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Nutrient digestibility coefficients of diets with varying energy to protein ratio for Japanese flounder, *Paralichthys olivaceus*

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Abstract
A laboratory trial was conducted in a sea water recirculatory system to study the nutrient digestibility coefficients of diets with varying energy to protein ratios in Japanese flounder *Paralicthys olivaceus*. Six different experimental diets with two protein levels (45 and 55%) having six different energy to protein ratio of 87, 90, 94, 107, 110 and 114 were formulated using white fish meal and casein as protein sources. The results of the study showed that the apparent protein digestibility (APD) value ranged between 90.59 to 91.61% and there was no significant differences (P> 0.05) between the APD values of diets 1, 2, 3, 4 and 6. The apparent lipid digestibility (ALD) values of diets ranged between 88.24 to 90.18%. The apparent energy digestibility (AED) values ranged between 80.55 to 87.52% with diet 3 producing significantly the highest AED value. In general, except in diet 1 the ALD and AED values increased with the increase of dietary lipid at both protein levels. The results of the present investigation indicated that Japanese flounder can efficiently digest the dietary nutrients at varying energy to protein ratios.

Key words: Nutrient digestibility, Energy-protein ratio, Japanese flounder

Introduction

One of the most important aspects in the evaluation of the effectiveness of a feed is the determination of its digestibility. This measures the ability of the fish to digest and absorb the nutrients it is fed. Besides, the measurement of the digestible crude protein, the determination of digestibility of the organic matter or lipid and energy content of feed is also important.

Measurement with chromic oxide as an exogenous indicator is the most frequently used method of digestibility determination (NRC 1983). But faecal collection methods pose serious technical problems in obtaining correct value. For example, faeces have been collected by several methods including dissection (Nose 1967), faecal stripping (Inaba *et al.* 1962) and suction (Windell *et al.* 1978a), collection in special chamber (Cho and Slinger 1979), settling of faeces (Law 1984) and continuous filtration of effluent water (Choubert *et al.* 1979). In the present study, a specially designed floating net cage

with tank underneath was used to collect faeces by sedimentation.

Japanese flounder (*Paralichthys olivaceus*) is one of the most important aquaculture species in Japan and production of cultured flounder ranked fourth among marine cultured fish in Japan (Kikuchi *et al.* 1997). The fish is highly carnivorous and needs higher dietary protein for growth. The growth of Japanese flounder with different protein sources has been studied (Kikuchi *et al.* 1997). The present study was undertaken to determine the nutrient digestibility coefficients of various diets with different energy to protein ratio for Japanese flounder (*P. olivaceus*).

Materials and methods

Experimental system

The experiment was conducted in Abiko Research Laboratory, Chiba, Japan in a specially designed faeces collection system. The floating net cages made of HDPE (high density polyethylene) were set in a 2000*l* volume sea water recirculating system fibre glass tank. The water depth in the tank was maintained at 0.6 m. There were 6 net cages (30×45×30 cm) set in the tank. Each of the floating net cages were fitted with a conical bottom rectangular perspex glass tank which served as faecal settling tank.

Experimental procedure

About one year old juvenile Japanese flounder (*P. olivaceus*) were obtained from the Abiko Research Laboratory. These fish were originally collected from a commercial hatchery (Anagawa Shokusan) in Mie Prefecture (average wt. 1.0g) and transported to Abiko Research Laboratory in Chiba Prefecture. The fish were reared in a 2000*l* volumes sea water tank with a commercial diet used for Japanese flounder (Higashimaru Foods Inc) at 20^{0}C until the start of the experiment.

Since there were 6 faeces collection tanks, the faeces collection experiment was run twice to have replicate values. Eight fish with mean body weight of 105-107g were stocked in each floating net cages. Faeces were collected twice - morning and evening for two weeks in each run. Natural light-dark was maintained and the water temperature was kept at 20±1^{0}C. Adequate oxygen supply was ensured by artificial aeration. The pH of water was maintained between 7.0 and 8.0 by occasional addition of $NaHCO_3$. The salinity was maintained at 35ppt. In order to adjust the salinity of rearing water, freshwater was added corresponding to evaporated volume.

Experimental diets

Six experimental diets were formulated using white fish meal (Nippon formula Feed Ltd.) and casein (New Zealand Milk Products) as the main protein sources (Table 1). The fish meal contained 68.8% protein, 13.9% lipid and 17.3% ash on dry matter basis. The casein contained 92.0% protein. The diets were formulated at two protein levels (55 and 45%) and each with three energy levels to have six different energy to protein ratio of 87, 90, 94, 107, 110 and 114. Pollack liver oil was used as lipid source to maintain the desired energy levels in different diets. Pollack liver oil was obtained from Riken

Vitamin Co. Ltd. Vitamin and minerals premixes were obtained from Nippon Formula Feed Mfg. Co. Ltd. The energy to protein ratio was calculated as gross energy per kg/crude protein (%) in diet. Chromic oxide was used at 0.5% level as an external marker for nutrient digestibility study. All the feedstuffs except the pollack liver oil were processed in a mixer after grinding and were formed into spheres of 4mm diameter using a twin extruder with addition of tap water and pollack oil. The prepared diets were dried in an air dryer at 25°C. These diets were stored at -35°C until use.

