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Research Article

Comparative Studies on Effects of Extruded Pellets and Dough Type Diets on Growth, Body Composition, Hematology and Gut Histology of Juvenile Japanese Eel, *Anguilla japonica* (Temminck et Schlegel)

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Abstract

Objective: The present study was conducted to evaluate the effects of two different sizes of extruded pellets and dough type diets on the growth performance, body composition, hematological parameters and gut histology of juvenile Japanese eel, *A. japonica*. **Methodology:** A 10-weeks feeding trial was conducted to evaluate the effects of two different sizes of extruded pellets (EP) and dough type diet on the growth performance, body composition, hematology and gut histology of juvenile Japanese eel, *Anguilla japonica*. Fish with average weight 21.3±1.0 g (means±SD) were fed 3 experimental diets: One dough type diet and two different sizes of Extruded Pellets (EP_1- 2 and EP_2- 3 mm) in triplicate groups. **Results:** At the end of feeding trial, the estimated weight gain, specific growth rate and feed efficiency of fish fed EP diets were significantly higher than those of fish fed dough diet ($p<0.05$). Water quality parameters, such as turbidity, total ammonia nitrogen and total phosphorus from tanks of fish fed EP_1 and EP_2 were significantly lower than those from tanks of fish fed dough diet indicating that EP diet was probably pollution free and environmentally friendly. Plasma glutamic oxaloacetic transaminase (GOT) and glucose concentrations were significantly higher in fish fed dough diet compared with those of fish fed EP diets ($p<0.05$). Whole body amino acid composition of fish was not significantly affected by the experimental diets ($p>0.05$). No differences in intestinal morphology or development of pathological lesions were evident in fish fed the diets. **Conclusion:** The results demonstrated that EP_2 diet could be a more efficient diet to promote improved growth performance and ensure better water quality parameters in Japanese eel aquaculture.

Key words: Japanese eel, extruded pellet, dough feed, growth, hematology, histology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Eel aquaculture has been considered as one of the most profitable industry in the world due to overwhelming demand in domestic as well as overseas seafood market. The Japanese eel, *Anguilla japonica* (Temminck et Schlegel) has been the predominant freshwater aquaculture species in Asia for last three decades. In Korea, the total production of this fish species reached to 5,149 metric tons in 2013 FAO[1]. However, due to lack of standard seed production technology and heavy dependence on wild catch, aquaculture of Japanese eel has become one of the toughest and challenging aquaculture industries. In order to increase its capture production, some studies have been conducted to boost aquaculture production and to meet the ever-increasing demand for this valuable species[2].

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[3,4]. During the last decade, there has been a marked increase in the use of extruded diets for feeding fish. These diets have superior water stability, better floating properties and higher energy content than other pelleted diets[5-7]. The main effects of feeding extruded diets on fish are: an increase in fish growth and an improvement in feed conversion ratio[8,9].

Furthermore, 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. Sub-optimal size pellets or high amount of pellets may cause feed wastage, as fish may be unable to catch large numbers of pellets before they sink through the net pen[10]. Fish farmers use different sizes of feed pellets during grow out period according to the size of fishes. The optimal size of pellets for different age group of eel is very important for maximum growth. Pellet size was also found to be a limiting factor for fish to ingest enough pellets to maintain the maximum growth[11,12].

Although, several studies have been done in the context of nutrient requirements and feeding methods of Japanese eel, limited information on the effects of extruded pellets and their size for juvenile Japanese eel is available. Therefore, the present study was conducted to evaluate the effects of two different sizes of extruded pellets and dough type diets on the growth performance, body composition, hematological parameters and gut histology of juvenile Japanese eel, *A. japonica*.

MATERIALS AND METHODS

Experimental diets: In this experiment, dough type diet from a domestic feed company (Woosung Feed Co., Ltd., Busan, Korea) and two different sizes of commercially available extruded pellets such as EP_1 (2 mm) and EP_2 (3 mm) were imported from Canada (EWOS Canada Ltd., BC, Canada). Proximate compositions of the experimental diets are shown in Table 1. All diets were stored in air tight bags at -20°C until use.

