

Biological Estimation of Waste Products from Olive Flounder (*Paralichthys olivaceus*) Fed on Three Different Feed Types

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Waste products were biologically estimated from juvenile flounder (*Paralichthys olivaceus*) on three diet types—raw fish-based moist pellets, moist pellets, and extruded pellets. Total solid and soluble wastes were estimated by determining nutrient digestibility and accumulation in juvenile flounders through growth trials. Total solid wastes produced were 20%-23% of the organic matter supplied. Soluble excretions ranged from 45% to 49%. Soluble nitrogenous excretions ranged from 36.4% to 46.2%. These results indicate that about 30.2%-35.9% of supplied feed is retained in the fishes' bodies while the remainder of feed is excreted into culture systems or the surrounding environment.

Key words: Waste products, Flounder, *Paralichthys olivaceus*, Feed waste, Fecal solids, Nitrogenous excretion.

Introduction

Mariculture areas have been increased to meet requirements for high-quality seafood production, but the consequent increase in aquaculture waste introduced into surrounding environments has led to many types of aquaculture being viewed as environmentally unfriendly. In all aquaculture systems, most circulated or effluent wastes originate primarily from feed (Cripps and Bergheim, 2000). For this reason, proper understanding and estimation of total waste production, in the form of solid waste as feed waste and fecal solids, and soluble waste as metabolites and nitrogenous wastes from supplied feed, are essential to formulate appropriate strategies for both environmentally sound coastal use and cost-effective aquaculture. Note in this context that most estimations of fish waste from culture systems (Leung et al., 1999; Zhu et al., 1999; Pagand et al., 2000; Crear et al.,

2002; Xu et al., 2007; Lam et al., 2008; Sun and Chen, 2009) have been undertaken using direct chemical methods. The chemical analysis of water quality is useful for examining concentrations in a unit volume of water. However, since fish waste per unit volume of effluent varies greatly according to the time of day, month, or year, depending on the culture system and its management, obtaining accurate, representative, reproducible samples for waste is extremely difficult without the use of homogeneously mixed samples taken from throughout the system (Cho et al., 1991). The chemical composition of waste solids in a recirculating aquaculture system also varies according to the sampling site (Lee, 2004). In contrast, the mass balance of nutrients in feed through fish feeding and growth can provide accurate data on waste products for the management of total allowable mass and carrying capacity. Moreover, this nutritional method is more economical than the traditionally used chemical analysis (Cho et al., 1991).

The olive flounder, *Paralichthys olivaceus*, is a

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commercially important aquaculture and fisheries species in Korea, Japan, and China. Most flounder farmers usually feed flounder with raw fish-based moist pellets, moist pellets, or extruded pellets. These types of pellets are made in part or entirely from a basic powder compound that is commercially formulated and supplied by feed companies (Lee and Jo, 2008). However, little information exists on solid and soluble waste production from flounder feeds under experimental conditions. Therefore, we estimated the waste products from juvenile flounder fed on three different diets commonly used in flounder farms, with the aim of providing useful information for the large-scale feeding and waste management of flounder culture.

Materials and Methods

Experimental Conditions

The experiment was conducted in water flow culture systems consisting of rearing tanks and solid collection systems (Fig. 1). Flounder purchased from a private flounder farm were acclimatized for 3 weeks to experimental conditions. During this period, the fish were fed the same type of extruded feed that was used at the farm. Subsequently, a series of waste collection, digestibility, and growth experiments were conducted using three replicates for each experimental diet in 250-L circular PVC tanks (40 fish per tank) for 6 weeks. Sand-filtered seawater was continuously pumped into the culture system. The water temperature and salinity were maintained at $19 \pm 1^\circ\text{C}$ and 34 ppt, respectively, during the experiment. The juvenile fish (initial average body weight of 3.4 g) were hand-fed twice daily until satiated.

The diets contained varying amounts of formulated powder compound from 100% in the moist pellets to 65% in the raw fish-based moist pellets. The formulation, ingredients, and manufacturing methods are detailed in Lee and Jo (2008), and the chemical composition of the diets is shown in Table 1. All diets contained 0.5% chromic oxide (Cr_2O_3).