Table 1. Formulation of experimental diets with different ratios of energy to protein for Japanese flounder

Ingredient (%)	Diet No.					
	1	2	3	4	5	6
White fish meal	52.1	52.1	52.1	42.5	42.5	42.5
Casein	22.3	22.3	22.3	18.2	18.2	18.2
Potato starch	8.5	8.5	8.5	8.5	8.5	8.5
Pollack liver oil*	3.0	7.0	11.0	7.0	12.0	17.0
Cellulose	8.1	4.1	0.1	17.8	12.8	7.8
Mineral mixture**	2.8	2.8	2.8	2.8	2.8	2.8
Vitamin mixture**	2.7	2.7	2.7	2.7	2.7	2.7
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100

**Riken Vitamin Co., Ltd. (Feed oil Ω)*
***Nippon formula Feed Mfg. Co., Ltd.*

Feeding and faeces collection

Fish were acclimated to the experimental system for 7 days before the start of the experiment. Fish were fed to satiation twice a day on each of the experimental diets. Faeces collection started 4 days after feeding to allow evacuation of all previously ingested materials. After feeding the fish, specially designed faeces collection tanks were attached beneath each of the net cages with hooks so that the faeces voided in net cages could be settled at the conical bottom of the attached tank. Before collecting faeces, the attached tanks were unhooked carefully and the settled faeces were pipetted out in centrifuge tubes. Then the faeces were collected by centrifuging in a refrigerated centrifuge at 5,000 rpm for 15 minutes. The collected faeces were stored at -35°C until sufficient amount was collected. At the end of the experiment, the collected faeces were dried in a vacuum freeze dryer before biochemical analysis was done.

Analytical methods

The proximate composition of the feed ingredients, feed and faeces samples were analysed according to AOAC (1980) with NFE (nitrogen free extract) being determined by difference. The gross energy content in feed and faeces samples were analysed by a bomb calorimeter (Shimadzu, autocalculating bomb calorimeter, Model CA-4P). Chromic oxide was determined by using the wet-digestion method of Furukawa and Tsukuhara (1966). Differences in digestibility coefficients were tested for significance

($P<0.05$) by Duncan's Multiple Range Test (Duncan 1955).

Results

The results of the proximate composition analysis of the experimental diets are shown in Table 2. Crude protein, lipid and energy content ranged between 45.4 to 56.5%, 9.8 to 20.3% and 485 to 532 Kcal/100g respectively. Protein, lipid, energy and chromic oxide content in faeces of fish fed experimental diets are shown in Table 3. The protein content was highest (19.12%) in faeces of fish fed diet 3 whilst fish fed diet 4 produced the lowest faecal protein (11.82%). Similarly, lipid content in faeces of fish fed diet 6 was highest and that of diet 1 was the lowest. The energy content in faeces of fishes fed different diets ranged between 263 to 305 kcal/100g

Table 2. Analysed proximate composition of the experimental diets (% dry matter basis)

Ingredients (%)	Diet No.					
	1	2	3	4	5	6
Dry matter	97.0	97.3	97.0	96.8	97.0	96.9
Crude protein	56.5	56.3	56.3	45.4	46.0	46.3
Crude lipid	9.8	14.9	17.2	12.7	16.0	20.3
Ash	11.2	11.5	11.6	9.5	9.8	9.7
Crude fibre	8.5	6.0	3.0	17.5	13.1	8.2
NFE*	14.0	11.3	11.9	14.9	15.1	15.5
Chromic oxide	0.46	0.47	0.49	0.46	0.46	0.45
Gross energy (Kcal/100gdiet)	492	510	532	485	508	530
GE/CP**	87	90	96	107	113	114

**Nitrogen free extract calculated as 100-% (moisture+ protein+ lipid+ ash+ crude fibre)*

*** Gross energy in per kg diet/crude protein (%)*

Table 3. Nutrient and chromic oxide contents in fish faeces fed diets with different energy to protein ratio

Components	Diet No.					
	1	2	3	4	5	6
Protein (%)	16.41	17.00	19.12	11.82	13.06	15.19
Lipid (%)	3.38	5.33	6.76	4.19	5.36	7.81
Energy (Kcal/100g)	263	279	266	265	293	305
Chromic oxide (%)	1.59	1.63	1.96	1.29	1.39	1.60

The apparent nutrient digestibility of protein, lipid and energy of the experimental diets are shown in Table 4. The results of the present study showed that all the nutrients under investigation were well digested by the Japanese flounder. The apparent protein digestibility (APD) values ranged between 90.59 to 91.61% and there was no significant difference ($P>0.05$) between the APD values of diets 1,2,3,4 and 6. However, diet 5 showed significantly the lowest APD value (90.59%). The apparent lipid digestibility

(ALD) values of different experimental diets ranged between 88.24 to 90.18%. There was no significant difference (P>0.05) between the ALD values of diets 1, 2, 3 & 4; 2, 5, & 6 and 4 & 5 respectively. The apparent energy digestibility (AED) values ranged between 80.55 to 87.52% with diet 3 producing significantly the highest AED value. There was no significant difference between the AED values of diets 1, 2, & 6 and 4 & 5 respectively.