Table 1: Proximate composition of the experimental diet (DM basis, percentage unless otherwise stated)

	Diets[1]		
Items	EP_1	EP_2	Dough
Moisture	6.99	7.02	7.09
Crude ash	10.00	10.42	10.50
Crude lipid	12.54	12.27	12.36
Crude protein	52.02	51.97	52.01
Crude fiber	2.12	2.09	1.93
Carbohydrate	16.33	16.23	16.11
Gross energy (kJ g^{-1})	16.15	16.02	16.07

[1]EP: Extruded pellet from EWOS Canada Ltd., BC: Canada, Dough: Feed from Woosung Feed Ltd., Busan, Korea

Experimental fish and feeding trial: The feeding trial was conducted at Inland Aquaculture Research Institute, National Institute of Fisheries Science (NIFS), Jinhae, Korea. Prior to the feeding trial, fish were fed commercial dough diet for 2 weeks to acclimate to environmental condition. The acclimation was followed by 24 h starving. A total of 180 juvenile Japanese eel with an average initial weight of 21.3±1.0 g (means±SD) were randomly distributed to each (20 fish/tank) of 9 circular plastic tanks (300 L per tank) in triplicate groups. All experimental tanks were equipped with an aeration system and water was heated by electric heaters in the central tank. Water temperature was maintained at 26±0.5°C and water flow rate was at 1 L min^{-1}. Each experimental diet was fed to triplicate groups at apparent satiation with a feeding rate of 2-4% per wet body weight, where the amount of feeds required for each day was divided into 2 parts and fed twice per day for 10 weeks. Total fish weight in each tank was determined after every 2 weeks and the amount of diet fed to fish was adjusted accordingly.

Water quality analysis: Water samples from the fish tanks were monitored daily just after 2 h of feeding of each meal. 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[13].

Sample collection and analysis: At the end of the 10 weeks of feeding trial, fish were starved for 24 h and they were then counted and weighed to calculate the weight gain

(WG), specific growth rate (SGR), feed efficiency (FE) and survival rate following standard methods. 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[14]. To determine the moisture content, the diet and fish samples were dried to maintain constant weights at 105°C for 24 h. Ash content was determined using a muffle furnace (550°C for 4 h). Crude lipid content was determined by the soxhlet extraction method using soxtec system 1046 (Foss, Hoganas, Sweden) and crude protein content by Kjeldahl method (N×6.25) after acid digestion. Carbohydrate content was calculated by subtracting total percentage of nutrient contents from 100. Gross energies of experimental diets were measured through combustion in a bomb calorimeter (Parr 1351, Parr Instrument Co., Moline, IL, USA). Blood samples were taken by using heparinized syringes from the caudal vein of 5 randomly chosen fish per tank. Plasma was collected from the blood after centrifugation at 3,000×g for 10 min at room temperature and stored at -70°C in order to analyze glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glucose, total protein, cholesterol and triglycerides. Plasma analysis were performed by using the kits of DRI-CHEM 4000i- Fuji Dri-chem Slide- 3150 (Minato-ku, Tokyo, Japan). Whole body amino acid analysis was performed by ninhydrin method (Sykam Amino Acid Analyzer S433, Sykam, Eresing, Germany). For histological study, three representative fish of each treatment were first dissected and then anterior parts of intestine were collected. The intestinal samples were fixed in 10% neutral buffered formalin, dehydrated in Bouin's solution and finally embedded with paraffin. Transverse sections of embedded intestines were taken at thickness of 5~7 μm using microtome machine (Leica, Germany). Tissue sections were stained with Harris's hematoxylin and 0.5% eosin (H and E) for histological examinations. The slides were then examined under a compound light microscope (CarlZeiss, Germany) and the pictures of the intestinal tissues were recorded for further analysis.

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, a least significant difference (LSD) *post hoc* test was used to compare means. Treatment effects were considered at $p<0.05$ level of significance.

RESULTS

Water quality parameters including turbidity, TAN and TP are presented in Table 2. Overall performance of water quality parameters recorded to be more favorable for the groups of fish fed EP_1 and EP_2 diets compared with dough diet.

Growth performance of juvenile Japanese eel fed different experimental diets for 10 weeks is shown in Table 3. At the end of experiment, WG, SGR and FE of fish fed EP_2 diet were significantly higher than those of groups fed EP_1 and dough diet. However, no significant differences in these parameters were observed among the fish fed EP_1 and dough diets. Survival rate ranged from 87.7-98.2% without any statistical difference among different treatments.

Whole body proximate compositions of juvenile Japanese eel fed different experimental diets for 10 weeks are summarized in Table 4. There were no statistical differences in moisture, crude ash, crude lipid and crude protein content among the fish fed different experimental diets.

