.

Whole-body proximate composition of fish indicated higher content of water and ash and lower content of lipid in the unfed fish than in those that received feed in most of these trials. While lipid seemed to accumulate with feeding rate in the 9 g fish the trend was not so clear in the 5 g fish; this discrepancy follows in other fish sizes in these trials. Whole-body proximate composition seems to be a weak parameter in determining feeding rate. Although Wang et al. (2007) reported increase in moisture and ash contents with corresponding decrease in lipid content of cuneat drum, *Nibea miichthioides*, with feeding rate such a relationship was never observed in olive flounder (Cho et al. 2007). Cho et al. (2006) observed no relationship between feeding rate and proximate composition of flounder except for crude protein. There was a trend towards decreased moisture content and increased protein and lipid with age of fish in the first two trials, as shown by the proximate composition of fish although this trend was not pronounced. Deng et al. 2003 recorded a similar trend as larval white sturgeon, *Acipenser transmontanus*, grew during the first four weeks after initiation of feeding. But this trend was inconsistent in larger fish in the current trials.

In conclusion, based on broken line analysis of WG the optimum feeding rates for 5, 9, 20, 30, 40 and 50 g juvenile olive flounder, *P. olivaceus*, could be 4.83, 4.52, 3.52, 3.19, 3.02 and 2.38% BW/day, respectively. Broken line analysis of WG indicated the optimum feeding rates in 97, 240 and 317 g growing olive flounder, *P. olivaceus*, to be 2.23, 1.09 and 0.99% BW/day, respectively while those in 384 g and 525 g sub-adult fish could be 0.74 and 0.70% BW/day, respectively.

Chapter 3. Development and application of the computer simulated feeding program

I. Introduction

The development and use of computer tools for aquaculture has shown considerable advancements due to the rapid expansion of both aquaculture production and computer technology in the last few decades (Ernst and Nath 2000). Products are available for a wide variety of purposes. Computer monitoring and automation has been employed in algae and food production (Rusch and Malone 1991, 1993), feed management (Hoy 1985), filtration systems (Whitson et al. 1993; Lee et al. 1995, 2000; Turk et al. 1997), vision systems (Whitsell and Lee 1994; Whitsell et al. 1997), environmental monitoring and control (Hansen 1987; Ebeling 1991, 1993; Munasinghe et al. 1993) and integrated system management (Lee 1991, 1993; Lee et al. 1995, 2000; Turk et al. 1997).

Of particular interest is the use of custom-designed control systems in feed delivery. A microcomputer was used to estimate and control feeding in a fish hatchery system as well as to measure and control temperature (Hoy1985). A microcomputer-based feed delivery system that allowed controlled experiments on feeding ratio and frequency was described by Ruohonen (1987). Grimsen et al. (1987) reported a feeding system that allowed feed delivery to each compartment of a lobster culture system and the automated recording of feeding rate per individual lobster. This system was composed of a custom-programmed microcomputer and air-actuated feeders controlled by the computer. Feed was continuously delivered to rainbow trout using a production-scale automated feeding system (Widmyer and Widmyer 1993). A central digital controller set the motor speed of the auger-driven feed hoppers so that feed delivery rate as well as feeding frequency could be controlled.

Some of the feed delivery systems, however, could be too sophisticated or expensive to be used on an average farm. Moreover, the feeding rates used in design of these programs are often based on feeding charts issued by the feed suppliers, usually in conjunction with the practical experience of farmers and researchers (Ballestrazzi et al. 1998; Morris and Robert 2001). These charts prepared under idealized environmental conditions are not always appropriate due to several variables such as the peculiarity of farms and farmers, fish species, fish sizes and size distribution, stocking rate, water temperature, other water quality parameters and feeding frequency. Hence, it would be helpful if a program is designed to calculate the daily feed allowance based on practical feeding rate trials in various sizes of a particular species, and to allow farmers the option of installing automated feeders or manually supplying the feed based on results of the computation. This program would make it possible for an average farmer with some knowledge of the use of a computer to calculate the daily feed allowance and hand-feed fish based on the result of the calculation without the need to install complicated feeding equipment. This program could also be incorporated into a fully automated feeding system.

This study was carried out to propose a computer simulated feeding program for olive flounder based on results of optimum feeding rate trials at different growth stages of this species at the optimum temperature. The proposed program was designed to be used to calculate the feeding rates based on real situation on individual farms. Input from the operator includes fish weight, number of fish and water temperature. Results are displayed as feeding rate in percent and actual weight of fish per tank in kilograms. Available features allow for easy data management and printing of results.

II. Materials and methods

1. Program development

This simulated feeding program was developed based on the feeding trials conducted to determine the optimum feeding rates in olive flounder, *P. olivaceus*, at the optimum temperature. Besides olive flounder, Korean rockfish, *Sebastes schlegeli*; common sea bream, *Sparus aurata*; and black sea bream, *Acanthopagrus schlegeli* were included to allow for adaptation of the program to farms that culture other species. The acquired results were used to develop the program to collect information from users and estimate the optimum feeding rate for fish in real farm situations in percent and the total amount of feed to be fed in kilogram. Users are prompted to enter actual values for a particular tank on the farm such as the fish species, fish weight, number of fish, and rearing water temperature. Built in features enable individual farms to be registered and daily feeding rates for various tanks to be calculated and saved by group. Results can be presented as lists, charts and printed copies. The first screen of the program is the welcome screen, which displays the name of the program. On top of the screen is the menu bar that contains menus that could be clicked on to go to various pages of the program. Basically, the program consists of the feeding rate calculation, database, group management, actual data and farm management (Fig. 23).

(1) Feeding rate calculation

The "Feeding Rate Calculation" page of the simulated feeding program includes features and functions to allow users enter basic information such as fish species, temperature and weight of fish. The program is designed to use this information to automatically calculate the feeding rate and store results of the calculation in a database. The algorithm for calculation of the feeding rate is shown in Fig. 24.

(2) Calculation list

This page lists results of calculations previously done on the “Feeding Rate Calculation” page. The page was designed to sort entries by the automatic filtering system and display individual entries in the form of charts and print.

(3) Group management

The Group Management page is used to sort lists of actual data by group. It allows for registration, modification and deletion of groups.

(4) Actual data entry

This was designed using actual data from experiments. It calculates and records actual data entered by a user.

(5) Actual data management

This page enables management of saved data list from “Actual Data Entry” page. From this page, data can be presented as charts and print.

(6) Farm management

By registration of new farms, this page enables efficient organization of feeding rate computations by farm. The page has various functions that can calculate the daily feed allowance with the automatic feeding rate calculating system. Through sampling, this page was designed to calculate the next feed allowance per tank based on feed conversion ratio, mortality and specific growth rate of fish. After calculating and saving each daily feed allowance the feed conversion ratio, mortality and specific growth rate of the fish is updated.

2. Program description and application

At the top of the program are the Start Screen, Feeding Rate Calculation, Calculation List, Group Management, Actual Data Entry, Actual Data Management, Farm Management, Current Water Temperature, Program Information, and End menus. Clicking on any of these menus takes you to the indicated page. To test-run the developed program, some hypothetical values were entered and feeding rates were calculated base on the entered data.

(1) Start screen

The “Start Screen”, shown in Fig. 25 is the opening screen of the program. It displays the name of the program.

(2) Feeding rate calculation

This page is for automatically calculating the optimum feeding rate using some parameters entered by the user. There are features for selecting fish species and water temperature as well as entering fish weight and number of fish in the tank. To calculate the optimum feeding rate, the user is prompted to enter this information as explained below using our hypothetical values.

a. Selection of Species

Clicking on the “Species” button opens a dialog box displaying four species of fish: olive flounder, *P. olivaceus*; Korean rockfish, *Sebastes schlegeli*; common sea bream, *Sparus aurata*; and black sea bream, *Acanthopagrus schlegeli*. Here the species of interest can be selected. In the trial run, olive flounder was selected as shown in Fig. 26.

b. Selection of the rearing water temperature

Water temperature can be selected by clicking on the “Temperature” button. This displays a list of temperatures from which users can select according to the temperature of the rearing water in their tank. In the current study 20 °C was selected (Fig. 27).

c. Fish weight

To enter fish weight, a user clicks on the “Fish Weight” button and types the weight directly with the keyboard. In Fig. 28 the weight of the fish, 20 g, was typed in the text box.

d. Number of fish

Similar to fish weight, a user clicks on “Number of Fish” button and types the total number of fish in the rearing tank directly in the text box with the keyboard. One fish was used in this calculation; hence, 1 was typed in the text box (Fig. 29).

e. Feeding rate calculation

After making the selections and entering the necessary data, “Feeding Rate Calculation” button can be clicked on. This displays the optimum feeding rate in terms of percent feeding rate, weight of feed per fish and total weight of feed for the tank of interest. Fig. 30 showed the optimum feeding rate to be 3.69% and the weight of feed to be supplied as 0.74 g or 0.00074 kg.

(3) Calculation list

This is the page where results of computations done on the feeding rate calculation page are displayed. It lists all calculations by the date and time they were saved. The current page, Fig. 31, shows calculations done at different times on November 15, 2009. Information contained here include species, temperature, fish weight, number of fish, optimum feeding rate in percent, daily feed allowance per fish in gram and total feed allowance per tank in kilogram. Entries in this list can be sorted by species, temperature and weight. Furthermore, lists can be sorted by the dates of creation/modification by selecting “Recent Record” from the “Sort” drop down list. Individual entries can be presented as charts by clicking on the “Chart” button. Clicking on the “Print Current Page” button at the bottom right corner of the screen opens the “Print” dialog box. Clicking on the “Print” button in the dialog box in turn displays the “Print Option” dialog box. The current list can be printed by finally clicking on the “Print” button in the Print Option dialog box. Conversely, clicking on “Cancel” button closes the box without printing the page.

(4) Group management

This enables the grouping of data entries. The “Group Registration” button opens a dialog box to type the name of the group to be registered. Data for this group can be subsequently entered and processed on the appropriate pages. Two groups were registered during the study as shown in Fig. 32.

(5) Actual data entry

This is for entering actual data from farms. Fig. 33 shows the hypothetical data entered under a group called Jeju-do for 20 g olive flounder reared at 20 °C. The data entry method detailed below is basically the same as that for feeding rate calculation explained in the previous section.

a. Group selection

Clicking on the “Group” button displays a list of groups previously entered on the “Group Management” page. The group for which data is to be entered (Jeju-do in the present study) can be selected from this list.

b. Species selection

The "Species" button opens a dialog box containing the four species previously mentioned. The species of interest (olive flounder) can be selected from this box.

c. Temperature selection

A list of available temperatures can be selected by clicking on the "Temperature" button. This opens a dialog box containing the available water temperatures. Users can select the temperature closest to the rearing water temperature in their tank.

d. Fish weight

A dialog box opens on clicking on "Fish Weight" button. The average weight of the fish whose feeding rate is to be calculated can be directly entered here with the keyboard.

e. Optimum feeding rate

Clicking on the "Optimum Feeding Rate" button opens a dialog box into which the optimum feeding rate of the fish can be entered. This is the feeding rate obtained by the optimum feeding rate trial at the optimum temperature.

f. Automatic calculation of feeding rate

The feeding rate can be automatically calculated by clicking on the "Calculate Feeding Rate" button.

g. Saving actual data

Entered data can be saved by clicking on the "Save Data" button. The saved data can be managed by clicking on the "Data Management" menu at the top of the screen.

(6) Actual data management

Saved data entered on the “Actual Data Entry” page can be managed on the “Actual Data Management” page.

(7) Farm management

This is where farms are registered and data for each farm is managed as shown in Fig. 34 for the fish farm called “test” and explained below.

a. Registration of new farms

At the top right side of the Farm Management page is the “New Farm Registration” button. The dialog box that pops up on clicking on this button can be moved by clicking and dragging with the mouse.

b. Species selection

The species to be raised on the new farm can be selected from the available species in the dialog box that opens on clicking on “Species” button.

c. Temperature selection

The proposed rearing water temperature of the new farm can be selected from a list of available temperatures by clicking on the “Temperature” button.

d. Register

After all selections are made, “Register” button can be clicked on to have the new farm registered.

e. Description of the farm list page

The farm list page consists of the “View Details”, “Diary”, and “Delete” buttons. The farm list page is automatically updated on registration of a new diary.

f. Farm details

Clicking on the “Details View” button opens a dialog box that shows detailed information about the farm. From this dialog box, information such as the farm name, fish species and temperature can be modified.

g. Description of the diary list

The “Diary List” window opens on clicking on the “Diary” button. The “Prepare Diary” button at the top right corner of the window can then be clicked on to prepare a diary.

h. Description of the “Prepare Diary” dialog box

Information about the fish for which the diary is prepared can be entered here. This includes the water temperature, fish weight, number of fish, etc.

i. Diary registration

By default, on opening the “Prepare Diary” dialog box, all information is automatically displayed. This information can then be saved.

j. Update and close

As previously mentioned, the diary list is automatically updated on preparation of a new diary. In addition to this, farm information is automatically updated. After processing of entered data, results of computations are used to modify information about the fish such as the estimated fish weight, mortality, specific growth rate and feeding rate.

III. Results and summary

The intended computer simulated feeding program was successfully proposed using results obtained from studies conducted to determine the optimum feeding rates in olive flounder at the juvenile, growing and sub-adult stages of this species. The program was subsequently run using some hypothetical values. At present, the user interface is designed in Korean Language.

Using a hypothetical farm and group called test and Jeju-do, respectively, various functions and features of the designed program were tested. The farm and group were successfully registered, data entered, and the feeding rate determined for 20 g juvenile olive flounder reared at 20 °C. The registered group, fish species and rearing water temperature were selected; fish weight and number of fish to be reared typed in the appropriate text boxes; and results of the calculation successfully displayed as designed. Using this and other information input into the computer various features and functions were tested.

The optimum feeding rate in 20 g juvenile olive flounder reared at 20 °C was determined by the program to be 3.69% body weight (BW)/day and the actual amount of feed to be fed this fish per day was 0.74 g or 0.00074 kg according to the program. The calculated feeding rate, though higher, is not much different from the 3.52% BW/day for 20 g juvenile olive flounder determined by the feeding rate trial. Granted that the slight difference in feeding rate translates into a lot of money on the large scale production, it is quite acceptable for the test run. It is expected that subsequent modifications would reconcile the discrepancy between the calculated feeding rate and that obtained by the actual feeding trial.

