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Effects of extruded pellet and moist pellet on growth performance, body composition, and hematology of juvenile olive flounder, *Paralichthys olivaceus*

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Abstract

A feeding trial was conducted to evaluate the effects of two different sizes of extruded pellets (EP) (EP_1 - 3 mm or EP_2 - 5 mm) and a moist pellet (MP) in olive flounder, *Paralichthys olivaceus*, reared in semi-recirculation system. A total of 450 fish with an average initial weight of 5.0 ± 0.2 g (mean ± SD) were fed one of the three experimental diets in triplicate groups. At the end of a 6-week feeding trial, weight gain, specific growth rate, and feed efficiency of fish fed EP diets were significantly higher than those of fish fed MP ($P < 0.05$). Water quality parameters like turbidity, total ammonia nitrogen, and total phosphorous from tanks of fish fed EP_1 and EP_2 were significantly lower than those from tanks of fish fed MP. Blood plasma glutamic oxaloacetic transaminase and glucose concentration were significantly higher in fish fed MP diet compared to fish fed EP diets ($P < 0.05$). Whole body crude protein contents in fish fed EP diets were higher than those from the fish fed MP diet. Whole body amino acid content like threonine, aspartic acid, serine, tyrosine, and cystine were found to be significantly higher in fish fed EP diets than those in fish fed MP diet. In considering overall performance of olive flounder, EP_2 diet could be recommended for the successful aquaculture of this important fish species.

Keywords: Extruded pellet, Moist pellet, Growth, Hematology, Juvenile olive flounder

Background

Fish feeding is one of the most important factors in commercial fish farming because feeding regime may have consequences on both growth performance and feed wastage (Tsevis et al. 1992; Azzaydi et al. 2000). During the last decade, there has been a marked increase in the use of extruded pellet (EP) for feeding fish. It has been well documented that EP diets have superior water stability, better floating properties, and higher energy content than other pelleted diets (Hilton et al. 1981; Johnsen and Wandsvik 1991; Ammar 2008). However, the size of feed pellets and the rate at which they are delivered may affect the amount of feed that an individual fish can ingest over a period of time. Undesirable size of pellets or a high amount of pellets may cause feed wastage, as fish may be unable to ingest the required amount of feeds (Bailey et al. 2003).

Olive flounder, *Paralichthys olivaceus*, is one of the most commercially important marine aquaculture species in Korea. Production of the olive flounder was 42,133 metric tons, ranked it first among Korean mariculture finfish species in 2014 (KOSTAT 2015). The suitable type and size of pellets for different age groups of olive flounder are very important for maximum growth. Most of the flounder production has been sourced from use of frozen raw fish (sardine or mackerel) or raw fish-based moist pellets (MP) composed of frozen raw fish and commercially available binder meal at a certain ratio (Cho and Cho 2009).

There are several studies that have been done in the context of nutrient requirements and feeding technology

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of olive flounder. However, information on the effects of extruded pellets and their size for juvenile olive flounder is scarce. Therefore, the present experiment was conducted to evaluate the effects of two different sizes of extruded pellets and a moist pellet on the water quality, growth performance, body composition, hematological characteristics, and gut histology of juvenile olive flounder, *P. olivaceus*.

Methods

Experimental diets

In this study, the MP diet was prepared from frozen sardine and commercial wheat flour at the ratio of 3:1 (wet weight basis) and the EP_1 (3 mm) and EP_2 (5 mm) feeds were provided by EWOS Canada Ltd. EP was designed to maintain the same level of protein (56 %) and lipid (10 %). Proximate compositions of the experimental diets are shown in Table 1. Diets were stored at −20 °C (wet pellets) until use.

Experimental fish and feeding trial

Juvenile olive flounder, *P. olivaceus*, was obtained from Tong-yeong, Republic of Korea. Prior to the feeding trial, the fish were fed different experimental diets for 2 weeks to allow them to adjust to the experimental diets and conditions. Feeding trial was conducted in a semi re-circulating system with 250-L aquaria each having a water flow rate of 1.5 L/min. Supplemental aeration was provided to maintain dissolved oxygen near saturation. Water temperature was maintained at 21 ± 1 °C (mean ± SD). Salinity was maintained at 31 ± 1 g/L (mean ± SD). Fish averaging 5.0 ± 0.2 g (mean ± SD) randomly distributed to each aquarium as groups of 50 fish and fed the experimental diets in triplicate at a rate of 2.5~4.5 % wet body weight per day for 6 weeks. Total fish weight in each aquarium was determined every 2 weeks, and the amount of diets fed to fish was adjusted accordingly.

