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Dietary Protein and Energy Requirements of Juvenile Japanese Flounder, *Paralichthys olivaceus*

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Abstract: Eight test diets of four protein (41, 44, 47 and 50%) and two energy levels (20 kJ g^{-1} and 19 kJ g^{-1}) were formulated to investigate the proper dietary protein and energy levels for the growth of juvenile Japanese flounder, *Paralichthys olivaceus*. Squid liver oil concentration was used to adjust energy levels and brown fishmeal was used as the protein source. Weighing about 5.9 g, each duplicate group of flounder was fed test diets twice a day to apparent satiation, for 45 days. Performance of fish fed the different diets was evaluated for survival, percent weight gain, relative growth rate, feed efficiency and protein efficiency rate. Survival was over 85% for all treatments. Growth and feed efficiency of flounder increased as dietary protein increased in both energy levels of 19 kJ g^{-1} and 20 kJ g^{-1} diet, but no evidence of reaching a plateau for growth data was found. Lowest rates of cumulative ammonia nitrogen excretion as proportion of ingested nitrogen were recorded in fish fed the 50% protein-20 kJ g^{-1} and 50% protein-19 kJ g^{-1} diet (2.78 and 2.60%, respectively). The digestibility rates of the experimental diets with the high energy level (20 kJ g^{-1}) were higher than those with the lower levels (19 kJ g^{-1}). Digestion efficiencies in all experimental groups ranged from 89 to 92% for protein, from 60 to 85% for lipid and from 73 to 89% for energy, while those for the total digestibility ranged from 51 to 72%. The results indicate that Japanese flounder juveniles with 6 g mean weight need at least 50% dietary protein for best growth when brown fishmeal is the sole protein source. Furthermore, it can be concluded that Japanese flounder juveniles can utilize dietary energy up to 20 kJ g^{-1} efficiently, under the conditions applied in this study.

Key words: Protein, energy, nutrition, flounder, *Paralichthys olivaceus*

INTRODUCTION

Commercial production of cultured Japanese flounder *Paralichthys olivaceus* is one of the rapidly increased industries in Japan due to the high market value of this species[1]. Although some nutritional studies have been carried out on this species[2-6], knowledge about nutritional requirements is still incomplete. In order to formulate cost-effective diets for Japanese flounder, the dietary requirements for protein and energy need to be defined.

Reported protein requirements for Japanese flounder are high in the studies of Kikuchi *et al.*[2-3], while lower protein and energy requirements were reported by Lee *et al.*[4-6]. Different findings for the protein requirement of Japanese flounder reported in the previous studies show that those findings are still not conclusive. Therefore it is necessary to re-evaluate the protein and energy requirements of Japanese flounder.

Since protein sources are among the most expensive feed ingredients, it is economically desirable to keep protein concentration low, but high enough to sustain good growth. The question is, to which extent protein can be replaced with other macronutrients, such as lipids.

The objective of study was to evaluate the effects of different dietary protein and energy levels on growth performance, feed digestibility and nitrogenous excretion rates of Japanese flounder and determine the protein and energy requirements of this species under laboratory conditions.

MATERIALS AND METHODS

Experimental fish and diets: Japanese flounder juveniles were obtained from a commercial fish farm, transported to Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan and maintained

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Table 1: Feed ingredients (g/100 g) and nutritional composition (%) of test diets