In conclusion, the proposed feeding program performed as designed, although hypothetical data was used. Feeding rate obtained by this program was not much different from that from actual feeding rate trial for fish of the same species and weight and at similar temperatures.

IV. Future development

As previously mentioned, this program was tested using some hypothetical data. In the future the program will be tested on the farm to see how well it could work in the real life situation. Problems arising from this application could then be documented for debugging and further improvement.

Basic functions are installed in the current form of the program. However, on installation of the program on a test farm, some other functions that might be of help in feed and farm management are likely to be realized. Moreover, compatibility issues may arise and these need to be addressed. For now the program runs smoothly with popular operating system and application software.

At present, the feeding rate for only four species can be determined using the feeding program. Also, the program provides a few values of temperature. Furthermore, the program was designed using sample weights from growth stages of the fish. Future development will look into the expansion of the program to include more species, temperatures and results obtained from feeding rate trials in other sizes of fish not included in this initial design.

For wider use of this program, future development would look into the translation of the program into other languages, with features for language selection built in. With the dramatic increase in the use of English in Korea and the expansion of trade relationship with various countries whose native language is English, there is the need to translate this program into English. Subsequently, other languages such as Chinese and Japanese could be incorporated considering the closeness of China and Japan to Korea and the trade and cultural relationships among them. In the future, it is expected that the program will be perfected and made available in other languages besides those already mentioned.

Chapter 4. General discussion and summary

I. Discussion

Feeding trials to determine the optimum feeding rates for olive flounder, *Paralichthys olivaceus*, fed extruded pellets at the optimum temperature were successfully carried out and results obtained from the trials were used in proposing the intended computer simulated feeding program.

Growth of olive flounder, *P. olivaceus*, improved with feeding rate in all experiments. Both weight gain (WG) and specific growth rate (SGR) increased with feeding rate up to satiation. Feed utilization on the other hand leveled out after a certain feeding rate in almost all feeding trials. The optimum feeding rates varied with fish size, being higher for younger fish than for the older ones. Growth performance was found to respond better to the treatments than did proximate composition, histological response or serological characteristics of fish in these feeding trials.

The proposed computer simulated feeding program was successfully designed using results obtained from the feeding trials. Hypothetical values were entered to test the program, simulating real-farm situations. The built in features and functions performed well. The feeding rate obtained using the hypothetical data compared well with the value obtained for fish of the same weight and species in the feeding trial.

II. Summary

The proposed feeding trials to determine the optimum feeding rates for olive flounder, *P. olivaceus*, fed extruded pellets at the optimum temperature were successfully carried out and results obtained from the trials were used in proposing the intended computer simulated feeding program. Broken line

analysis of weight gain indicated that the optimum feeding rates for 5, 9, 20, 30, 40, 50, 97, 240, 317, 384 and 525 g olive flounder, *P. olivaceus*, could be 4.83, 4.52, 3.52, 3.19, 3.02, 2.38, 2.23, 1.09, 0.99, 0.74 and 0.70% body weight (BW)/day, respectively. Optimum feeding rate determined for 20 g fish by the proposed feeding program was 3.69% BW/day. It was recommended that the feeding program be tried on the farm with real data. The inclusion of other languages, features and functions was also recommended.

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Tables

Table 1. Proximate composition of the experimental diet for 5 g juvenile olive flounder, *Paralichthys olivaceus* (dry matter basis)

	Composition
Moisture (%)	8.23
Crude protein (%)	60.6
Crude lipid (%)	13.3
Crude ash (%)	13.4
Gross energy (MJ/kg)	17.1
Diet size (mm)	2.0~2.2

Table 2. Effects of feeding rates on growth performance of 5 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets							Pooled
	0%	3%	4%	4.25%	4.5%	4.75%	S[2]	SEM[9]
IW[3]	5.03	4.90	4.99	4.96	4.98	5.01	4.92	0.02
FW[4]	3.96	7.32	8.72	9.17	9.17	9.35	10.7	0.39
WG[5]	-21.3[d]	49.5[c]	74.8[b]	84.9[b]	84.6[b]	86.7[b]	115.9[a]	8.02
SGR[6]	-1.84[d]	3.09[c]	4.29[b]	4.72[b]	4.70[b]	4.80[b]	5.90[a]	0.46
FE[7]	-	123[b]	132[ab]	141[a]	134[ab]	133[ab]	121[b]	2.16
PER[8]	-	2.25[b]	2.42[ab]	2.59[a]	2.45[ab]	2.43[ab]	2.22[b]	0.04
Survival (%)	93.3[b]	98.8[a]	97.5[ab]	98.8[a]	100[a]	100[a]	100[a]	0.65

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (5.52%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 3. Whole-body proximate composition of 5 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks (%)[1]

	Diets							Pooled
	0%	3%	4%	4.25%	4.5%	4.75%	S[2]	SEM[3]
Moisture	81.5[a]	78.1[bc]	78.2[bc]	78.5[b]	78.0[cd]	77.5[e]	77.7[de]	0.24
Crude protein	71.2[b]	76.7[a]	69.7[bc]	70.8[bc]	71.1[bc]	70.4[bc]	69.3[c]	0.49
Crude lipid	2.67[e]	4.65[d]	6.79[c]	7.38[bc]	7.77[ab]	8.07[a]	7.42[b]	0.36
Crude ash	32.5[a]	20.4[b]	18.8[c]	18.5[cd]	18.0[de]	17.9[de]	17.6[e]	0.96

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (5.52%)

[3]Pooled standard error of means: SD/√n

Table 4. Proximate composition of the experimental diet for 9 g juvenile olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	8.00
Crude protein (%)	56.8
Crude lipid (%)	14.4
Crude ash (%)	11.8
Gross energy (MJ/kg)	17.6 MJ/kg
Diet size (mm)	4.0~4.3 mm

Table 5. Effects of feeding rates on growth performance of 9 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets							Pooled SEM[9]
	0%	2.0%	3.0%	3.5%	4.0%	4.5%	S[2]	
IW[3]	9.19	9.22	9.28	9.14	9.21	9.07	9.13	0.02
FW[4]	7.68	11.8	14.5	15.3	16.9	17.7	18.8	0.67
WG[5]	-16.6[g]	27.5[f]	56.6[e]	67.9[d]	83.4[c]	94.9[b]	114.0[a]	7.57
SGR[6]	-1.39[g]	1.85[f]	3.45[e]	3.98[d]	4.67[c]	5.13[b]	5.87[a]	0.45
FE[7]	-	94.2[b]	130[a]	129[a]	133[a]	132[a]	137[a]	3.33
PER[8]	-	1.73[b]	2.38[a]	2.37[a]	2.44[a]	2.43[a]	2.51[a]	0.06
Survival (%)	100[a]	93.3[b]	100[a]	96.7[ab]	98.3[ab]	98.3[ab]	96.7[ab]	7.57

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (4.64%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 6. Whole-body proximate composition of 9 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks (%)[1]

	Diets							Pooled
	0%	2.0%	3.0%	3.5%	4.0%	4.5%	S[2]	SEM[3]
Moisture	79.0[a]	77.0[b]	75.7[cd]	76.5[bc]	75.2[d]	75.5[cd]	75.5[cd]	0.22
Crude protein	69.4[b]	75.3[a]	74.7[a]	73.2[a]	73.8[a]	73.3[a]	75.2[a]	0.44
Crude lipid	2.98[e]	4.80[d]	7.23[c]	7.49[c]	8.81[b]	10.3[a]	10.2[a]	0.40
Crude ash	26.9[a]	19.2[b]	17.6[c]	17.5[c]	16.6[cd]	16.0[d]	15.3[d]	0.59

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (4.64%)

[3]Pooled standard error of means: SD/√n

Table 7. Proximate composition of the experimental diet for 20 and 30 g juvenile olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	8.00
Crude protein (%)	56.8
Crude lipid (%)	14.4
Crude ash (%)	11.8
Gross energy (MJ/kg)	17.6 MJ/kg
Diet size (mm)	4.0~4.3 mm

Table 8. Effects of feeding rates on growth performance of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	2.0%	3.0%	3.5%	S[2]	SEM[9]
IW (g/fish)[3]	19.9	20.6	20.1	20.5	20.1	19.9	0.13
FW (g/fish)[4]	19.2	23.6	26.7	30.0	32.0	32.0	1.18
WG (%)[5]	-3.0[e]	14.8[d]	33.0[c]	46.5[b]	59.1[a]	61.2[a]	5.81
SGR %/day[6]	-0.26[e]	1.15[d]	2.35[c]	3.17[b]	3.87[a]	3.98[a]	0.38
FE (%)[7]	-	89.3[c]	109[b]	128[a]	132[a]	131[a]	4.45
PER[8]	-	0.74[d]	1.46[c]	2.15[b]	2.52[ab]	2.64[a]	0.18
Survival (%)	96.6	96.5	98.9	98.9	96.6	98.9	0.62

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (4.12%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 9. Effects of feeding rates on serological characteristics of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	2.0%	3.0%	3.5%	S[2]	SEM[9]
HCT[3]	28.00[a]	23.17[b]	21.50[b]	25.00[ab]	21.17[b]	25.50[ab]	0.76
Hb[4]	5.11	5.20	5.04	4.62	4.63	4.52	0.15
GOT[5]	61.49[a]	26.84[bc]	21.70[c]	34.65[b]	22.52[c]	28.10[bc]	4.23
GPT[6]	3.26[b]	12.69[ab]	21.67[a]	11.15[ab]	9.06[ab]	7.38[b]	1.97
Glucose[7]	20.28	25.65	25.63	27.21	21.57	28.84	1.55
Total protein[8]	2.57[c]	3.33[ab]	2.89[bc]	3.10[ab]	3.49[a]	3.27[ab]	0.09

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (4.12%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 10. Whole-body proximate composition of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks (%)[1]

	Diets						Pooled
	0%	1.0%	2.0%	3.0%	3.5%	S[2]	SEM[3]
Moisture	77.2[a]	76.1[b]	75.4[e]	75.7[d]	73.9[f]	75.9[c]	0.21
Crude protein	77.5[a]	76.0[b]	74.4[c]	73.2[d]	72.0[e]	74.2[c]	0.38
Crude lipid	1.93[e]	7.35[d]	8.73[b]	7.99[c]	11.3[a]	7.39[d]	0.59
Crude ash	19.7[a]	16.9[b]	15.6[c]	15.8[c]	14.7[d]	15.8[c]	0.34

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (4.12%)

[3]Pooled standard error of means: SD/√n

Table 11. Effects of feeding rates on growth performance of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	2.0%	2.6%	3.2%	S[2]	SEM[9]
IW (g/fish)[3]	29.7	29.9	29.3	30.1	29.8	30.0	0.12
FW (g/fish)[4]	28.9	35.2	41.6	44.4	43.1	50.3	1.69
WG (%)[5]	-2.64[d]	17.5[c]	42.4[b]	43.6[b]	46.4[b]	68.1[a]	5.57
SGR %/day[6]	-0.19[d]	1.15[c]	2.51[b]	2.58[b]	2.72[b]	3.71[a]	0.31
FE (%)[7]	-	87.1[c]	107[b]	126[a]	130[a]	129[a]	3.26
PER[8]	-	1.74[c]	2.14[b]	2.52[a]	2.60[a]	2.58[a]	0.16
Survival (%)	80.0[b]	95.6[a]	91.1[ab]	97.8[a]	100[a]	95.6[a]	2.22

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (3.53%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 12. Effects of feeding rates on serological characteristics of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	2.0%	2.6%	3.2%	S[2]	SEM[9]
HCT[3]	32.33	29.17	28.33	30.67	29.33	30.17	0.87
Hb[4]	5.42[a]	4.99[ab]	4.45[b]	5.05[ab]	4.97[ab]	4.92[ab]	0.12
GOT[5]	22.55	28.69	24.39	30.38	30.90	31.57	1.80
GPT[6]	10.50	11.48	12.39	10.86	11.45	12.22	0.42
Glucose[7]	23.07[a]	18.72[b]	20.59[ab]	19.19[b]	18.87[b]	18.79[b]	0.50
Total protein[8]	3.05[d]	3.46[cd]	3.70[cb]	4.03[ab]	4.25[a]	4.25[a]	0.12

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (3.53%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 13. Whole-body proximate composition of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks (%)[1]

	Diets						Pooled
	0%	1.0%	2.0%	2.6%	3.2%	S[2]	SEM[3]
Moisture	77.8[a]	74.5[c]	74.6[c]	75.7[b]	74.4[cd]	74.1[d]	0.27
Crude protein	74.8[a]	72.0[bcd]	70.3[d]	72.8[bc]	71.0[cd]	73.0[ab]	0.39
Crude lipid	5.33[e]	10.2[c]	13.5[a]	9.99[c]	11.1[b]	7.24[d]	0.56
Crude ash	18.7[a]	15.4[d]	15.1[d]	16.5[c]	16.4[c]	17.3[b]	0.26

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (3.53%)

[3]Pooled standard error of means: SD/√n

Table 14. Proximate composition of the experimental diet for 40 and 50 g juvenile olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	8.51
Crude protein (%)	59.5
Crude lipid (%)	12.7
Crude ash (%)	11.3
Gross energy (MJ/kg)	17.3 MJ/kg
Diet size (mm)	6.0~6.3 mm

Table 15. Effects of feeding rates on growth performance of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	2.0%	2.4%	2.8%	S[2]	SEM[9]
IW (g/fish)[3]	40.1	40.2	40.5	40.1	41.1	40.4	0.16
FW (g/fish)[4]	37.8	45.7	51.5	52.2	55.4	56.3	1.63
WG (%)[5]	-5.68^{d}	13.6^{c}	27.1^{b}	30.1^{ab}	34.8^{ab}	39.5^{a}	3.47
SGR %/day[6]	0.05^{d}	0.91^{c}	1.71^{b}	1.88^{ab}	2.12^{ab}	2.38^{a}	0.21
FE (%)[7]	-	97.0^{b}	118^{a}	117^{a}	127^{a}	121^{a}	2.98
PER[8]	-	1.94^{b}	2.36^{a}	2.36^{a}	2.36^{a}	2.42^{a}	0.02
Survival (%)	97.8	100	100	100	100	95.6	0.81