Table 1 Proximate composition of the experimental diet (percentage dry matter basis)

Items	Diets		
	EP_1^a	EP_2^a	MP[b]
Moisture (%)	7.39	7.17	65.15
Crude ash (%)	10.91	11.07	12.08
Crude lipid (%)	10.18	10.04	10.40
Crude protein (%)	56.79	56.91	57.07
Crude fiber (%)	2.38	2.30	1.61
Carbohydrate (%)	14.35	14.34	13.47
Gross energy (kcal/g)	3.76	3.75	3.76

[a]*EP* extruded pellet; EWOS Canada Ltd.
[b]*MP* moist pellet; composed of frozen sardine and wheat flour (3:1)

Water quality analysis

Water samples from the fish tanks were monitored just after 2 h of feeding. Turbidity, total ammonia nitrogen (TAN), and total phosphorous (TP) were determined from the water of the each experimental tank. The concentration of turbidity, TAN, and TP were recorded according to the standard methods for marine environmental analysis (Ministry of Land Transport and Maritime Affairs 2010).

Sample collection and analysis

At the end of the feeding trial, fish were starved for 24 h and they were counted and weighed to calculate the weight gain (WG), specific growth rate (SGR), feed efficiency (FE), and survival rate. After the final weighing, three fish from each aquarium were analyzed for whole body proximate composition. Proximate composition of the experimental diets and fish bodies were performed by the standard methods of AOAC (1995). To determine the moisture content of diets and fish, they were dried to maintain constant weight at 135 °C for 2 h. Ash content was determined using a muffle furnace (550 °C for 4 h). Crude lipid content was determined by the soxhlet extraction method by using Soxtec system 1046 (Foss, Hoganas, Sweden) and crude protein content by Kjeldahl method (N × 6.25) after acid digestion, distillation, and titration of samples. Fiber content was analyzed with a fiber analyzer (FT122 Fibertec™, Foss, Hillerød, Denmark). Carbohydrate content was calculated by subtracting total percentage of nutrient contents from 100 %. Gross energy of experimental diets were calculated based on the calculation of 16.7, 16.7, and 37.7 kJ/g for protein, carbohydrate, and lipid, respectively (Halver and Hardy 2002). Blood samples were taken by using heparinized syringes from the caudal vein of five randomly chosen fish per tank. Plasma was collected after centrifugation at 3000 rpm for 10 min and stored at −70 °C in order to analyze glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glucose, total protein (T-protein), cholesterol, and triglycerides. Plasma analyses were performed at the National Fisheries Research and Development Institute (NFRDI), Gijang, Busan, Korea, by using the kits of DRI-CHEM 4000i- Fuji Dri-Chem Slide- 3150 (Minato-ku, Tokyo, Japan). Amino acid analysis of edible body parts was performed by ninhydrin method (Sykam Amino Acid Analyzer S433, Sykam, Eresing, Germany).

Statistical analysis

All data were analyzed by one-way ANOVA (Statistix 3.1; Analytical Software, St. Paul, MN, USA) to test the effects of the dietary treatments. When a significant treatment effect was observed, an LSD test was used to compare means. Treatment effects were considered at $P < 0.05$ level of significance.

Results and discussion

Water quality has been acknowledged to have profound effects on the growth performance and health of aquaculture fish species. In the present experiment, water quality parameters were affected by the experimental diets (Table 2). The observed water quality parameters especially turbidity was significantly lower among the group of fish fed extruded pellet diets. Turbidity caused by suspended solids has been reported to have great effects on fish metabolism in terms of fish growth and survival. After 2 h of feeding, turbidity, TAN, and TP were recorded to be significantly higher in the group of fish fed MP diet than those of fish fed EP diets. These results may indicate that MP diet was easily soluble in water before consumption by fish, whereas extruded pellets were more stable in water and their leaching rate in water was comparatively prolonged. Folke and Kautsky (1989) reported that water pollution by fish feeding is caused largely by increasing turbidity, as well as ammonia and phosphorus loading through uneaten feeds and feces. From an on-farm experiment with flounder, Kim and Lee (2000) reported that the excretion of nitrogen (N) ranged from 48 to 70 g and phosphorus (P) from 10 to 12 g per kilogram weight gain. However, under practical feeding conditions, flounder excreted much higher N (114 g) and P (28 g) per kilogram weight gain, suggesting a substantial waste of feed (Kim et al. 2002). Likewise, similar findings have been reported by Cha et al. (2008) in their experiment with olive flounder fed MP and chitosan-based extruded pellets.