	Diet No.							
Ingredients (g/100 g)	1	2	3	4	5	6	7	8
Brown fishmeal[a]	67	62	57	52	67	62	57	52
Squid liver oil[b]	10	10	10	10	5	5	5	5
Dextrin[c]	3	3	3	3	3	3	3	3
α-Starch[d]	3	3	3	3	3	3	3	3
Vitamin mix[e]	3	3	3	3	3	3	3	3
Mineral mix[f]	3	3	3	3	3	3	3	3
Activated gluten[g]	5	5	5	5	5	5	5	5
Attractants[h]	1	1	1	1	1	1	1	1
α-Cellulose[i]	5	10	15	20	10	15	20	25
Total	100	100	100	100	100	100	100	100
Nutritional composition (% dry matter, except for moisture)								
Moisture	7.05	7.72	7.97	6.96	8.10	6.48	6.85	5.77
Crude protein	49.70	47.00	43.50	40.90	50.50	47.30	44.30	41.40
Digestible protein[o]	45.70	43.30	40.10	37.80	46.10	42.60	39.40	37.00
Crude lipid	16.10	15.60	15.50	15.10	11.10	10.80	10.70	9.40
Digestible lipid[o]	13.40	13.40	13.30	12.90	8.40	7.80	7.30	5.70
Crude ash	12.60	11.80	10.90	10.20	12.50	11.80	10.70	10.60
NFE[j]	14.60	17.80	22.20	26.80	17.90	23.60	27.40	32.90
GE (kJ g^{-1} diet)[k]	20.50	20.30	20.10	20.20	19.30	19.40	19.30	19.10
DE (kJ g^{-1} diet)[l]	14.80	14.00	12.70	12.70	13.00	11.20	10.70	9.70
P:E (mg/kJ)[m]	24.20	23.20	21.60	20.30	26.10	24.30	22.90	21.70
DP/DE ratio[n]	30.90	30.90	31.60	29.80	35.50	38.00	36.80	38.10

[a]Mackerel meal; [b]Riken Vitamin Co., Ltd., Japan; [c]Dextrin Hydrate, Kanto Chemicals Co., Ltd., Japan; [d]alfa Starch-Okanol; [e]Vitamin mix., 2.8 (Kadai Vitamin); Vit. C, 0.2 (Phospitan C, Showa Denko, Japan); [f]Kadai Mineral, Japan; [g]A-guru SS, Glico, Japan; [h]DL-α-Alanin, 0.3; Betaine Monohydrate (99%), 0.3; Taurine, 0.3; L(-)-Proline (min. 99% Assay), 0.1 (Nacalai Tesque, Inc., Kyoto, Japan); [i]Sigma α-Cellulose, Sigma-Aldrich Chemie Gmbh, Germany; [j]Nitrogen-free extracts, calculated by difference; [k]Gross energy determined according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} NFE; [l]Digestible or available energy calculated from (GE x Total digestibility,%); [m]P:E = crude protein/gross energy; [n]DP/DE ratio (mg digestible protein/kJ digestible energy); [o]Values calculated from Table 5

on a commercial formulated diet (Higashimaru Foods, Kagoshima, Japan) until the start of the experiment.

Eight test diets (Table 1) were formulated to contain four dietary protein levels of 41, 44, 47 and 50% and two energy levels of 19 kJ g^{-1} and 20 kJ g^{-1}, respectively. Squid liver oil concentrations were adjusted to change dietary energy levels. Brown fishmeal was used as the single protein source and α-cellulose was added to make up the total ingredients to 100%.

Dry ingredients were sieved to remove particles larger than 250 μm in diameter and were then mixed in a blender for 10 min. Then the lipids and lipid soluble vitamins were added and finally the ingredients were mixed with water (35% of the dry weight) for 20 min. Pellets of ca. 3 mm diameter were then prepared using a meat grinder. Diets were dried in a dry air mechanical connection oven at 70°C for 1 h and were then stored in a freezer at –25°C until being used. The crude protein and lipid contents of the test diets were determined by the Kjeldahl method and the Bligh and Dyer[7] method, respectively. Ash and moisture contents were analysed by Association of Official Analytical Chemists[8] method and dietary energy content was estimated according to nutritional fuels of 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17 kJ g^{-1} for nitrogen free extracts. The analyses of feed samples were carried out on air-dried material, while of the feces and fish whole body samples were done on freeze-dried material. All samples were homogenized in a grinder before analysis.