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (3.04%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 16. Effects of feeding rates on serological characteristics of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	2.0%	2.4%	2.8%	S[2]	SEM[9]
HCT[3]	27.00[a]	19.33[bc]	23.50[ab]	23.00[ab]	18.00[c]	23.33[ab]	0.90
Hb[4]	4.86[a]	4.62[ab]	4.40[ab]	4.52[ab]	4.01[b]	4.22[ab]	0.11
GOT[5]	22.73[a]	16.04[bc]	18.97[ab]	12.36[c]	16.50[bc]	15.51[bc]	1.05
GPT[6]	8.04	6.82	7.33	8.19	7.46	7.80	0.33
Glucose[7]	26.74[b]	23.00[b]	25.62[b]	30.47[b]	38.39[ab]	19.68[c]	2.03
Total protein[8]	5.94[ab]	3.98[b]	6.46[ab]	9.38[a]	9.00[a]	8.45[a]	0.64

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (3.04%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 17. Whole-body proximate composition of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks (%)[1]

	Diets						Pooled
	0%	1.0%	2.0%	2.4%	2.8%	S[2]	SEM[3]
Moisture	76.2[a]	75.4[c]	75.9[b]	72.6[e]	73.9[d]	76.2[a]	0.28
Crude protein	76.5[a]	73.5[b]	76.2[a]	73.6[b]	74.6[ab]	76.2[a]	0.37
Crude lipid	6.29[d]	8.59[c]	8.59[c]	10.5[b]	11.4[a]	10.3[b]	0.36
Crude ash	18.5[a]	17.6[b]	16.7[c]	14.9[e]	16.0[d]	15.1[e]	0.28

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (3.04%)

[3]Pooled standard error of means: SD/√n

Table 18. Effects of feeding rates on growth performance of 50 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	1.5%	2.0%	2.5%	S[2]	SEM[9]
IW (g/fish)[3]	50.0	50.2	50.4	50.1	50.2	50.1	0.11
FW (g/fish)[4]	48.0	60.5	66.7	72.1	78.1	79.6	2.69
WG (%)[5]	-4.0[e]	20.5[d]	32.4[c]	43.9[b]	55.5[a]	59.0[a]	5.32
SGR %/day[6]	-0.29[e]	1.33[d]	2.00[c]	2.59[b]	3.15[a]	3.31[a]	0.30
FE (%)[7]	-	107[b]	128[a]	128[a]	137[a]	131[a]	3.38
PER[8]	-	0.19[d]	0.31[c]	0.42[b]	0.53[a]	0.56[a]	0.03
Survival (%)	100	86.7	100	95.6	97.8	97.8	1.64

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (2.73%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 19. Effects of feeding rates on serological characteristics of 50 g juvenile olive flounder, *P. olivaceus*, for 2 weeks[1]

	Diets						Pooled
	0%	1.0%	1.5%	2.0%	2.5%	S[2]	SEM[9]
HCT[3]	20.7	22.7	23.7	22.7	22.3	24.0	0.54
Hb[4]	3.64[c]	4.20[abc]	4.47[ab]	3.81[bc]	4.37[abc]	4.63[a]	0.11
GOT[5]	25.2[a]	17.3[b]	19.1[ab]	16.1[b]	18.3[b]	20.5[ab]	0.81
GPT[6]	10.3	10.1	11.6	10.2	10.2	10.8	0.41
Glucose[7]	20.4[b]	21.4[b]	19.0[b]	21.6[b]	28.0[ab]	35.5[a]	2.00
Total protein[8]	2.45[b]	3.35[a]	3.58[a]	3.46[a]	3.84[a]	3.93[a]	0.08

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2] Satiation (2.73%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 20. Whole-body proximate composition of 50 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 2 weeks (%)[1]

	Diets						Pooled
	0%	1.0%	1.5%	2.0%	2.5%	S[2]	SEM[3]
Moisture	77.7[a]	75.8[b]	74.9[d]	75.1[c]	75.1[c]	74.3[e]	0.23
Crude protein	76.3[a]	75.9[a]	74.3[ab]	71.1[c]	72.6[bc]	74.6[a]	0.45
Crude lipid	5.16[d]	8.06[c]	12.1[b]	12.2[b]	13.1[a]	12.5[b]	0.61
Crude ash	19.2[a]	17.4[b]	15.5[d]	16.2[c]	14.7[e]	15.2[de]	0.33

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2] Satiation (2.73%)

[3] Pooled standard error of means: SD/√n

Table 21. Proximate composition of the experimental diet for 97 and 240 g growing olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	8.49
Crude protein (%)	58.6
Crude lipid (%)	14.5
Crude ash (%)	12.7
Gross energy (MJ/kg)	17.5
Diet size (mm)	9.0~9.4

Table 22. Effects of feeding rates on growth performance of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks[1]

	Diets					Pooled
	0%	1.0%	1.65%	2.3%	S[2]	SEM[9]
IW (g/fish)[3]	95.5	96.5	96.5	102	94.0	0.90
FW (g/fish)[4]	83.6	113	135	160	160	9.85
WG (%)[5]	-12.5[d]	16.9[c]	39.1[b]	56.9[ab]	70.2[a]	9.91
SGR %/day[6]	-0.74[d]	0.86[c]	1.84[b]	2.50[ab]	2.93[a]	0.44
FE (%)[7]	-	101.7[c]	122.6[b]	118.0[b]	139.3[a]	7.84
PER[8]	-	0.32[d]	0.76[c]	1.09[b]	1.34[a]	0.19
Survival (%)	97.5	97.5	95.0	87.5	95.0	2.52

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (2.52%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 23. Effects of feeding rates on serological characteristics of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks[1]

	Diets					Pooled
	0%	1.0%	1.65%	2.3%	S[2]	SEM[9]
HCT[3]	29.00	29.50	26.50	30.00	29.75	0.69
Hb[4]	4.97[ab]	5.34[a]	4.52[b]	5.44[a]	4.77[ab]	0.12
GOT[5]	19.17[b]	32.24[a]	14.53[bc]	12.54[c]	33.53[a]	2.98
GPT[6]	9.83	9.57	9.08	9.81	8.80	0.8
Glucose[7]	22.75[ab]	20.70[ab]	13.52[b]	28.17[a]	19.35[ab]	1.96
Total protein[8]	3.85[ab]	3.02[b]	4.40[a]	4.00[ab]	4.09[a]	0.18

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (2.52%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 24. Whole-body proximate composition of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks (%)[1]

	Diets					Pooled
	0%	1.0%	1.65%	2.3%	S[2]	SEM[3]
Moisture	75.7[a]	74.6[d]	75.0[b]	74.8[c]	73.8[e]	0.14
Crude protein	71.3[c]	82.0[a]	68.8[d]	72.7[b]	72.3[b]	1.04
Crude lipid	14.5[a]	1.65[d]	13.7[b]	10.9[c]	14.0[b]	1.11
Crude ash	12.1[e]	19.2[a]	12.8[d]	16.0[b]	14.4[c]	0.58

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (2.52%)

[3]Pooled standard error of means: SD/√n

Table 25. Effects of feeding rates on growth performance of 240 g growing olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks[1]

	Diets					Pooled
	0%	0.5%	0.75%	1.0%	S[2]	SEM[9]
IW (g/fish)[3]	237	236	251	226	252	3.86
FW (g/fish)[4]	218	265	287	282	319	13.7
WG (%)[5]	-8.1[d]	12.2[c]	14.5[bc]	24.7[ab]	26.6[a]	5.19
SGR %/day[6]	-0.40[c]	0.55[b]	0.64[ab]	1.05[a]	1.12[a]	0.21
FE (%)[7]	-	94.5	90.7	136	108	10.3
PER[8]	-	1.75	1.68	2.52	1.99	0.19
Survival (%)	87.5	85.0	85.0	97.5	95.0	2.76

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (1.25%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 26. Effects of feeding rates on serological characteristics of 240 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks[1]

	Diets					Pooled
	0%	0.5%	0.75%	1.0%	S[2]	SEM[9]
HCT[3]	32.8	33.3	32.3	29.8	25.0	1.54
Hb[4]	3.08	3.25	2.70	2.71	2.59	0.11
GOT[5]	23.83[b]	18.67[b]	17.93[b]	36.73[a]	18.74[b]	2.59
GPT[6]	8.91	9.62	9.09	10.98	9.54	0.32
Glucose[7]	16.1	28.9	20.2	19.9	25.2	2.69
Total protein[8]	5.04[a]	2.65[b]	3.96[ab]	3.84[ab]	3.82[ab]	0.28

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (1.25%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 27. Whole-body proximate composition of 240 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks (%)[1]

	Diets					Pooled
	0%	0.5%	0.75%	1.0%	S[2]	SEM[3]
Moisture	71.9[ab]	72.9[a]	72.5[a]	71.9[ab]	71.0[b]	0.22
Crude protein	65.3[b]	68.5[a]	69.3[a]	65.0[b]	59.1[c]	1.20
Crude lipid	23.3[b]	18.0[e]	18.7[d]	22.2[c]	26.9[a]	1.08
Crude ash	12.7[bc]	13.5[ab]	13.6[a]	11.6[d]	11.9[cd]	0.27

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (1.25%)

[9]Pooled standard error of means: SD/√n

Table 28. Proximate composition of the experimental diet for 317 g growing olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	8.51
Crude protein (%)	56.5
Crude lipid (%)	14.6
Crude ash (%)	13.2
Gross energy (MJ/kg)	17.4
Diet size (mm)	11.0~11.4

Table 29. Effects of feeding rates on growth performance of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks[1]

	Diets					Pooled
	0%	0.4%	0.6%	0.8%	S[2]	SEM[9]
IW (g/fish)[3]	316.7	318.9	322.9	306.4	318.6	2.52
FW (g/fish)[4]	279.5[c]	348.6[b]	377.5[b]	355.0[b]	409.0[a]	14.53
WG (%)[5]	-10.6[c]	9.3[b]	16.9[b]	15.9[b]	28.4[a]	4.37
SGR %/day[6]	-0.66[c]	0.52[b]	0.91[b]	0.87[b]	1.47[a]	0.24
FE (%)[7]	-	112.6	131.6	104.8	128.6	8.76
PER[8]	-	2.13	2.48	1.98	2.43	0.17
Survival (%)	85.7	82.1	85.7	85.7	82.1	1.06

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (1.0%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 30. Effects of feeding rates on serological characteristics of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks[1]

	Diets					Pooled
	0%	0.4%	0.6%	0.8%	S[2]	SEM[9]
HCT[3]	23.5[a]	25.4[a]	23.8[a]	25.6[a]	22.1[b]	0.60
Hb[4]	6.63	6.17	6.36	7.49	3.53	0.48
GOT[5]	22.5	25.3	27.8	28.9	13.1	2.79
GPT[6]	69.5[a]	22.8[ab]	17.4[ab]	7.0[b]	11.5[ab]	9.18
Glucose[7]	14.8	25.7	19.5	10.6	19.1	2.88
Total protein[8]	2.94[b]	2.06[b]	3.04[ab]	4.80[a]	3.59[ab]	0.34

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2] Satiation (1.0%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 31. Whole-body proximate composition of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks (%)[1]

	Diets					Pooled
	0%	0.4%	0.6%	0.8%	S[2]	SEM[3]
Moisture	72.0	73.7	74.3	71.7	72.1	0.43
Crude protein	68.2[b]	68.1[b]	70.3[a]	65.4[c]	66.6[c]	0.57
Crude lipid	20.7[b]	20.9[b]	15.9[d]	22.6[a]	19.6[c]	0.74
Crude ash	9.9[c]	11.2[bc]	13.3[a]	11.9[b]	14.2[a]	0.53

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2] Satiation (1.0%)

[3] Pooled standard error of means: SD/√n

Table 32. Proximate composition of the experimental diet for 384 g sub-adult olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	4.50
Crude protein (%)	58.1
Crude lipid (%)	13.2
Crude ash (%)	12.5
Gross energy (MJ/kg)	17.3
Diet size (mm)	13.0~13.4

Table 33. Effects of feeding rates on growth performance of 384 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks[1]

	Diets					Pooled
	0%	0.3%	0.5%	0.7%	S[2]	SEM[9]
IW (g/fish)[3]	392.0	388.9	380.7	378.5	380.7	2.84
FW (g/fish)[4]	337.5	411.7	414.5	447.5	452.8	13.9
WG (%)[5]	-13.9[c]	5.9[b]	8.9[b]	18.2[a]	18.9[a]	4.01
SGR %/day[6]	-0.79[c]	0.3[b]	0.44[b]	0.88[a]	0.91[a]	0.21
FE (%)[7]	-	85.0	73.0	120	99.0	9.66
PER[8]	-	1.52	1.31	2.14	1.77	0.17
Survival (%)	88.9	88.9	85.2	85.2	92.6	2.53

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (0.9%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 34. Effects of feeding rates on serological characteristics of 384 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks[1]

	Diets					Pooled
	0%	0.3%	0.5%	0.7%	S[2]	SEM[9]
HCT[3]	34.9	39.4	34.8	34.4	34.8	
Hb[4]	6.5	6.3	6.2	6.0	5.7	
GOT[5]	13.61[b]	14.95[b]	22.58[a]	13.82[b]	19.86[ab]	
GPT[6]	7.48[ab]	8.80[ab]	7.13[b]	7.77[ab]	9.3[a]	
Glucose[7]	34.4	66.9	92.5	46.7	88.9	
Total protein[8]	4.7[b]	4.7[b]	6.2[a]	4.3[b]	5.1[ab]	

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (0.9%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 35. Whole-body proximate composition of 384 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks (%)[1]

	Diets					Pooled
	0%	0.3%	0.5%	0.7%	S[2]	SEM[3]
Moisture	73.8	72.1	72.9	72.8	74.0	0.36
Crude protein	68.2[b]	68.4[b]	68.5[b]	68.6[b]	72.2[a]	0.52
Crude lipid	15.0[c]	19.1[a]	17.4[b]	19.6[a]	13.5[d]	0.79
Crude ash	15.1[a]	11.6[bc]	12.9[b]	11.2[c]	11.9[bc]	0.48

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (0.9%)

[3]Pooled standard error of means: SD/√n

Table 36. Proximate composition of the experimental diet for 525 g sub-adult olive flounder, *P. olivaceus* (dry matter basis)