In the present study, significantly higher growth performance was observed for the group of fish fed EP_2 than those of fish fed MP diet (Table 3). At the end of experiment, WG, SGR, and FE of fish fed EP_2 diet were significantly higher than those of fish fed EP_1 and MP diet. However, there were no significant differences in these parameters among the fish fed EP_1 and MP diets. Survival rate ranged from 94 to 97 % without any statistical differences among different treatments. Likewise, Cho and Cho (2009) reported that an extruded pellet is more recommendable than a moist pellet for the growth performance in flounder aquaculture. In our study, the results of lower feed efficiency in fish fed MP diet could be due to high leaching properties of the MP diet before the ingestion of feed by the fish. It is well documented that extruded pellets have superior water stability, better floating properties, and a higher energy content among the pelleted diets (Hilton et al. 1981; Johnsen and Wandsvik 1991; Ammar 2008). Aqua feed technology is moving in tandem with the aquaculture growth with the usage of extrusion procedures for the improvement of digestibility (Umar et al. 2013). Chang and Wang (1999) stated the advantages of extrusion cooking process for aquaculture feed production including improved feed conversion ratio, control of pellet density, greater feed stability in water, better production efficiency, and versatility. During extrusion cooking, various reactions take place including thermal treatment, gelatinization, protein denaturation, hydration, texture alteration, partial dehydration, and destruction of microorganisms and other toxic compounds (Kannadhason et al. 2011). According to Chang and Wang (1999), the gelatinization that occurs during extrusion process improves durability of the feed rations and digestibility of starch. In the present study, result for the fish fed EP_2 concordantly supported the various reports that extruded pellets are having a better efficiency over MP diet for juvenile olive flounder growth. However, it is difficult to attribute any reason for the observed lower weight gain for the group of fish fed EP_1 diet in the present experiment.

The present experiment clearly demonstrated the beneficial effects of pellet size of extruded pellets on the performance of olive flounder. Interestingly, we observed a lower growth rate for the group of fish fed EP_1 than did fish fed EP_2 diet. Feed pellet size will obviously have an effect on fish performance, and there are indications of this effect presented in the study. Usually, for a pellet that is larger than the mouth gape of fish, handling time becomes a limiting factor in the fish's ability to ingest enough pellets to maintain good growth which will

Table 2 Water quality parameters after 2 h of feeding of juvenile olive flounder fed with the different experimental diets for 6 weeks

Parameters	Diets			Pooled SEM
	EP_1	EP_2	MP	
Turbidity (NTU)	0.57^b	0.56^b	2.63^a	0.34
TAN (mg/L)	0.33^b	0.31^b	0.53^a	0.04
TP (mg/L)	0.04^b	0.04^b	0.07^a	0.01

Value are means from groups ($n = 3$) of samples where the values in each row with different superscripts are significantly different ($P < 0.05$)
TAN total ammonia nitrogen, *TP* total phosphorus, *Pooled SEM* pooled standard error of mean (SD/√n)

Table 3 Growth performances of juvenile olive flounder fed with the different experimental diets for 6 weeks

Variables	Diets			Pooled SEM
	EP_1	EP_2	MP	
WG[§]	130.8^b	142.1^a	128.4^b	2.01
SGR[¶]	2.78^b	2.95^a	2.75^b	0.03
FE[¤]	101.1^b	103.1^a	101.4^b	0.41
SR*	97.3	96.0	94.0	1.15

Values are means from groups ($n = 3$) of fish where the values in each row with different superscripts are significantly different ($P < 0.05$)
Pooled SEM pooled standard error of mean (SD/√n)
[§]Weight gain (%): (final wt. – initial wt.) × 100/initial wt.
[¶]Specific growth rate (%/day): (ln final wt. – ln initial wt.) × 100/days
[¤]Feed efficiency (%): (wet weight gain/dry feed intake) × 100
*Survival rate = (total fish – dead fish) × 100/total fish

clearly have negative effects. However, in our study, EP_2 feed was well accepted by the fish even though its size was larger than EP_1 probably because EP_2 was more suitable in relation to mouth gape size of fish than those of EP_1 diet.