Table 5 shows the whole body amino acid composition of juvenile Japanese eel fed different experimental diets for 10 weeks. Although, significant differences were recorded in few amino acids but no clear trend could be drawn.

Table 2: Water quality parameters after 2 h of feeding of juvenile Japanese eel fed different experimental diets for 10 weeks

Items	Diets[1] EP_1	EP_2	Dough	Pooled SEM[2]
Turbidity (NTU)	0.44^b	0.46^b	1.51^a	0.18
TAN (mg L^{-1})	0.68^b	0.67^b	1.16^a	0.08
TP (mg L^{-1})	0.05^b	0.05^b	0.29^a	0.04

TAN: Total ammonia nitrogen, TP: Total phosphorus, [a,b]Within a row means from the groups (n = 3) with different superscripts are significantly different ($p<0.005$), [1]EP: Extruded pellet from EWOS Canada Ltd., BC: Canada, Dough: Feed from Woosung Feed Ltd., Busan, Korea and [2]Pooled SEM: Pooled standard error of mean (SD/√n)

Table 3: Growth performances of juvenile Japanese eel fed different experimental diets for 10 weeks

Items	Diets[1] EP_1	EP_2	Dough	Pooled SEM[2]
WG[3]	124.4^b	129.2^a	123.2^b	1.02
SGR[4]	1.56^b	1.63^a	1.58^b	0.01
FE[5]	86.4^b	88.5^a	86.3^b	0.35
SR[6]	98.2	94.7	87.7	1.77

WG: Weight gain, SGR: Specific growth rate, FE: Feed efficiency, SR: Survival rate, [a,b]Within a row means from the groups (n = 3) with different superscripts are significantly different ($p<0.005$), [1]EP: Extruded pellet from EWOS Canada Ltd., BC: Canada, Dough: Feed from Woosung Feed Ltd., Busan, Korea, [2]Pooled SEM: Pooled standard error of mean (SD/√n), [3]Weight gain (%) = (Final weight-Initial weight)×100/Initial weight, [4]Specific growth rate (%/day) = (Log_e final weight-Log_e initial weight)×100/Days, [5]Feed efficiency (%) = (Wet weight gain/Dry feed intake)×100 and [6]Survival rate = (Total fish-dead fish)×100/Total fish

Table 4: Whole body proximate composition of juvenile Japanese eel fed different experimental diets for 10 weeks (percentage of dry matter basis)

	Diets[1]			
Items	EP_1	EP_2	Dough	Pooled SEM[2]
Moisture	61.56	61.52	61.98	0.30
Crude ash	5.340	5.170	5.390	0.06
Crude lipid	46.37	46.00	45.71	0.23
Crude protein	46.12	45.92	46.40	0.25

[a,b]Within a row means from the groups (n = 3) with different superscripts are significantly different (p<0.005), [1]EP: Extruded pellet from EWOS Canada Ltd., BC: Canada, Dough: Feed from Woosung Feed Ltd., Busan, Korea and [2]Pooled SEM: Pooled standard error of mean (SD/√n)

Table 5: Whole body amino acid composition of juvenile Japanese eel fed different experimental diets for 10 weeks (percentage of dry matter basis)

	Diets[1]			
Amino acids	EP_1	EP_2	Dough	Pooled SEM[2]
Essential amino acids				
Methionine	1.27	1.28	1.19	0.02
Leucine	2.97	3.10	3.16	0.07
Isoleucine	1.80	1.91	1.94	0.06
Arginine	3.05	2.93	3.03	0.03
Histidine	2.00	2.04	2.07	0.04
Lysine	3.07	3.20	3.08	0.08
Phenylalanine	1.76	1.72	1.85	0.06
Threonine	1.78	1.90	1.87	0.05
Valine	1.94^b	2.10^{ab}	2.34^a	0.07
Non-essential amino acids				
Alanine	3.18	3.22	3.27	0.04
Glycine	3.89	3.78	3.94	0.07
Aspartate	4.82	4.77	4.80	0.07
Proline	2.96	3.15	3.02	0.06
Serine	1.77	1.83	1.76	0.04
Glutamate	5.88	6.05	5.95	0.11
Tyrosine	1.02	1.09	1.19	0.06
Cystine	1.02	1.12	1.03	0.03

[a,b]Within a row means from the groups (n = 3) with different superscripts are significantly different (p<0.005), [1]EP: Extruded pellet from EWOS Canada Ltd., BC: Canada, Dough: Feed from Woosung Feed Ltd., Busan, Korea and [2]Pooled SEM: Pooled standard error of mean (SD/√n)

The hematological parameters of juvenile Japanese eel fed different experimental diets are presented in Table 6. Plasma GOT and glucose of fish fed dough diet were significantly higher than those of fish fed EP_1 and EP_2 diets. However, no significant differences were found in these parameters among the fish fed EP_1 and EP_2 diets. Similarly, there were no significant differences noticed in GPT, total protein, cholesterol and triglycerides contents among all the experimental groups.