	Composition
Moisture (%)	7.04
Crude protein (%)	56.0
Crude lipid (%)	14.3
Crude ash (%)	11.4
Gross energy (MJ/kg)	17.6
Diet size (mm)	15.0~15.4

Table 37. Effects of feeding rates on growth performance of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks[1]

	Diets					Pooled
	0%	0.2%	0.4%	0.6%	S[2]	SEM[9]
IW (g/fish)[3]	525.3	523.2	522.0	518.7	537.3	8.73
FW (g/fish)[4]	393.2	549.4	575.7	581.5	628.7	27.1
WG (%)[5]	-20.7[c]	5.0[b]	10.3[ab]	12.1[ab]	17.0[a]	4.49
SGR %/day[6]	-1.37[c]	0.29[b]	0.58[ab]	0.67[ab]	0.92[a]	0.28
FE (%)[7]	-	102	113	111	112	8.92
PER[8]	-	1.83	2.02	1.99	2.00	0.16
Survival (%)	91.7	91.7	91.7	88.3	83.3	2.15

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (0.8%)

[3] Initial weight (g/fish)

[4]Final weight (g/fish)

[5]Weight gain (%) = (final weight - initial weight) × 100 / initial weight

[6] Specific growth rate (%) = 100 × ($\log_e$ final wt. - $\log_e$ initial wt.) / days

[7] Feed efficiency (%) = wet weight gain (g) × 100 / dry feed intake (g)

[8] Protein efficiency ratio = wet weight gain / protein intake

[9]Pooled standard error of means: SD/√n

Table 38. Effects of feeding rates on serological characteristics of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks[1]

	Diets					Pooled SEM[9]
	0%	0.2%	0.4%	0.6%	S[2]	
HCT[3]	21.0[b]	29.5[a]	29.9[a]	27.6[a]	22.8[b]	
Hb[4]	5.8	7.4	7.5	7.4	6.9	
GOT[5]	23.6	45.6	41.5	40.7	33.9	
GPT[6]	6.38	5.04	12.76	6.11	5.96	
Glucose[7]	10.7[c]	13.2[bc]	22.1[ab]	24.8[a]	13.4[bc]	
Total protein[8]	3.4	4.1	3.3	2.8	4.1	

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (0.8%)

[3]HCT (%): Hematocrit

[4]Hb (g/dL): Hemoglobin

[5]GOT (AST;U/L): Glutamic oxaloacetic transaminase (Aspartate transaminase)

[6]GPT (ALT; U/L): Glutamic pyruvic transaminase (Alanine transaminase)

[7]Glucose (mg/dL)

[8]Total protein (mg/dL)

[9]Pooled standard error of means: SD/√n

Table 39. Whole-body proximate composition of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates for 3 weeks (%)[1]

	Diets					Pooled
	0%	0.2%	0.4%	0.6%	S[2]	SEM[3]
Moisture	74.4[ab]	72.8[b]	74.0[ab]	72.8[b]	74.7[a]	0.29
Crude protein	67.8[b]	70.2[a]	64.9[c]	68.0[b]	69.2[ab]	0.61
Crude lipid	18.9[b]	19.2[b]	17.3[c]	20.6[a]	17.4[c]	0.42
Crude ash	12.2[b]	11.6[b]	12.3[b]	14.0[a]	12.6[b]	0.29

[1] Values are means from duplicate groups of fish where the values in each row with different superscripts are significantly different ($P < 0.05$).

[2]Satiation (0.8%)

[3]Pooled standard error of means: SD/√n

Figures

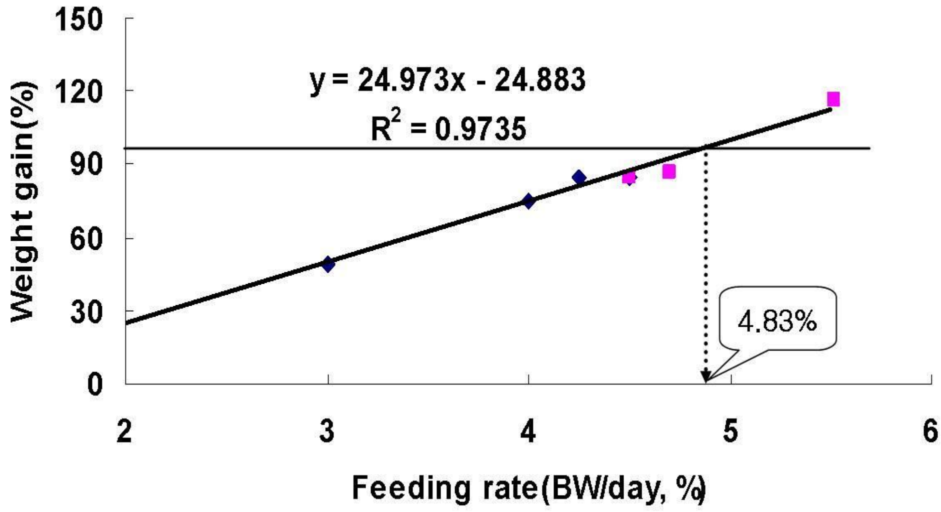

Fig. 1. Broken line analysis of weight gain of 5 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

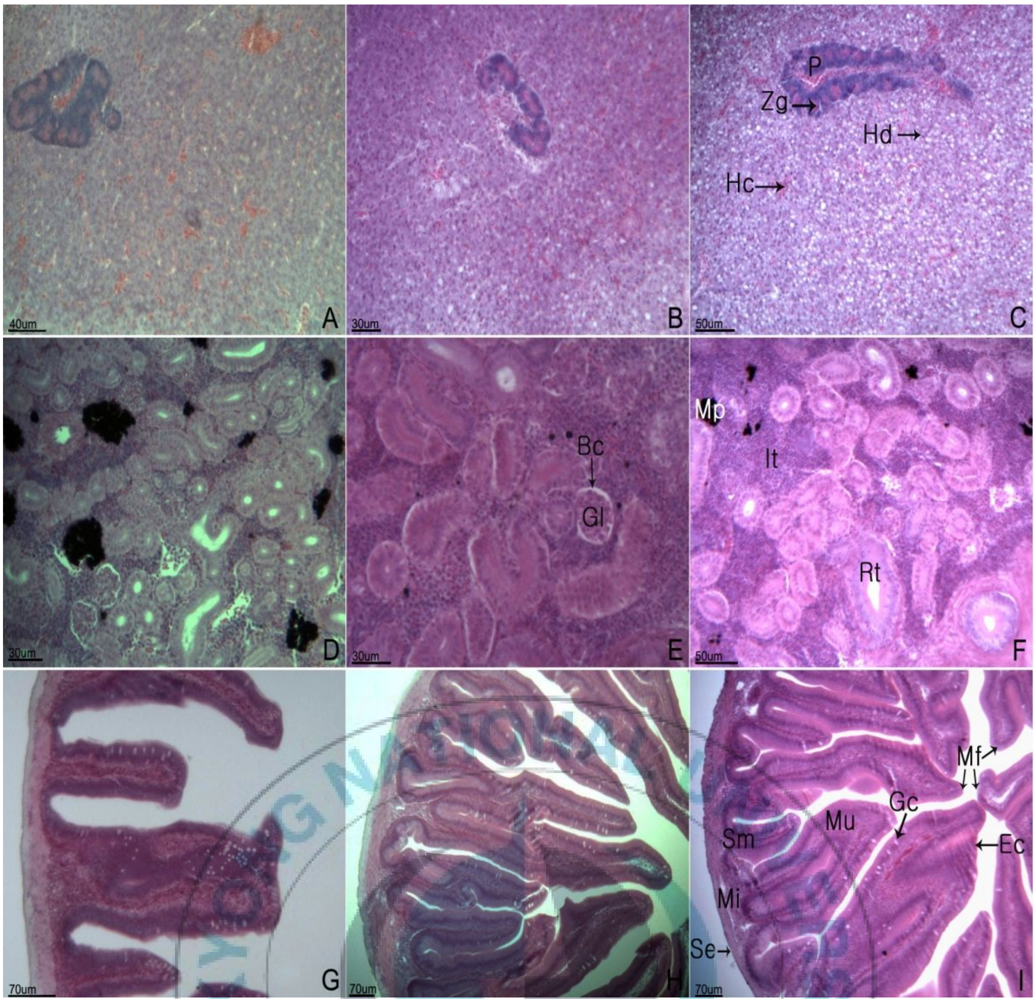

Fig. 2. Histological changes of the hepatopancreas, kidney and anterior intestine of 5 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 4 and 5.52% BW/day. Abbrivation: Bc, bouman's capsule; Ep, epithelial cell; Gc, goblet cell; Gl, glomerulus; Hc, hepatic cell; Hd, hepatic cord; It, interstitial tissue; Mf, mucosal fold; Ml, muscularis; P, pancreas; Rt, renal tubule; Se, serosa; Sm, submucosa; Zg, zymogen granule

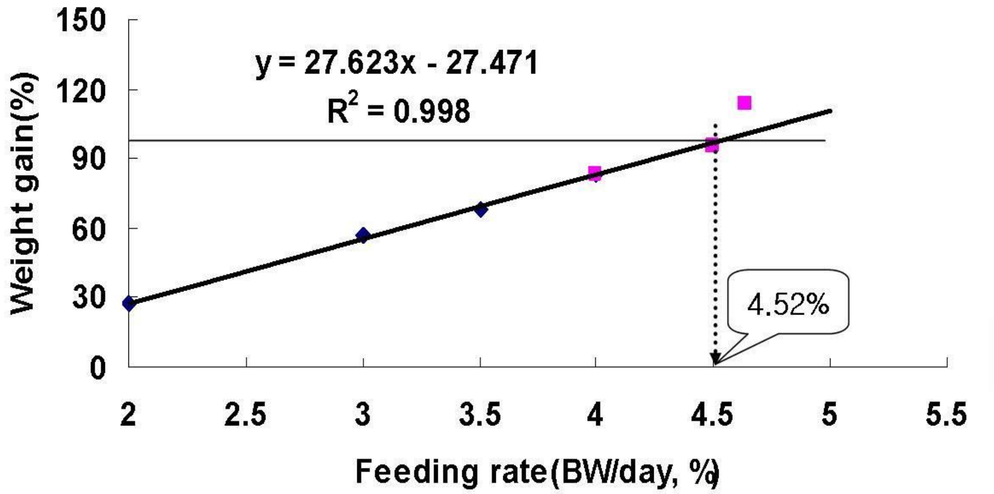

Fig. 3. Broken line analysis of weight gain of 9 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

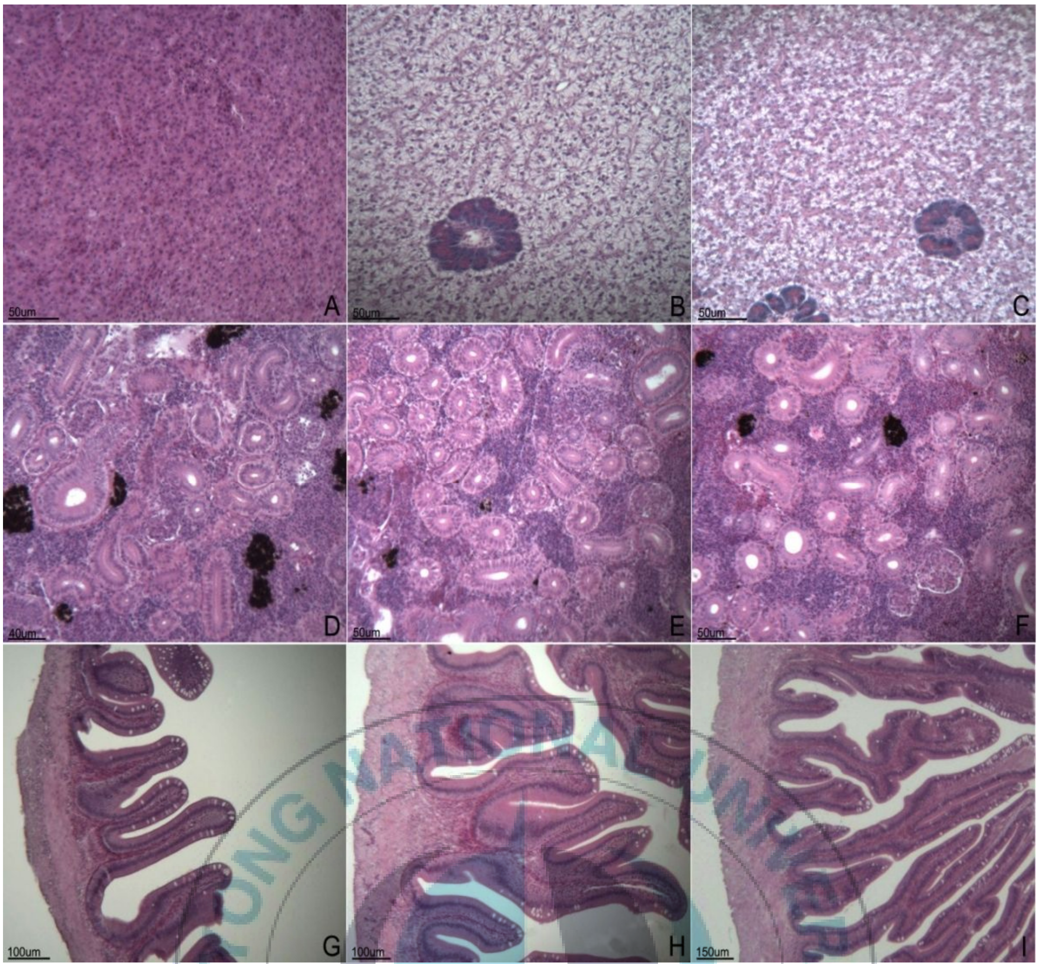

Fig. 4. Histological changes of the hepatopancreas, kidney and anterior intestine of 9 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 3.5% and 4.64% BW/day.