Waste Collection

Feed waste was collected from effluent in each rearing tank using a micro-screen ($<15 \mu\text{m}$) for 40 minutes while satiation feeding was being carried out. After the feed waste was collected, fecal solid collectors were connected to the fish tank. The fecal solid collector was designed to connect a fish tank, a collection column, and a settling bottle (300 mL) vertically to minimize the amount of fecal matter flushed out before it had settled (Fig. 1). According to

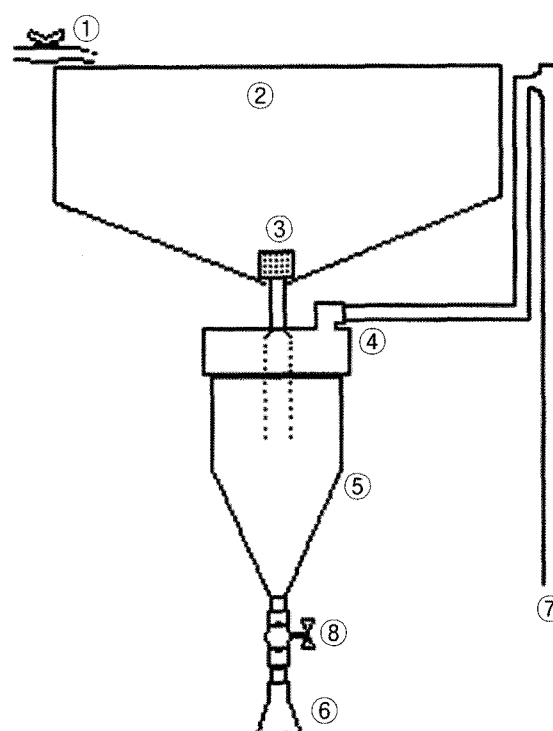


Fig. 1. Fish culture tank and feces collection system; 1, water inflow; 2, rearing tank (250 L); 3, screen; 4 detachable part; 5, collection column; 6, removable flask (300 mL); 7, water outflow; 8, valve.

Table 1. Chemical compositions (% dry matter basis) of the experimental diets

	Diets ¹		
	EP	RMP	MP
Crude protein	50.75	59.30	54.17
Crude lipid	9.51	8.70	5.41
Crude ash	18.20	17.66	20.20
Crude fiber	1.72	1.61	1.51
N-free extract ²	19.82	12.75	18.71
Gross energy (kJ/g) ³	19.14	19.62	18.14
Digestible protein ⁴	44.88	54.80	49.82
Digestible lipid ⁴	8.26	7.98	4.57
Digestible NFE ⁴	12.65	6.09	10.81
Digestible energy (kJ/g) ⁴	15.68	17.39	15.18

¹EP, extruded pellet; RMP, raw fish-based moist pellet; MP, moist pellet.

²Calculated by difference (Aksnes et al., 1997).

³Calculated using combustion values for protein, lipid and NFE of 23.6, 39.5 and 17.2 MJ/kg, respectively (Aksnes and Opstvedt, 1998).

⁴Calculated based on Table 2.

Hajen et al. (1993), nutrient leaching from feces in seawater was to be expected over 6 hours; thus, feces were collected within 3- and 5-hour intervals. The collected fecal matter was then dewatered, frozen, and freeze-dried, before being pooled over the 6-

week period prior to analysis.

Chemical Analysis

At the beginning and end of the experiment, 15 fish, taken from both the stock population and each rearing tank, were randomly sampled, and the chemical composition of the whole body was determined. Samples of the feed, feed waste, and feces were also used for the analysis of Cr_2O_3 (Furukawa and Tsukahara, 1966), protein (Kjeldahl; VAPODEST-5, No 6550; Gerhardt Co. Ltd., Germany), lipids (Soxhlet extraction), fiber (Fibertec automatic analyzer; Tecator, Hoganas, Sweden), and ash (at 550°C for 6 hours in a muffle furnace). The nitrogen-free extract (NFE) was calculated by means of the difference (Aksnes et al., 1997).