Histological differences caused by the different experimental diets are presented in Fig. 1. No significant visible differences were observed in the anterior intestine of fish fed different experimental diets. The stratum compactum, lamina propria and apical absorptive vacuoles were prominent in intestine of fish fed all experimental diets. However, fish fed EP showed slightly better and distinct size and shape of villi (small finger-like projections that protrude from the epithelial lining of the intestinal wall) than fish fed dough diet.

Table 6: Hematological parameters of juvenile Japanese eel fed different experimental diets for 10 weeks

	Diets[1]			
Parameters	EP_1	EP_2	Dough	Pooled SEM[2]
GOT (U L^{-1})	108.7^b	101.1^b	131.0^a	4.14
GPT (U L^{-1})	3.0	3.0	3.0	0.29
Glucose (mg dL^{-1})	85.0^b	84.0^b	125.3^a	7.62
Total protein (g dL^{-1})	3.0	2.9	3.0	0.10
Cholesterol (mg dL^{-1})	446.0	445.0	463.0	12.00
Triglycerides (mg dL^{-1})	474.0	528.0	509.0	15.60

GOT: Glutamic oxaloacetic transaminase, GPT: Glutamic pyruvic transaminase, [a,b]Within a row means from the groups (n = 3) with different superscripts are significantly different (p<0.005), [1]EP: Extruded pellet from EWOS Canada Ltd., BC: Canada, Dough: Feed from Woosung Feed Ltd., Busan, Korea and [2]Pooled SEM: Pooled standard error of mean (SD/√n)

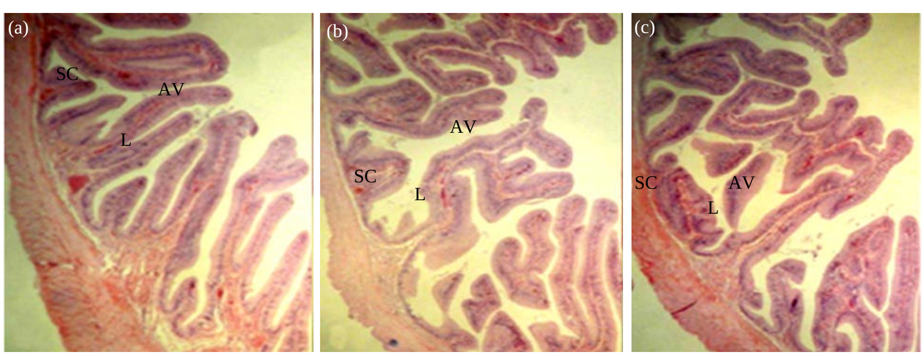

Fig. 1(a-c): Normal anterior intestine of juvenile Japanese eel fed different experimental diets for 10 weeks. The histological slides depicted mucosal epithelial cells with apical absorptive vacuoles (AV), lamina propria (L) and stratum compactum (SC) (H and E stain, ×400), (a) EP_1 group, (b) EP_2 group and (c) Dough diet group and EP: Extruded pellet

DISCUSSION

Results obtained from the present study shows the growth performance of Japanese eel fed the extruded pellets was significantly higher than those fed the dough diets. Furthermore, the present experiment clearly demonstrated the beneficial effects of pellet size of extruded pellets on the performance of Japanese eel. Similarly, Kim and Shin[15] reported that extruded pellets are better than paste diet for the growth performance and water quality management in eel aquaculture. In the present study, the results of lower feed efficiency in fish fed dough diet could be due to high leaching properties of it before the ingestion of feed by the fish.

Interestingly, it was observed that lower growth rate for the group of fish fed EP_1 compared with fish fed EP_2 diet. Aqua feed technology is moving in tandem with the aquaculture growth from the usage of extrusion procedures for the improvement of digestibility[16]. Chang and Wang[17] 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[18]. According to Chang and Wang[17], the gelatinization that occurs during extrusion process could improve durability of the feed rations and digestibility of starch. In the present study, results from the fish fed EP_2 diet, supported the various report that extruded pellets have a better efficiency over moist and dough diet for juvenile Japanese eel growth. However, it is difficult to attribute any reason for the observed lower weight gain for the group fed EP_1 diets in the present experiment.