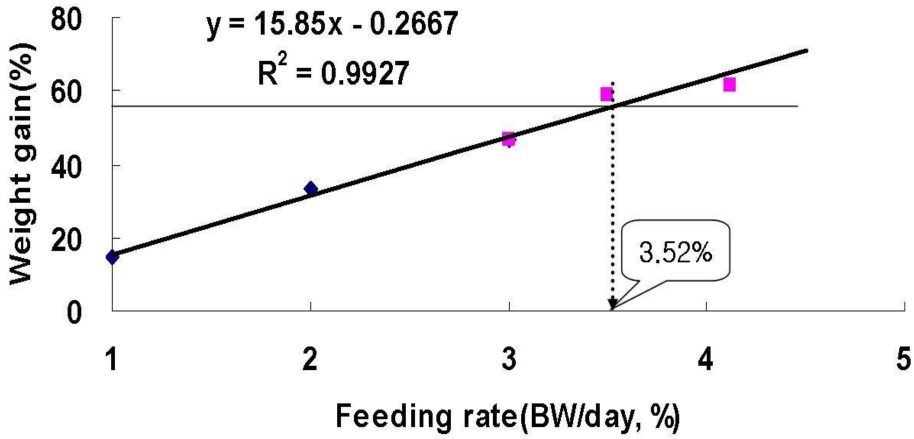

Fig. 5. Broken line analysis of weight gain of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

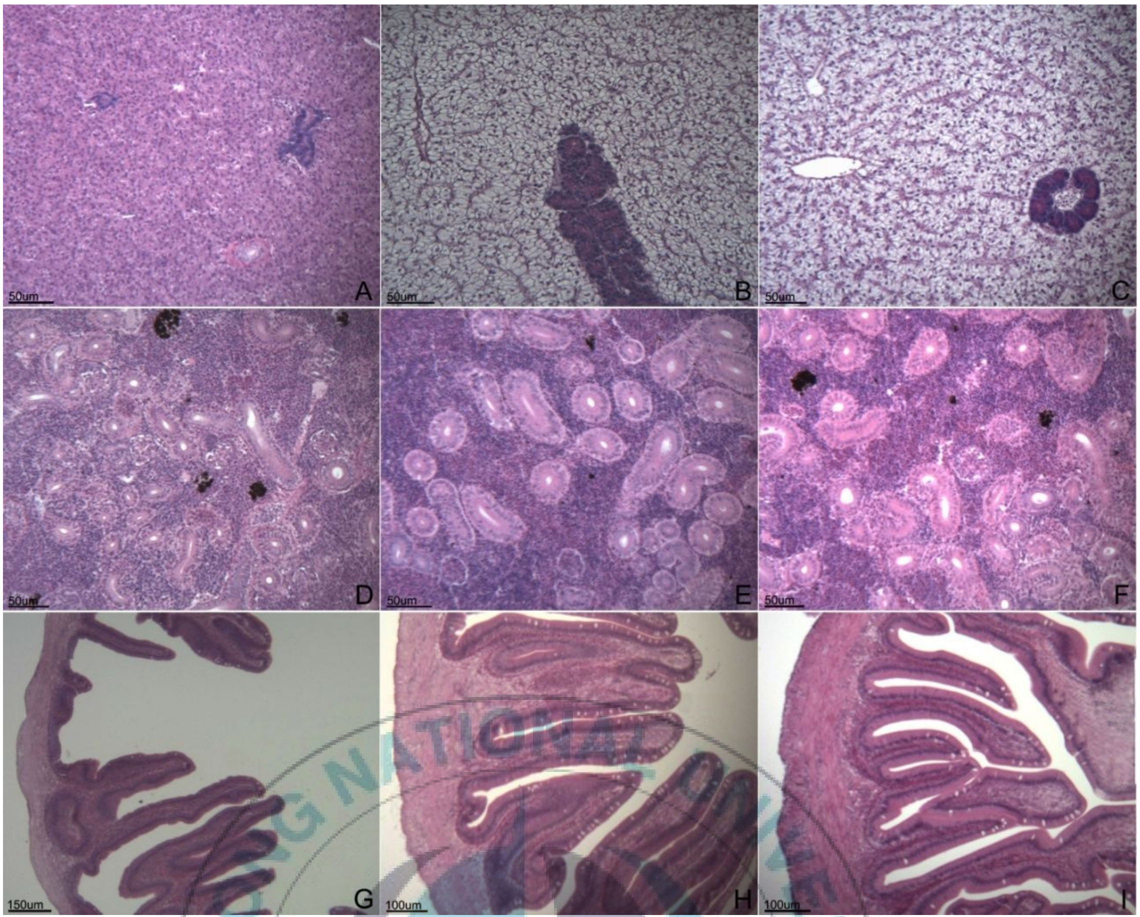

Fig. 6. Histological changes of the hepatopancreas, kidney and anterior intestine of 20 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 3.0 and 4.12% BW/day.

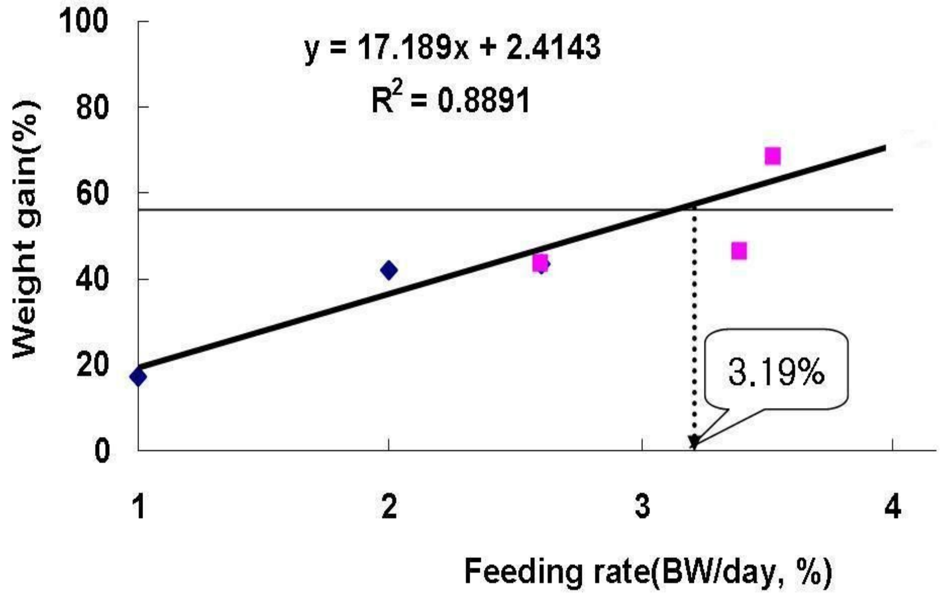

Fig. 7. Broken line analysis of weight gain of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

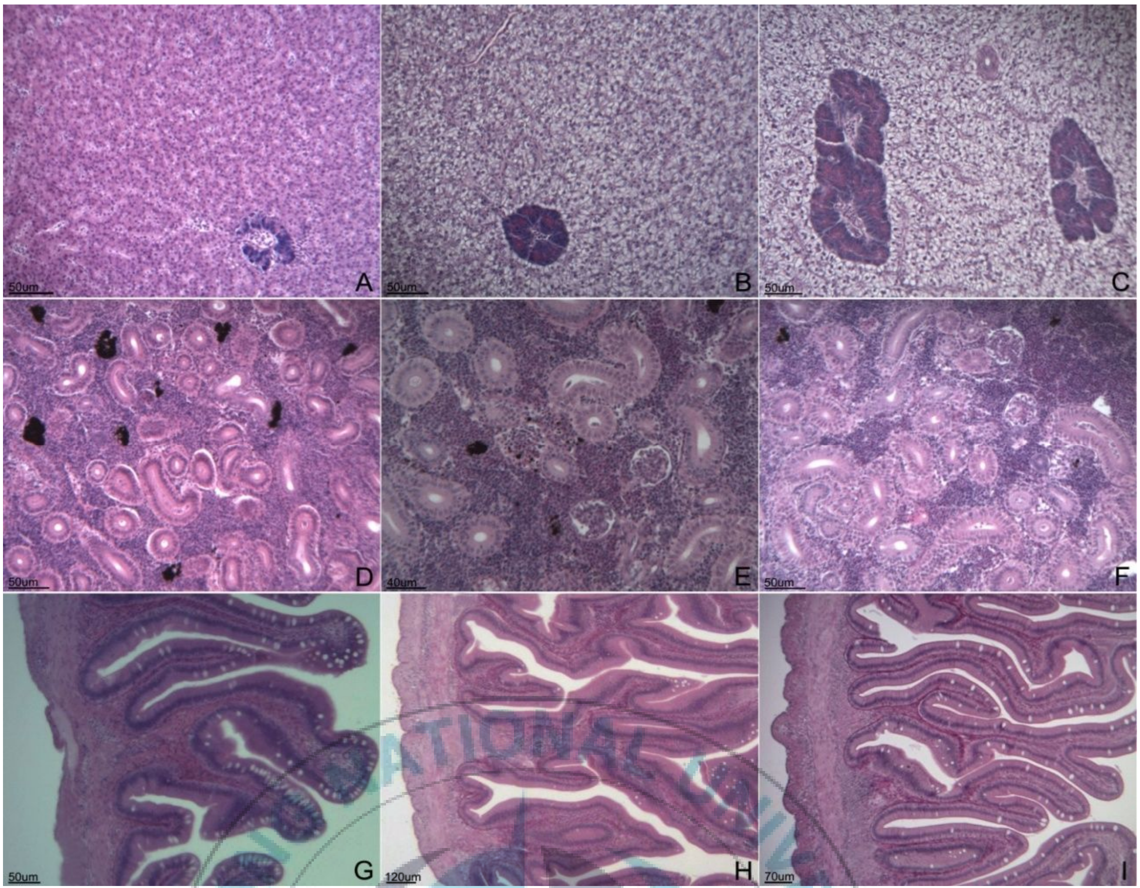

Fig. 8. Histological changes of the hepatopancreas, kidney and anterior intestine of 30 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 2.6 and 3.53% BW/day.

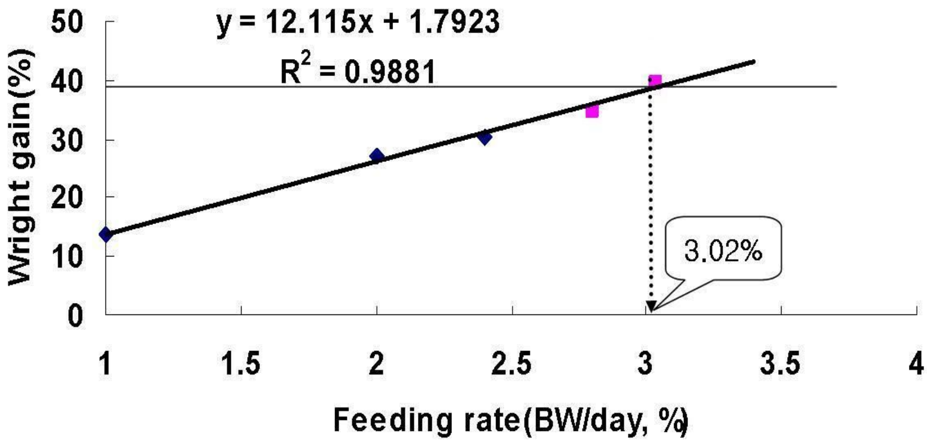

Fig. 9. Broken line analysis of weight gain of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

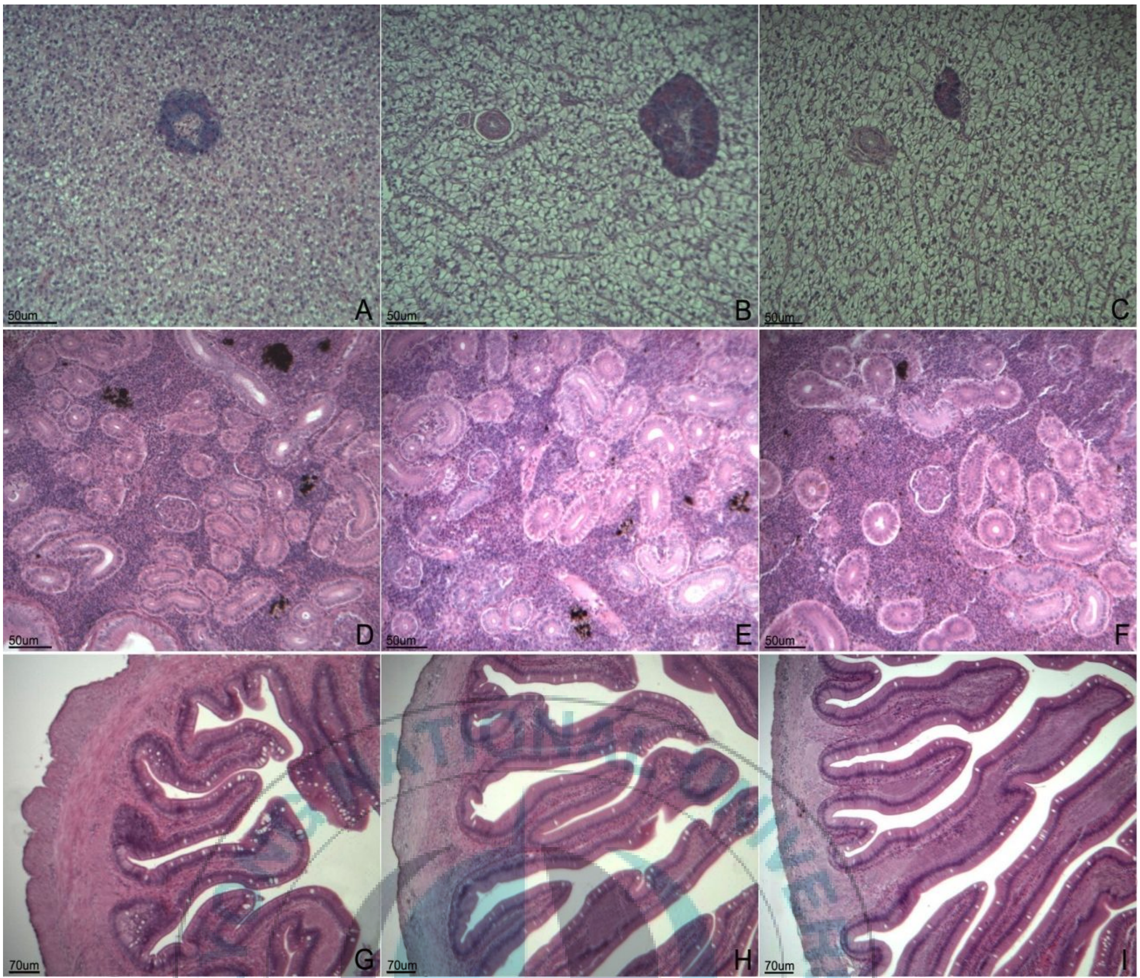

Fig. 10. Histological changes of the hepatopancreas, kidney and anterior intestine of 40 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 2.4 and 3.04% BW/day.