Calculations

The apparent digestibility coefficient (ADC), fecal solids, total soluble excretion, and nitrogenous excretion were calculated on a dry matter basis using the following equations:

$$\text{ADC of nutrients or energy (\%)} = [1 - (\text{dietary } \text{Cr}_2\text{O}_3 / \text{fecal } \text{Cr}_2\text{O}_3) \times (\text{fecal nutrient or energy} / \text{dietary nutrient or energy})] \times 100 \quad (\text{eq. 1})$$

$$\text{Fecal solids (\%)} = [\text{nutrients and fiber consumed in feed} \times (1 - \text{ADC of nutrients and fiber})] \div \text{nutrients and fiber in feed} \times 100 \quad (\text{eq. 2})$$

$$\text{Total soluble excretion (\%)} = [(\text{nutrients consumed in feed} \times \text{ADC of nutrients}) - \text{nutrients retained in fish body}] \div \text{nutrients consumed in feed} \times 100 \quad (\text{eq. 3})$$

$$\text{Nitrogenous excretion (\%)} = [(\text{nitrogen consumed in feed} \times \text{ADC of nitrogen}) - \text{nitrogen retained in fish body}] \div \text{nitrogen in feed} \times 100 \quad (\text{eq. 4})$$

Statistical Analysis

A one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to analyze the significance of the differences among the test groups at $P \leq 0.05$ using the SAS program (SAS Institute Inc., Cary, North Carolina, USA).

Results

Digestibility

The determination of apparent digestibility of nutrients is shown in Table 2. The ADC of protein in fish fed the extruded pellets was significantly ($P < 0.05$) lower than that of fish fed with raw fish-based moist pellets or moist pellets. However, the ADC of

NFE in the fish fed with extruded pellets was significantly ($P < 0.05$) higher than that of fish fed with raw fish-based moist pellets (Table 2). The ADC of lipids did not differ among diets ($P > 0.05$).

Table 2. Apparent digestibility of nutrients in juvenile flounder fed the experimental diets

Diets ¹	Apparent digestibility coefficient (%)		
	Crude protein	Crude lipid	NFE ²
EP	88.4 ± 0.15 ^b	86.9 ± 0.78 ^a	63.8 ± 0.53 ^a
RMP	92.4 ± 0.15 ^a	91.8 ± 4.25 ^a	48.1 ± 3.27 ^b
MP	92.0 ± 0.49 ^a	84.4 ± 5.99 ^a	57.7 ± 3.73 ^a

¹EP, extruded pellet; RMP, raw fish-based moist pellet; MP, moist pellet.

²NFE, nitrogen-free extract.

Values (mean ± SD of three replications) with different superscript within same column are significantly different ($P < 0.05$).

Growth Performance and Body Composition

The highest weight gain was achieved in the fish fed the raw fish-based moist pellets, but this was not significantly ($P > 0.05$) different from the weight gain in fish fed on extruded pellets (Table 3).

No significant differences in protein and ash contents of the whole body were found in fish fed with raw fish-based moist pellets, moist pellets, or extruded pellets ($P > 0.05$; Table 4). Fish fed on extruded pellets had significantly ($P < 0.05$) higher whole-body lipid content than fish fed on other diets.

Table 3. Growth performance of juvenile flounder fed the experimental diets

Diets ¹	Av. body wt. ± SD (g)		Weight gain ² (%)
	Initial	Final	
EP	3.2 ± 0.49 ^a	17.0 ± 0.23 ^b	414.4 ± 40.43 ^{ab}
RMP	3.5 ± 0.52 ^a	19.4 ± 0.40 ^a	452.4 ± 21.60 ^a
MP	3.4 ± 0.61 ^a	16.7 ± 0.17 ^b	394.7 ± 16.45 ^b

¹EP, extruded pellet; RMP, raw fish-based moist pellet; MP, moist pellet.

²[(Final fish weight - Initial fish weight + Fish loss weight) × 100] / Initial fish weight.

Values (mean ± SD of three replications) with different superscripts within each column are significantly different ($P < 0.05$).