It is recommended that pellet size should be approximately 20–30 % of the size of the fish species mouth gape (Craig 2009). Feeding too small a pellet results in inefficient feeding because more energy is used in finding and eating more pellets. Conversely, pellets that are too large will depress feeding and, in the extreme, cause choking. Therefore, it is better to select the largest sized feed that the fish will actively eat. Smith et al. (1995) reported that the length and diameter of pellets influence the detectability and/or attractiveness of pellets to salmonids. In another report, Irwin et al. (2002) reported that smaller sized turbot prefer to accept a bigger size of pellet (pellet size, 40 % of mouth gape) whereas the larger fish group prefers a smaller pellet size (pellet size, 20 % of mouth gape) which is higher than the preferred pellet size of salmonid species (Wankowski and Thorpe 1979; Brannas and Alanara 1992). The results may support the findings of the present study. However, feed range selectivity may be governed by hunger levels of fish (Croy and Hughes 1991). Ellis et al. (1997) reported that farmed turbot prefer pellets and due to their extended jaws they can engulf large prey items (Holmes and Gibson 1986). Some workers (Hjertnes et al. 1993; Tuene and Nortvedt 1995) have used larger pellet sizes in experiments with halibut than those recommended for Atlantic salmon, possibly because halibut have a larger mouth gape than salmonids of the same weight. In contrast, Stradmeyer et al. (1988) reported that adult salmon showed a more immediate response to larger pellets but that these were more likely to be rejected than pellets of a shorter length. However, the texture and hardness of pellets is an important issue. It has been seen that juvenile salmon can handle larger size of soft pellets than hard pellets (Mearns 1990). Tuene and Nortvedt (1995) fed 9-mm pellets to 90–662-g halibut and concluded that the high intra-individual (day-to-day) coefficient of variation of feed intake may have been caused by the large pellet size since the average consumption at each meal was less than two pellets per fish.

Whole body proximate composition data revealed significantly lower whole body crude protein contents for the group of fish fed MP diet than those of fish fed EP diet, whereas whole body crude lipid content was significantly higher among the group of fish fed MP diet (Table 4). Moisture contents for fish fed EP_2 were significantly lower than those of fish fed all other diets. However, there was no significant difference in whole body moisture content among the group of fish fed EP_1 and MP diets. Similar findings have been reported in various previous experiments. For instance, Cho and Cho (2009) reported from their experiments that proximate composition of whole body of flounder with and without liver, except for moisture content of liver, was not significantly affected by the different diets (extruded pellets, semi-moist pellets, and moist pellets). Results for whole body amino acids (Table 5) showing only four amino acids viz. aspartic acid (Asp), threonine (Thr), serine (Ser), and tyrosine (Tyr) were significantly lowest for the fish fed MP diet than those of fish fed other

Table 4 Whole body proximate composition of juvenile olive flounder fed with the different experimental diets for 6 weeks (percentage of DM basis)

Items	Diets			Pooled SEM
	EP_1	EP_2	MP	
Moisture	76.87[a]	75.36[b]	77.11[a]	0.30
Crude ash	17.53	16.96	18.45	0.47
Crude lipid	3.25[b]	5.34[ab]	7.50[a]	0.58
Crude protein	74.70[a]	74.16[a]	71.91[b]	0.53

Value are means from groups ($n = 3$) of fish where the values in each row with different superscripts are significantly different ($P < 0.05$)
Pooled SEM pooled standard error of mean (SD/√*n*)

Table 5 Whole body amino acid composition of juvenile olive flounder fed with the different experimental diets for 6 weeks (percentage of DM basis)

Amino acids (AA)	Diets			Pooled SEM
	EP_1	EP_2	MP	
Essential (EAA)				
Methionine	1.64	1.50	1.37	0.05
Leucine	5.07	4.84	4.82	0.05
Isoleucine	3.38	2.90	2.98	0.11
Arginine	4.37	4.39	4.01	0.06
Histidine	1.87	1.78	1.90	0.04
Lysine	5.56	5.19	5.25	0.06
Phenylalanine	2.72[a]	2.64[ab]	2.54[b]	0.03
Threonine	2.90[a]	2.82[a]	2.18[b]	0.09
Valine	3.39	3.27	3.35	0.03
Non-essential (NEAA)				
Alanine	4.59	4.71	4.33	0.07
Glycine	5.31	6.06	5.24	0.18
Aspartate	7.25[a]	7.14[a]	6.27[b]	0.13
Proline	3.44	3.69	3.33	0.07
Serine	2.93[a]	2.87[a]	1.50[b]	0.20
Glutamate	10.32	10.00	9.96	0.09
Tyrosine	1.94[a]	1.83[a]	1.08[b]	0.12
Cystine	1.29[a]	1.15[ab]	1.00[b]	0.04