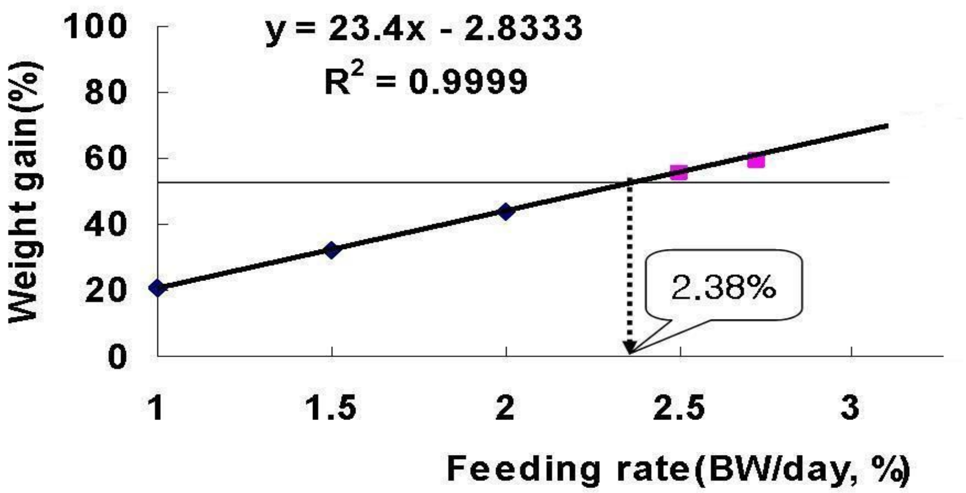

Fig. 11. Broken line analysis of weight gain of 50 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

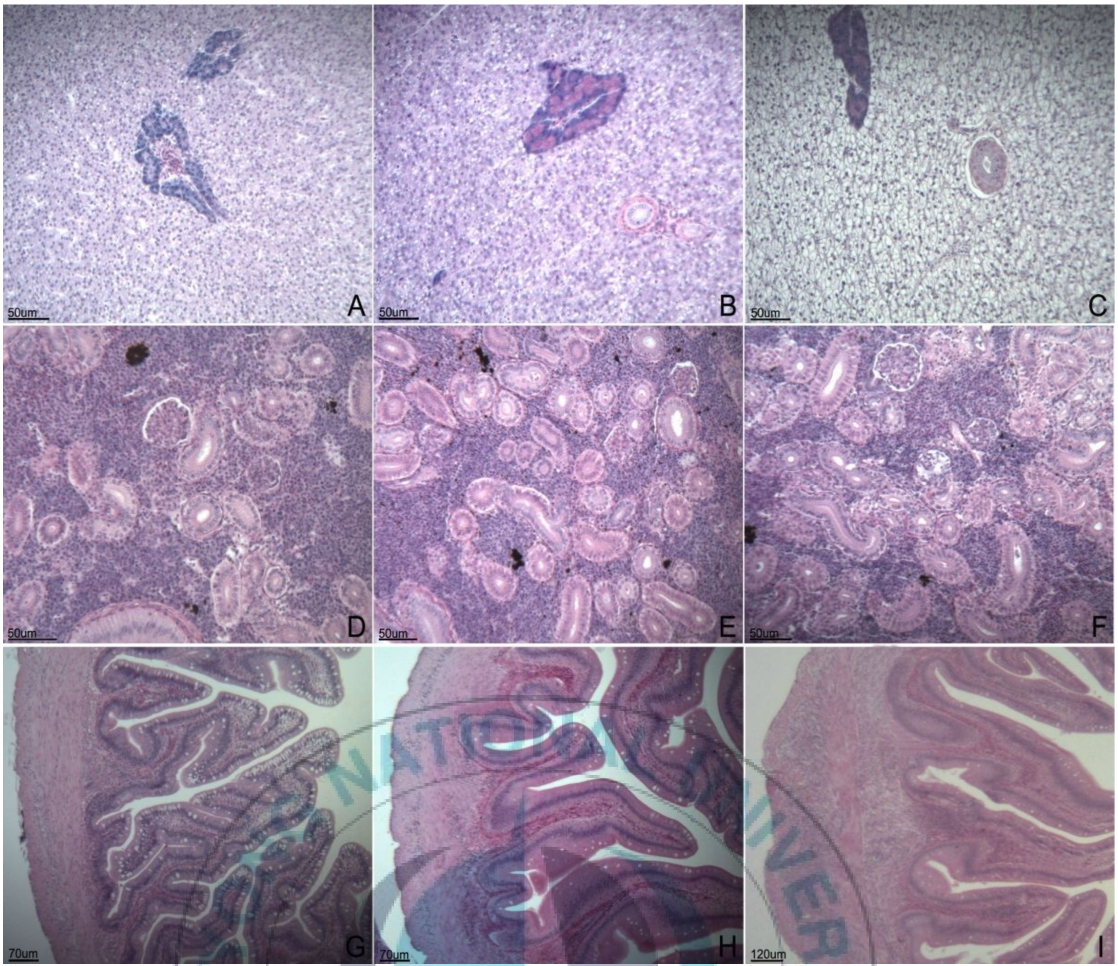

Fig. 12. Histological changes of the hepatopancreas, kidney and anterior intestine of 50 g juvenile olive flounder, *P. olivaceus*, fed the experimental diet for 2 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 2 and 2.73% BW/day.

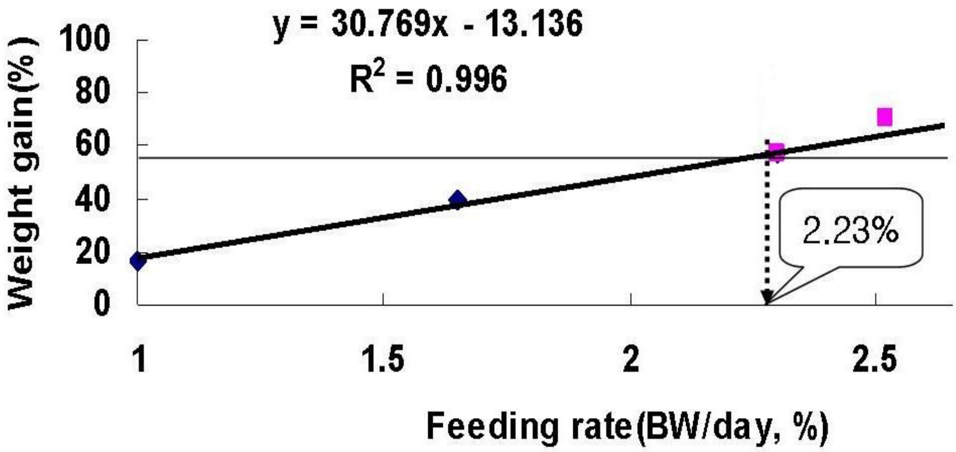

Fig. 13. Broken line analysis of weight gain of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

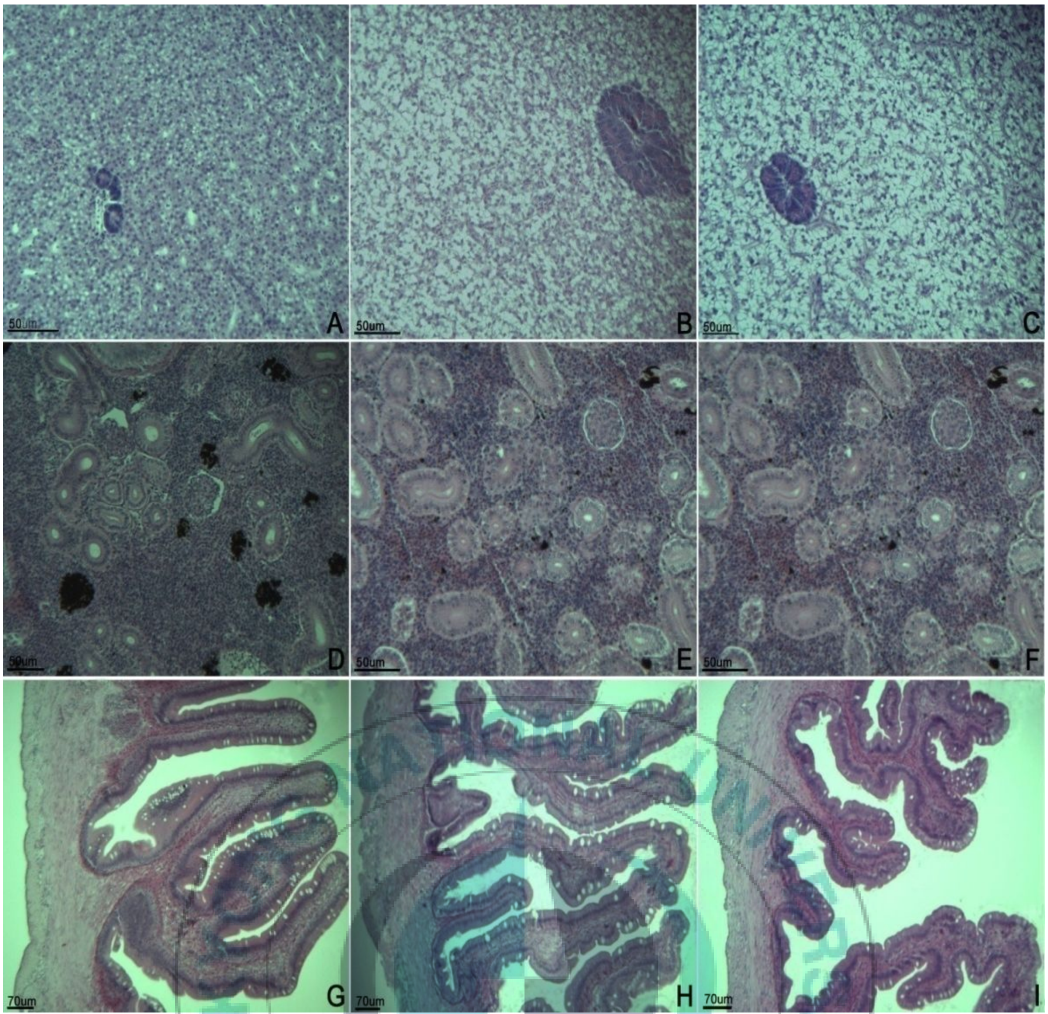

Fig. 14. Histological changes of the hepatopancreas, kidney and anterior intestine of 97 g growing olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 1 and 2.52% BW/day.

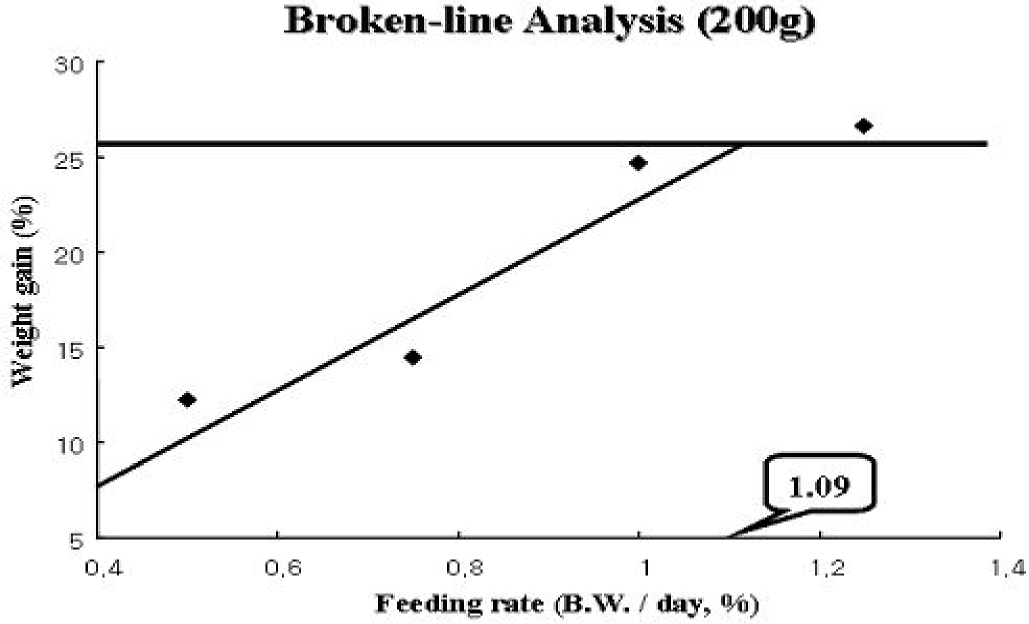

Fig. 15. Broken line analysis of weight gain of 240 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

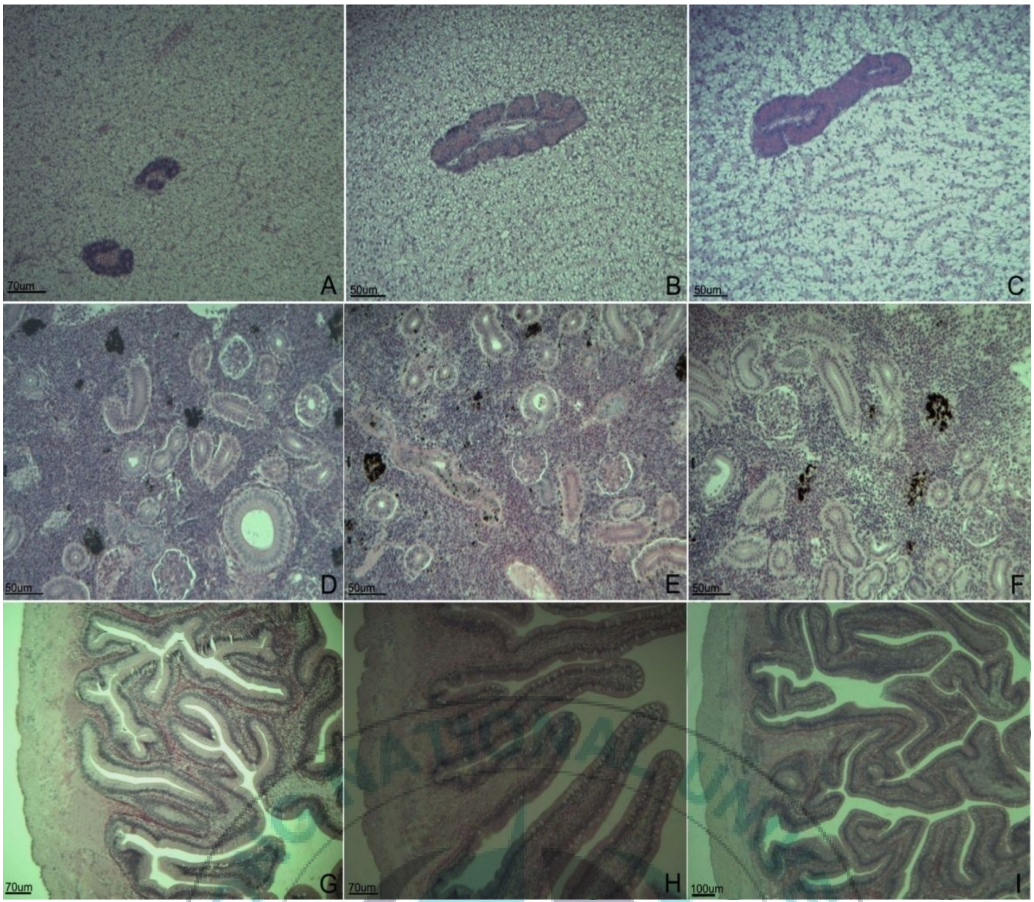

Fig. 16. Histological changes of the hepatopancreas, kidney and anterior intestine of 240 g growing olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 0.75 and 1.25% BW/day.