Total Solid Waste and Nitrogenous Solids

The amount of feed waste ranged from 5.9% to 6.8% of feed supplied (Table 5). The feed waste in fish fed on extruded pellets was slightly, but not significantly ($P > 0.05$), higher than that in fish fed on other diets. Fecal solid waste was estimated from undigested protein, lipids, and NFEs (eq. 2). Fecal solid waste ranged from 13.5% to 16.0% of feed supplied. The fecal solid waste from fish fed on

Table 4. Proximate composition of whole body in juvenile flounder fed the experimental diets

Diets ¹	Moisture	Crude protein	Crude lipid	Crude ash
EP	77.3 ± 0.44 ^a	15.7 ± 0.21 ^a	3.0 ± 0.10 ^a	3.2 ± 0.15 ^{ab}
RMP	77.2 ± 0.72 ^a	15.9 ± 0.25 ^a	2.5 ± 0.15 ^b	3.3 ± 0.33 ^{ab}
MP	78.6 ± 0.54 ^a	15.0 ± 0.46 ^a	2.2 ± 0.15 ^b	3.4 ± 0.18 ^a

¹EP, extruded pellet; RMP, row fish-based moist pellet; MP, moist pellet. Moisture, crude protein, crude lipid, and ash contents of initial fish were 74.1, 16.9, 3.3, and 3.3%, respectively. Values (mean ± SD of three replications) with different superscript within each column of criteria are significantly different ($P < 0.05$).

Table 5. The dry matter proportions of total solid and soluble wastes and their nitrogen excretion to feed supplied in juvenile flounder fed the experimental diets

Diets ¹	Total solid waste		Total soluble	Nitrogenous excretion		Nutrient
	Feed Waste	Feces	excretion	Total solid	Metabolite	Retained
EP	6.8 ± 1.46 ^a	16.0 ± 0.21 ^a	44.5 ± 0.79 ^b	18.3 ± 1.40 ^a	36.4 ± 1.42 ^b	32.7 ± 0.52 ^{ab}
RMP	6.0 ± 0.38 ^a	13.5 ± 0.87 ^{ab}	44.6 ± 2.28 ^b	13.6 ± 0.36 ^b	43.6 ± 1.85 ^a	35.9 ± 2.05 ^a
MP	5.9 ± 0.12 ^a	14.6 ± 0.30 ^b	49.3 ± 1.19 ^a	13.9 ± 0.42 ^b	46.2 ± 1.43 ^a	30.2 ± 0.97 ^b

¹EP, extruded pellet; RMP, row fish-based moist pellet; MP, moist pellet. Values (mean ± SD of three replications) with different superscript within each column are significantly different ($P < 0.05$).

extruded pellets was significantly ($P < 0.05$) higher than that of fish fed on other diets. Total nitrogenous solids ranged from 13.6% to 18.3%. The nitrogenous total solid waste of the fish fed extruded pellets was significantly ($P < 0.05$) higher than those of fish fed other diets (Table 5).

Total Soluble and Nitrogenous Metabolites

Total soluble and nitrogenous metabolites were calculated from equations 3 and 4, respectively. The total soluble metabolite excretion calculated from the organic matter balance was from 44.5% to 49.3% of feed supplied. The total soluble metabolite excretion of fish fed on moist pellets was significantly higher than that of fish fed on other diets ($P < 0.05$; Table 5). Nitrogenous metabolite excretions ranged from 36.4% to 46.2%. The proportion of nitrogenous metabolite excretion from fish fed on extruded pellets was significantly ($P < 0.05$) lower than that of fish fed on other diets (Table 5).

From these data, we calculate that about 30.2%–35.9% of the organic matter proportion of feeds will be accumulated in fish bodies as food products, while the remainder of the organic matter of supplied feed will be discharged into culture systems or surrounding environments as nutrient waste.

Discussion

Digestibility

Nutrient digestibility is strongly correlated with fish growth (Lee and Jo, 2008). Waste production estimates based on biological estimation suggest

economical and exact data because feed is the sole source of all forms of waste produced in fish culture systems (Cho et al., 1991). Since both feed digestibility and fish growth rate affect waste products as fecal solids and metabolites produced for a given feed, biological estimation of waste products will help to manage the carrying capacity, total allowable concentrations of aquaculture effluents, and culture water quality.

Feed ingredients, composition, and process methods will affect feed digestibility and waste production. Fish fed on extruded pellets showed higher NFE ADC than those fed on raw fish-based moist pellets. The extrusion process has been known to improve the availability of dietary starch in both marine (Jeong et al., 1991) and freshwater fish (Bergot and Breque, 1983; Takeuchi et al., 1990; Takeuchi et al., 1994).