Table 4. Apparent nutrient digestibility coefficients of diets with different energy to protein ratio in Japanese flounder

Components	Diet No.					
	1	2	3	4	5	6
Protein	91.60[a*]	91.51[a]	91.61[a]	90.72[ab]	90.59[b]	90.74[ab]
	(±0.28)**	(±0.13)	(±0.05)	(±0.16)	(±0.25)	(±0.72)
Lipid	90.04[a]	89.70[ab]	90.18[a]	88.24[c]	88.90[bc]	89.67[ab]
	(±0.18)	(±0.11)	(±0.06)	(±0.23)	(±0.27)	(±0.85)
Energy	84.54[b]	84.25[b]	87.52[a]	80.55[c]	80.91[c]	83.73[b]
	(±0.53)	(±0.71)	(±0.08)	(±0.04)	(±1.20)	(±1.17)

**Figures in the same row having the same superscripts are not significantly different (P>0.05)*

*** Values in the parenthesis are standard deviation of treatment means.*

Discussion

The result of the present study indicated that the protein, lipid and energy of the experimental diets with different energy to protein ratio were well digested by Japanese flounder. The APD value of all the diets are above 90%. This better digestibility might be related with the higher level of white fish meal used as dietary protein source. According to NRC (1983) fish can digest up to 95% of the protein in fish meal. However, this value can decrease to 80 to 85% depending on the origin and processing of the fish meal used (Ogino and Chen 1973). The fish meal used in the present study was prepared by low temperature processing. Sato (1999) reported slightly higher APD values of 95.1 to 96.6% in Japanese flounder (*P. olivaceus*) fed diets with 55 to 66% protein having different energy to protein ratios. The APD values obtained in the present study are similar to the APD value of white fish meal protein (90.7%) reported for rainbow trout by Smith *et al.* (1995). Kabir *et al.* (1998) also reported a similar APD value of 89.40% in rainbow trout fed a diet containing 50% protein.

In general, higher APD values were obtained in diets with higher protein contents. These results are in agreement with similar studies measuring APD in other species (Austreng and Refstie 1979, De Silva and Perera 1984, Hajen *et al.* 1993). The usual explanation offered has been that the proportion of metabolic faecal nitrogen decreases with the rise in protein content in the diet (Nose 1967, Ogino and Chen 1973, Jauncey 1982). It is also possible that an adaptation of digestive enzymes to the level of protein in the diet contributes to increasing the level of nitrogen (i.e. protein) digestibility when the level of protein in the diet increases (Kawai and Ikeda 1973).

The ALD values obtained in the present study are slightly lower than that of Sato

(1999) who observed ALD values of 90.1 to 95.7% with smaller sized (79g) Japanese flounder fed diets containing 55 to 60% protein. Ohta and Watanabe (1998) also reported crude lipid digestibility of 87.9 to 97.1% in rainbow trout fed 49% protein containing extruded pellet diet. However, Hossain *et al.* (1992) reported 89.96% and 93.24% digestibility of lipid in fish meal and soybean meal diet respectively for Tilapia (*Oreochromis mossambicus*) which are within the range of the values obtained in the present study.

The AED values of different experimental diets in the present study ranged between 80.55 to 87.52% and these values are higher than some reported values (74%, Windell *et al.* 1978b for rainbow trout) but lower than reported by others (91%, Cho *et al* 1982, 91.5%, Smith *et al.* 1980, 89.3%, Smith *et al.* 1995) for the same species. Sato (1999) also reported slightly higher AED values of 85 to 95.4% in Japanese flounder fed diets containing 55 to 66% protein. However, the values obtained in the present study are more or less similar to the value reported for grass carp (83%, Law 1986). The slightly lower AED values obtained in the present study with Japanese flounder compared to that of Sato (1999) might be related to the smaller size of the fish and higher level of dietary protein used.

The lower AED values obtained with different diets compared to diet 3 in the present study might be related to the higher level of cellulose in those diets. Hilton *et al* (1983) suggested that a higher dietary crude fibre content may accelerate the rate of passage of digesta through the intestinal tract thus reducing the digestibility of energy and protein. Smith and Lovell (1973) also reported that fibre is negatively correlated with digestibility of nutrients. The best nutrient digestibility obtained with diet 3 containing 55% protein and a energy to protein ratio of 96 supports the findings of Utsumi (1999) who observed better growth and feed utilization in Japanese flounder fed diet with similar protein and energy levels. The results of this study demonstrated that Japanese flounder can efficiently digest the dietary nutrients with varying energy to protein ratios.

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