Whole body proximate composition and whole body amino acids composition showed non-significant trends among different treatments, which agree well with that reported by Kim *et al.*[19] in olive flounder fed extruded pellet and moist pellet diets. In other words, whole body proximate and amino acids composition seems not to be reliable parameters in evaluating the efficiency of different extruded and dough diets in juvenile Japanese eel.

Water quality has been acknowledged to have profound effects on the growth performance and health of aquaculture fish species. In the present study, water quality parameters were recorded to fluctuate with the duration after feeding. Observed water quality parameters especially turbidity was significantly lower among the groups 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 highest among the group of fish fed dough diet than those of fish fed EP diets. Nevertheless, these water quality parameters may not be reliable factors directly affecting fish growth in a closed laboratory and flow through system. However, these results may indicate that dough diets were easily soluble in water before consumption by fish. On the other hand, extruded pellets were more stable in water and their leaching rate in water was comparatively prolonged. Water pollution by fish feeding is caused largely by increasing turbidity, as well as ammonia and phosphorus loading through uneaten feeds and feces[20]. From an on-farm experiment with flounder, Kim and Lee[21] reported that the excretion of nitrogen (N) ranged from 48-70 g and phosphorus (P) from 10-12 g kg^{-1} weight gain. However, under practical feeding conditions, flounder excreted much higher N of 114 g and P of 28 g kg^{-1} weight gain, suggesting a substantial waste of feed[22]. Likewise, Kim and Shin[15] reported significantly higher ammonia content in the eel fed paste feeds compared with that in pellet diets. The water quality parameters together with observed weight gain in the present experiment indicated that juvenile Japanese eel growth can be maximized under the environmentally friendly by using EP diet.

Hematological characteristics can be used as an index of health status of fish[23]. Hematological changes have been detected following different types of stress conditions like exposure to pollutants, diseases, hypoxia, etc.[24]. 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[25] and it may vary greatly depending on the physiological status of the animal[26]. Mommsen *et al.*[27] reported that plasma glucose values can increase, decrease or keep constant under high plasma cortisol. Plasma GOT and GPT activities may give information on liver injury or dysfunction[28]. They are also used as valuable diagnostic means of stress responses in several fish species[29,30]. The present study has been indicated that plasma GOT and glucose in the group of fish fed dough diet were significantly higher than those of fish fed EP diet because fish might be always in stressful condition due to feed competition (Table 6).

In the present study, no pathomorphological changes were observed in the anterior intestinal epithelium among the experimental diets. A similar finding was reported by Kim *et al.*[31] in Korean rockfish fed with extruded pellet, soft extruded pellet or moist pellet.

Moist pellet diets were used for salmon and trout production due to their better acceptance with soft texture and relatively low cost compared with dry diet[32]. However, in yellow tail and flounder culture, moist diet has demerits in causing water pollution from left-over feed, which ultimately increase the production cost by decreasing water quality and quantity of fish[15,33]. In this instance, extruded pellet diet could be a right choice to minimize water pollution and increase total production in eel aquaculture.

Moreover, pellet size obviously has an effect on fish performance and there are indications of this effect presented here. For example, a pellet that is larger than the mouth gape width of the fish or is so large that handling time becomes a limiting factor in the fish's ability to ingest enough pellets to maintain good growth will clearly have adverse effects. Smith *et al.*[11] examined fish feeding behavior at a commercial Atlantic salmon farm in Scotland. In concurrence with Stradmeyer *et al.*[12] found that adult salmon showed a more immediate response to larger pellets, but that these were more likely to be rejected than shorter pellets possibly due to their large mouth gape. Smith *et al.*[11] found that pellets slightly smaller than the ''normal'' commercial size were eaten at the fastest rate, thereby indicating that the salmons are perhaps adjusting their feeding behavior to compensate for smaller feed pellets.

CONCLUSION

In conclusion, results from the present study, showed the beneficial effects of extruded pellets and their diameters over commonly used dough diets in promoting growth of Japanese eel, suggesting the need to revise the feed and feeding technology for eel aquaculture. The findings also evidenced that fish fed EP_2 (3 mm) had improved growth performance and better water quality parameters than fish fed dough diet in juvenile eel aquaculture.

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