Value are means from groups ($n = 3$) of fish where the values in each row with different superscripts are significantly different ($P < 0.05$)
Pooled SEM pooled standard error of mean (SD/√*n*)

experimental diets. Although significant differences were recorded in whole body amino acids for other 13 amino acids, no clear trend could be drawn among different treatments. MP diet appeared to affect distinctly only these four amino acids. Due to the lack of reports on whole body amino acid contents in similar studies, it is difficult to compare the present observation with others.

Hematological characteristics can be used as an index of the health status of fish (Blaxhall 1972). Hematological changes have been detected following different types of stress conditions like exposure to pollutants, diseases, and hypoxia (Duthie and Tort 1985). Hence, it could be suggested that any unhealthy condition caused by poor nutrition could affect the hematological characteristics of fish. Plasma glucose concentration is one of the stress indicators in fish (Menezes et al. 2006) which may vary greatly depending on the physiological status of the animal (de Andrade et al. 2007). Mommsen et al. (1999) reported that plasma glucose values can increase, decrease, or keep constant under a high plasma cortisol level. Plasma GOT and GPT activities may give information on liver injury or dysfunction (Wells et al. 1986). They are also used as valuable diagnostic means of stress responses in several fish species (Almeida et al. 2002; Choi et al. 2007). The present study has been indicated that plasma GOT and glucose in the group of fish fed MP diet were significantly higher than those of fish fed EP diet because fish might be always in stress of feed competition (Table 6). However, no significant differences were found in GPT, T-protein, cholesterol, and triglyceride levels among the fish fed EP or MP diet.

In salmon aquaculture, MP diets were used due to their better acceptance with soft texture and relatively low cost compared to dry diet (Ghittino 1979). However, in yellow tail and flounder culture, MP diet has demerits in causing water pollution from leftover feed which ultimately increases production cost by decreasing water quality and quantity of fish (Kim and Shin 2006; Kim et al. 2007). In this instance, extruded pellet diet could be a right choice to minimize water pollution and increase total production in flounder aquaculture.

Table 6 Hematological parameters of juvenile olive flounder fed with the different experimental diets for 6 weeks

Parameters	Diets			Pooled SEM
	EP_1	EP_2	MP	
GOT (U/L)	34.0^b	34.7^b	44.9^a	1.96
GPT (U/L)	5.0	5.3	5.0	0.23
Glucose (mg/dL)	62.0^b	61.0^b	86.3^a	4.18
T-protein (g/dL)	3.4	3.3	3.0	0.06
Cholesterol (mg/dL)	238.0	265.5	238.6	10.5
Triglycerides (mg/dL)	259.4	308.2	325.4	28.0

Value are means from groups ($n = 3$) of samples where the values in each row with different superscripts are significantly different ($P < 0.05$)
Pooled SEM pooled standard error of mean (SD/√*n*), *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase

Conclusions

The results from the present study demonstrated the beneficial effects of EP and their diameter over commonly used MP diets in promoting growth of olive flounder, suggesting the need to revise the feed and feeding technology for flounder aquaculture. In the present experiment, the results evidenced that fish fed EP_2 (5 mm) had the better growth and water quality parameters than did fish fed MP diet in juvenile olive flounder.

Abbreviations

CL: Crude lipid; CP: Crude protein; EAA: Essential amino acids; EP: Extruded pellet; FE: Feed efficiency; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase; MP: Moist pellet; SGR: Specific growth rate; SR: Survival rate; WG: Weight gain

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. There is no additional data and materials to disclose.

Authors' contributions

SL conducted the research, analyzed the samples, and prepared the draft manuscript. MM helped to write the draft manuscript. JB, MS, and YS helped in the research conduction and statistical analysis. BKD helped in the research design and reviewed the manuscript. SCB designed and monitored the experiment and finalized the draft manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

The manuscript has been read and approved by the authors, and none of its parts have been submitted and published elsewhere. The authors also declare that nobody who qualifies for authorship has been excluded from the list of authors.

Ethics approval

The experiment was conducted under the guidelines of Animal Ethics Committee Regulations, No. 554, issued by the Pukyong National University, Busan, Republic of Korea.

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