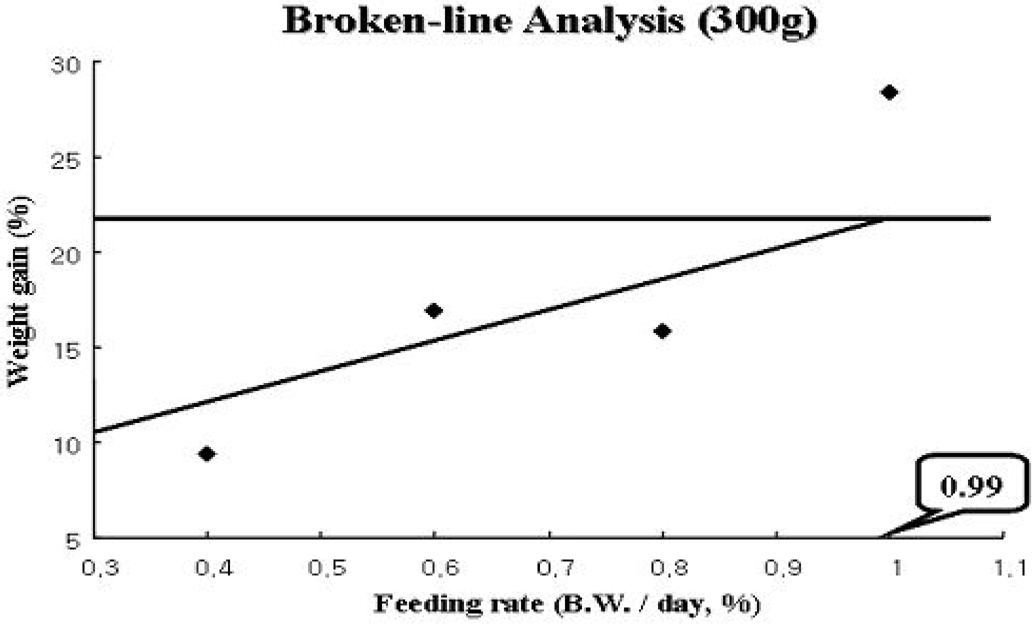

Fig. 17. Broken line analysis of weight gain of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

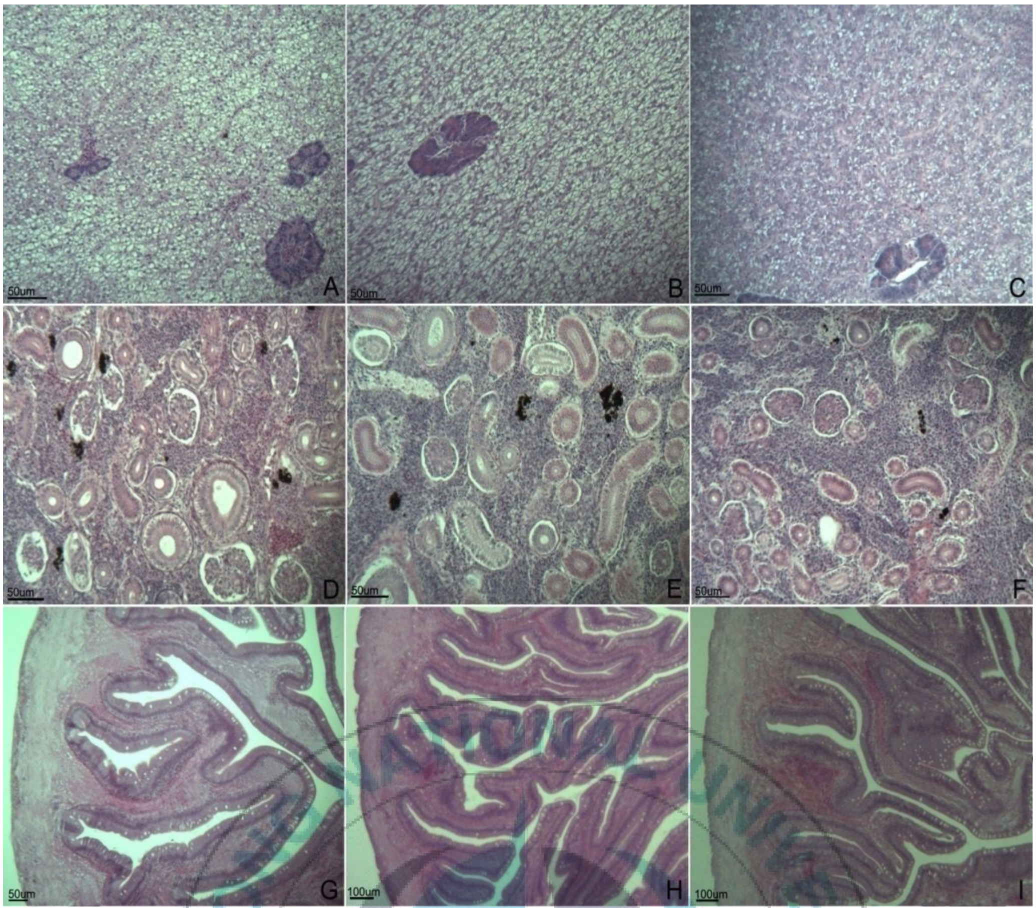

Fig. 18. Histological changes of the hepatopancreas, kidney and anterior intestine of 317 g growing olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 0.6 and 1.0% BW/day.

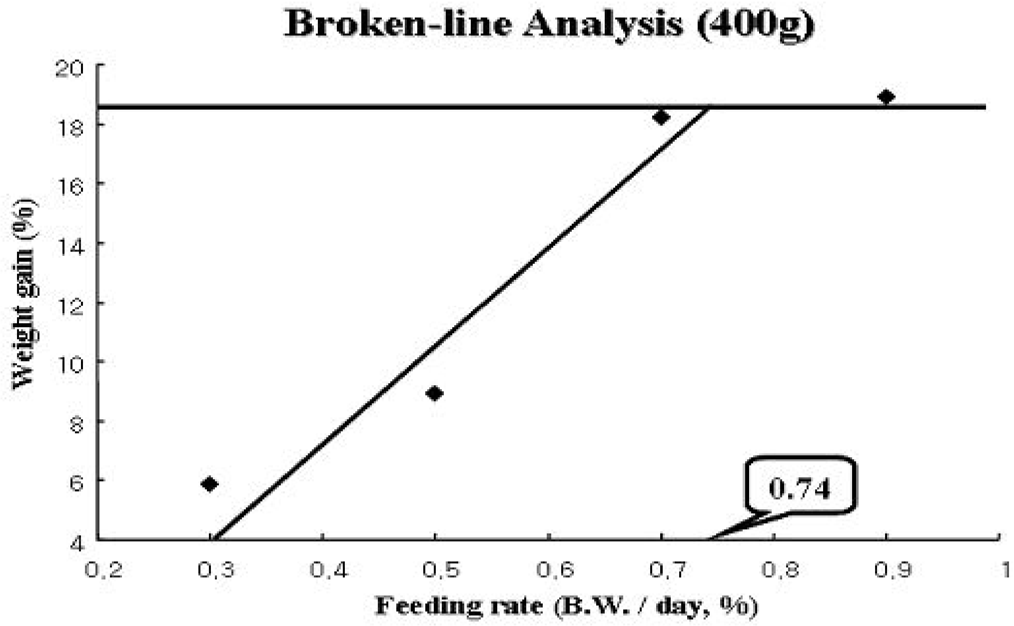

Fig. 19. Broken line analysis of weight gain of 384 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

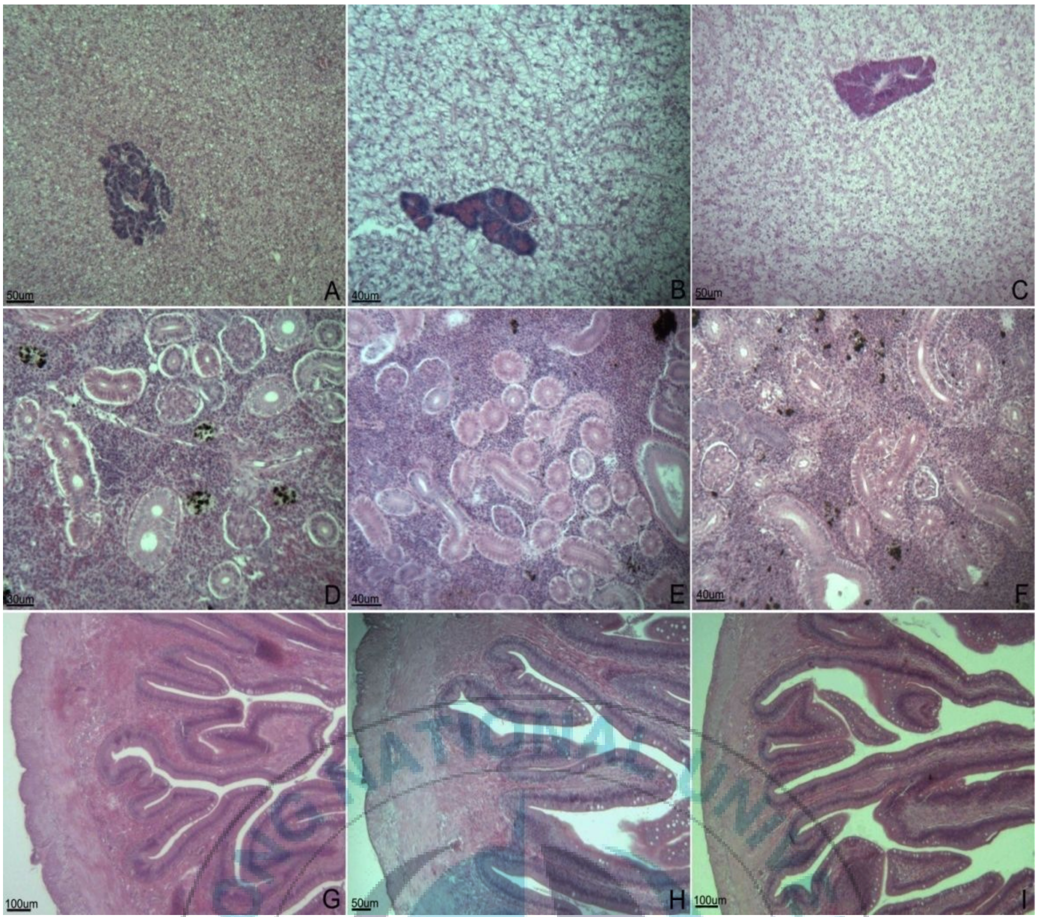

Fig. 20. Histological changes of the hepatopancreas, kidney and anterior intestine of 384 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 0.5 and 0.9% BW/day.

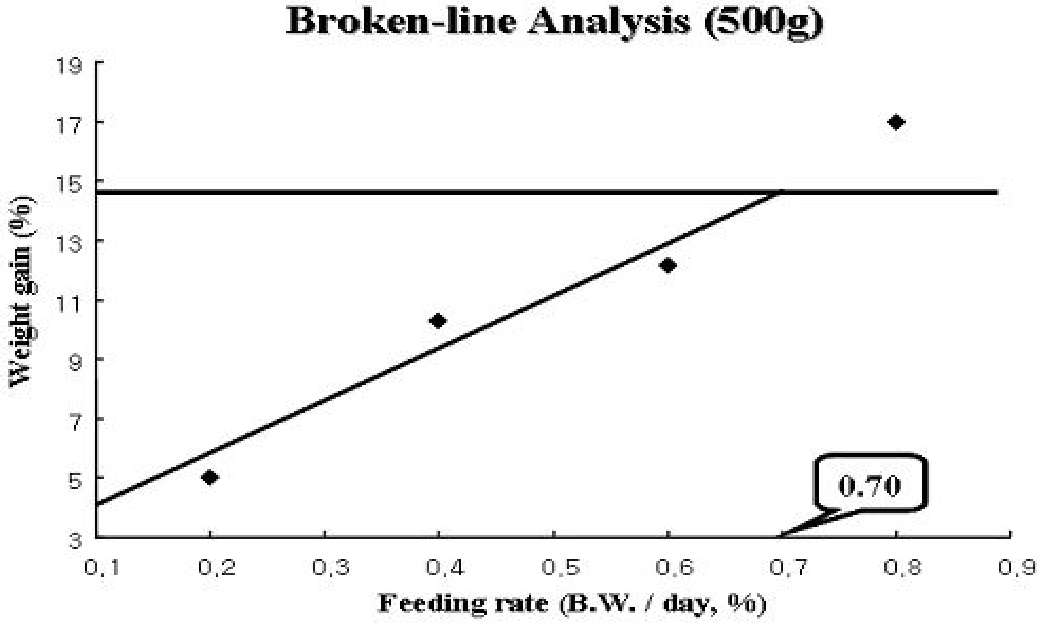

Fig. 21. Broken line analysis of weight gain of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet at different feeding rates.