Growth Performance and Body Composition

The growth performances of fish fed on the experimental diets used in this study and comparisons with other studies were detailed by Lee and Jo (2008). From carcass analysis, flounders seem to have a comparatively low lipid deposition capacity, as shown by Oku and Ogata (2000). Similarly, Berge and Storebakken (1991) reported no difference in the chemical composition of halibut fillets (weighing from 0.6 to 1.5 kg) when fish were fed dietary lipid levels ranging from 8% to 20%. As Ando et al. (1993) reported, flounders tend to accumulate lipids in the liver, and not in the muscles. Gholam et al. (1992) also noted that a higher HSI (Hepatosomatic index)

was observed in fish fed on diets with a higher E:P ratio. These results indicate that juvenile flounder cannot utilize high levels of dietary lipid for growth or other synthesis. Moreover, excessive dietary lipids will aggravate waste production. In the light of this, the effects of lipid content in flounder diets on waste production and protein sparing effects will be of interest.

Total Solid Waste and Nitrogenous Solids

Under appropriate culture conditions, factors including manual feeding skills (Kolsäter, 1995; Islam, 2005), fish feed activity and feed palatability, stability, and formulation (Hebb et al., 2003) affect the production of feed waste. Feed waste products can also vary according to feed manufacturing and handling.

In the present study, cautious hand-feeding resulted in feed waste of 6% to 7%. This is comparable to the feed waste of 5% suggested by Cho et al. (1991). However, the feed waste of 19% produced from yellow tail (*Seriola quinqueradiata*) fed raw fish-based moist pellets (Watanabe, 1991) is much higher than that of the fish fed on raw fish-based moist pellets in the present study. However, feed waste could be higher when the proportion of minced fish in the raw fish-based moist pellets is increased.

In the present study, ash was deliberately excluded from the calculation of fecal solid waste because fish have the ability to uptake and release a considerable amount of minerals from culture water. The mass imbalance of minerals from ash digestibility in Atlantic halibut (*Hippoglossus hippoglossus*) has been suggested by Grisdale-Helland and Helland (1998). However, they calculated similar rates (13.8%-24%) of fecal solid waste from organic matter digestibility.

Total Soluble and Nitrogenous Metabolites

According to Robaina et al. (1999), the ammonia N excretion rates for sea bass fed on extruded diets were lower than those for fish fed on pelleted diets. One possible explanation for this is that the extrusion process improved the availability of starch and consequent nitrogen utilization, as reported by Takeuchi et al. (1990), Jeong et al. (1991), and Takeuchi et al. (1994). According to Kikuchi et al. (1992, 1993) flounder excreted 24%-33% of consumed nitrogen as metabolic nitrogen. However, the present study and other studies by Jobling (1981), Kikuchi et al. (1991), and Porter et al. (1987), have found that metabolic nitrogen excretions are much lower. Gaumet et al. (1994) also noted that the flounder nitrogen

accumulation (62%) reported by Kikuchi et al. (1991, 1992) was much higher than that reported by Bromley (1980) on turbot, in which the author observed a 40% protein utilization coefficient. In contrast, the nitrogen accumulation ratios of 30% and 27% reported by Porter et al. (1987) and Nijhof (1994), respectively, were lower than those recorded for extruded pellets (45%), raw fish-based moist pellets (43%), and moist pellets (40%) in this study. Since a linear relationship between N intake and N excretion has been well established by many studies (Kaushik and Cowey, 1991; Kikuchi et al., 1992; Médale et al., 1995), higher metabolic N excretion rates are expected in fish fed on diets with higher protein content. The metabolic nitrogen excretions of the fish fed the raw fish-based moist pellets and the moist pellets, which contain dietary protein levels of 59.3% and 54.2%, respectively, were higher than those of fish fed on extruded pellets, which contained dietary protein of 50.8%.

Conclusions

In this study, we suggest a biological estimation of feed availability and waste products through measurements of digestibility, growth, and nutrient accumulation for the three feed types used by flounder farmers. The dry matter proportions of nutrients retained in the fish bodies were 33%, 36%, and 30% for the supplied diets of extruded pellets, raw fish-based moist pellets, and moist pellets, respectively. The remaining nutrients were excreted as solids and as soluble excretions. These data on waste products, obtained using a biological method, are valuable for predicting aquaculture waste outputs and for the development of low-pollution diets.

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