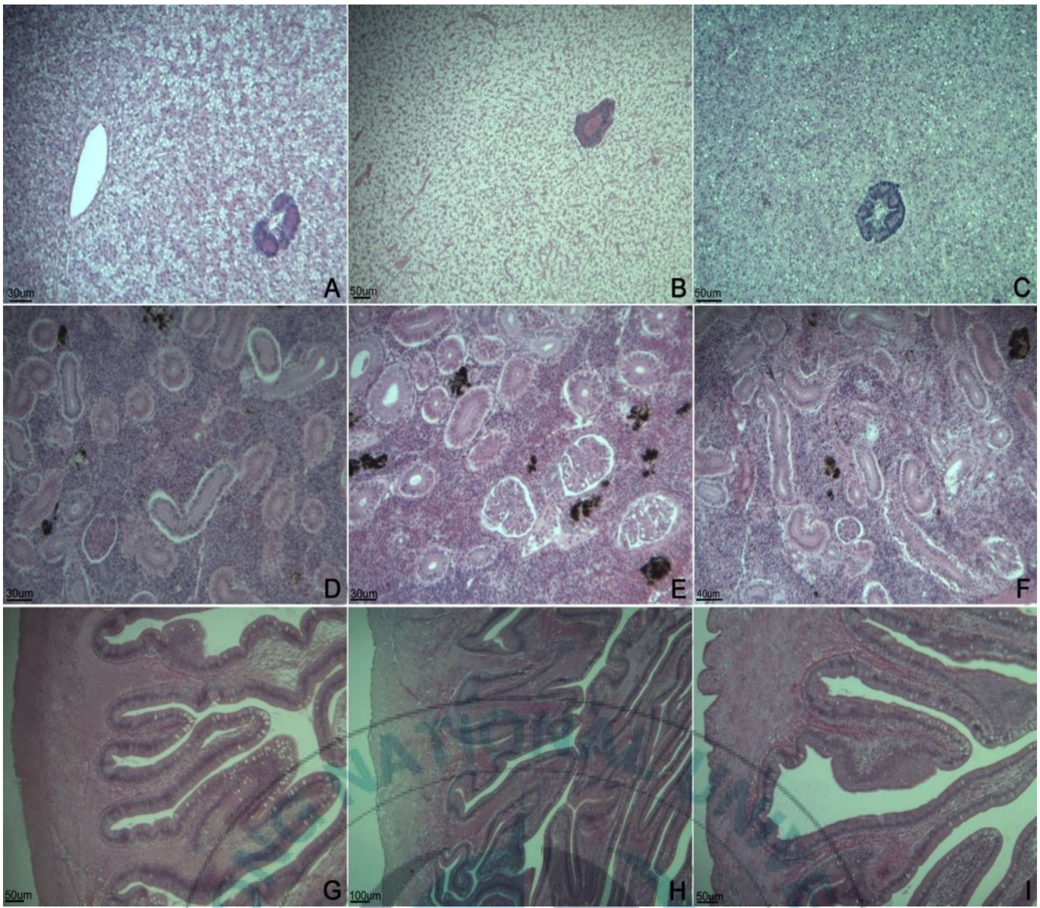

Fig. 22. Histological changes of the hepatopancreas, kidney and anterior intestine of 525 g sub-adult olive flounder, *P. olivaceus*, fed the experimental diet for 3 weeks. A-C: Hepatopancreas, D-F: Kidney, G-I: Anterior intestine. Columns A-G, B-H and C-I represent fish fed at 0, 0.4 and 0.8% BW/day.

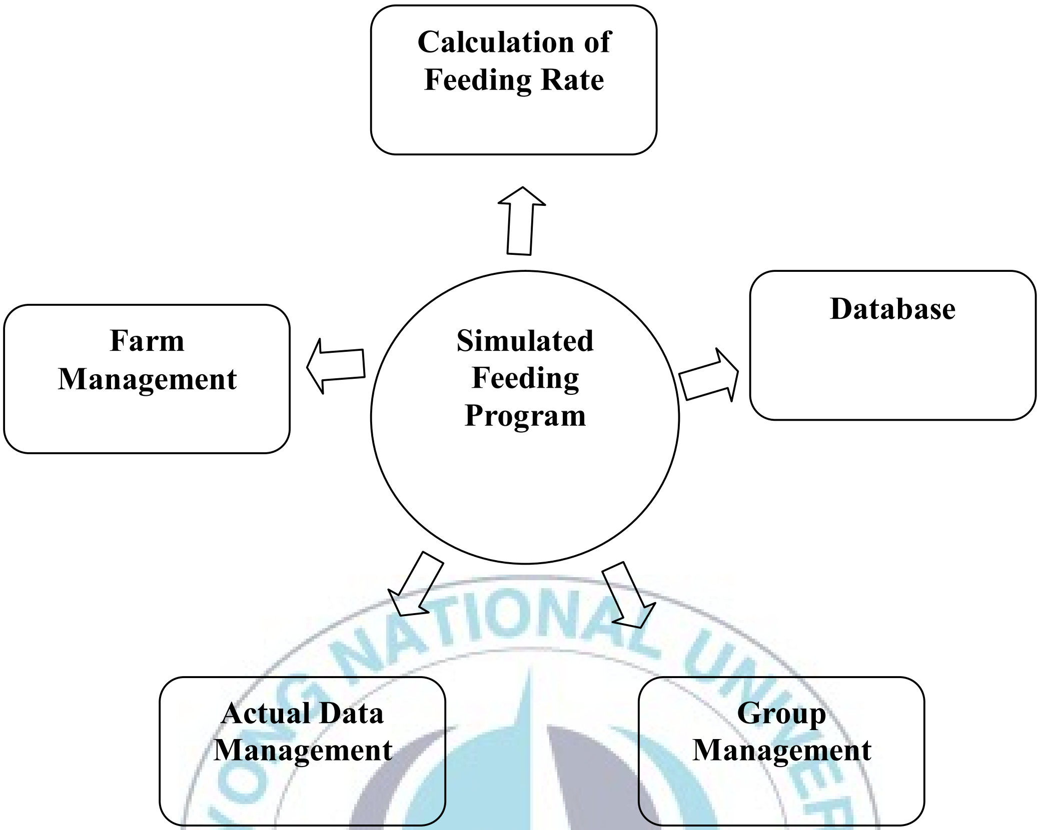

Fig. 23. Composition of the simulated feeding program

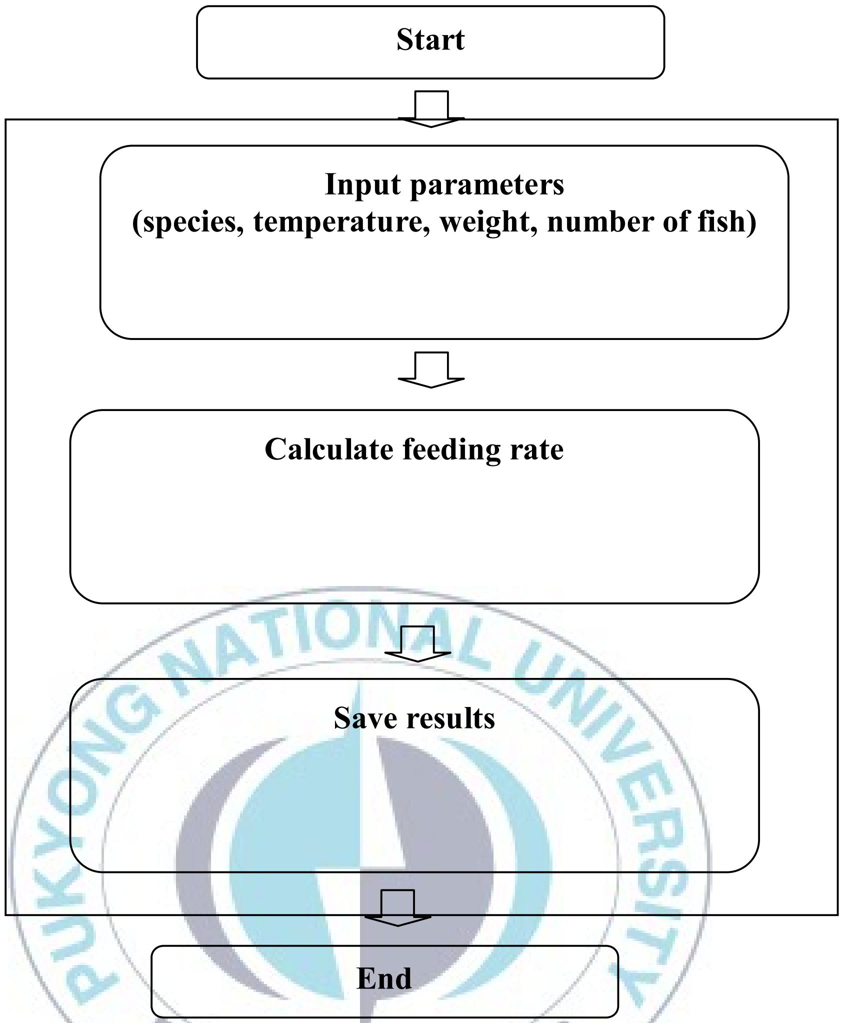

Fig. 24. Algorithm for calculation of the feeding rate

Fig. 25. The start screen

Fig. 26. Selection of species

Fig. 27. Selection of rearing water temperature

Fig. 28. Fish weight

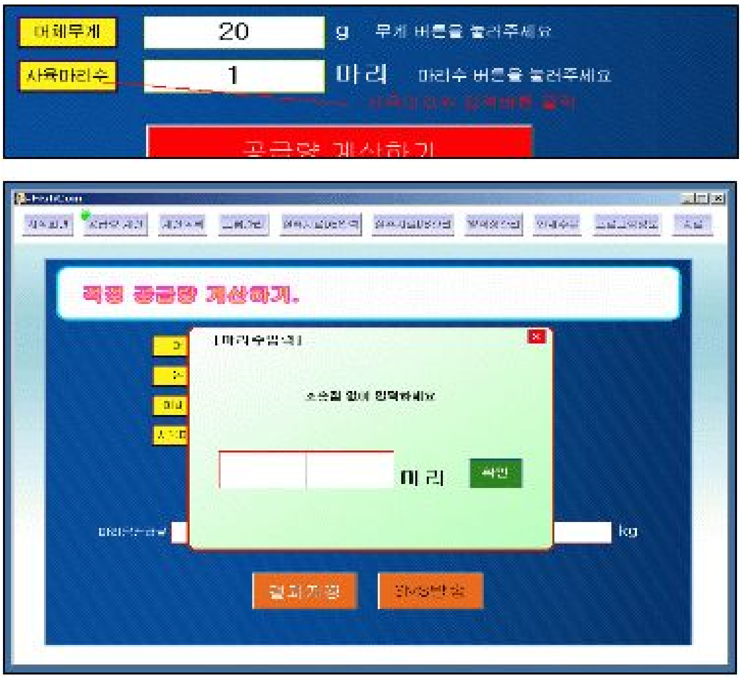

Fig. 29. Number of fish

Fig. 30. Calculation of the feeding rate

Fig. 31. Calculation list

Fig. 32. Group management

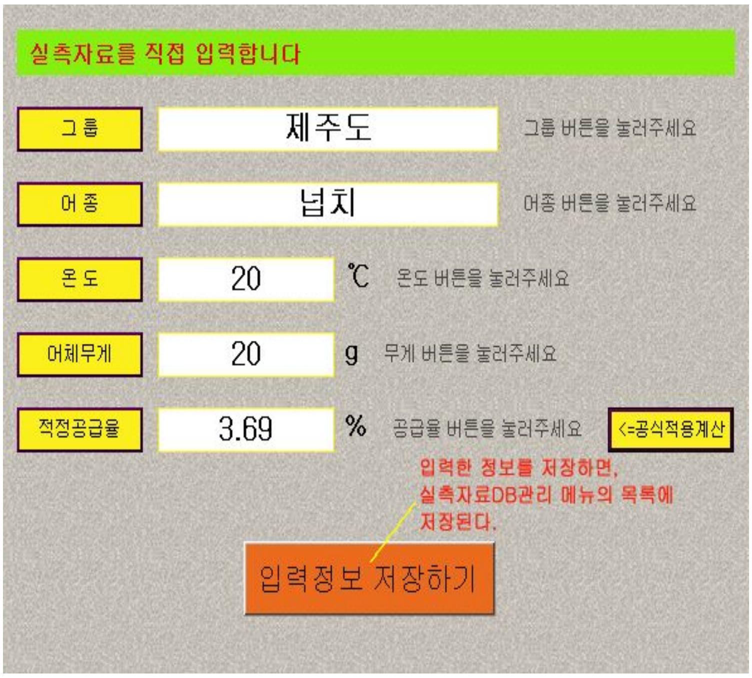

Fig. 33. Actual data entry

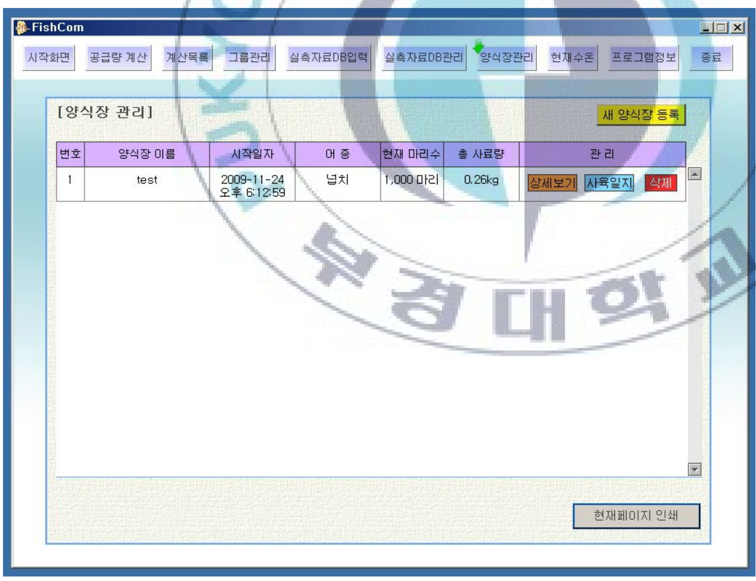

Fig. 34. Farm management