Growth trial: Japanese flounder juveniles were placed in a 500 L round holding tank and fed commercial feed (Higashimaru Co., Japan) under ambient water temperature (16-17°C) and salinity (32 ppt) conditions for 2 weeks until used in the experiment. Totally 128 juveniles (5.9±0.64 g) were used. The fish were individually weighed at the beginning and the end of the experiment. The fish were weighed in mass every 15 days during the trial. There were eight fish per tank with two replicate 100 L tanks per treatment. Water volume was set at 60 L and seawater was supplied at 420 mL min^{-1}. Temperature was seawater ambient and ranged between 18.0 and 22.1°C and fish were exposed to natural photoperiod during the experiment. The fish were fed to satiation twice a day for 45 days and feed intake was registered daily.

At the beginning of the trial, 15 fish were sampled for initial proximate analysis of whole body fish. At the end of the experiment, six fish were sampled from each treatment (three fish from each replicate) for subsequent analysis. All analyses were performed in duplicate.

Determination of digestibility: After termination of the growth experiment, feces were collected for digestibility determinations. Chromic oxide (Cr_2O_3) (0.5%) was mixed with each test diet to allow determination of apparent digestibility of lipid, protein and energy. Fish were allowed to feed on the test diets for 1 h in the same experimental tanks used for the growth experiment. Then the uneaten feeds were collected and the water of the tank replaced with new water in order to avoid collection of feed or other material with the feces. All tanks were observed continuously for 9 h and feces was immediately collected by siphoning, when a small particle of feces had been noticed. Apparent digestibility of lipid, protein, energy and the total digestibility were calculated as follows:

$$\text{Digestibility (\%)} = 100-\{100 \times \left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}}\right) \times \left(\frac{\% \text{ nutrient in feces}}{\% \text{ nutrient in feed}}\right)\}$$

$$\text{Total digestibility (\%)} = 100 - \{100 \times \left(\frac{\%\ Cr_2O_3 \text{ in feed}}{\%\ Cr_2O_3 \text{ in feces}}\right)\}$$

Feces were freeze dried after collection; protein, lipid, energy and chromium oxide contents were analyzed by the Kjeldahl, Bligh and Dyer[7], bomb calorimeter and Furukawa and Tsukahara[9] method, respectively.

Determination of ammonia-N excretion: After termination of the growth experiment and the digestibility experiment, 6 fish from each treatment were selected randomly for ammonia excretion rates. The composition of diets given to animals was the same as those used in the growth trial. Fish were allowed to feed on the test diets for 1 h and transferred immediately to 5 L chambers, each with a 5 mm mesh base (one fish per chamber). Filtered seawater was used and the water volume was held as 3 L/chamber. Ammonia excretion rates were determined based on the difference in ammonia concentration of filtered seawater and the concentration in chambers with fish. Ammonia concentrations were determined every 1 h over a 5 h period by the phenol-hypochlorite method of Strickland and Parsons[10] and ammonia-N excretion rates were expressed as mg-N/100 g fish/h.

Statistical analysis: Statistical significance of differences among parameters was computed using analysis of variance (ANOVA, SPSS 10.0 for Windows). Duncan's New Multiple Range Test (SPSS 10.0 for Windows, General Linear Model – Univariate procedure, Post Hoc Tests) was applied to determine significant differences between individual treatments when significance ($P<0.05$) of factors was detected by ANOVA.

RESULTS

Fish survival was high (over 85%) and did not differ between treatments, indicating that differences in dietary protein and energy content did not affect survival (Table 2). There was a significant influence of protein and energy levels on relative growth rate (RGR) (Table 2). RGR of fish fed the 50% protein-20 kJ g^{-1} diet was significantly ($P<0.05$) higher than those of other groups fed with lower protein levels, but same energy concentration. In the lower energy level of 19 kJ g^{-1} diet, however, RGR of fish fed the 50% protein diet was significantly higher than those of the 44 or 41% protein diets. RGR of fish fed the 50% protein-19 kJ g^{-1} diet was also higher than that of the 47% protein-19 kJ g^{-1} diet, but no significance was found between these two groups. In the experimental groups fed diets containing 47% protein, a decline, but not significant, was recorded in RGR as the energy level increased. However, this was not observed in other protein levels.

Feed efficiency of fish was influenced only by dietary protein level. Dietary energy levels had no significant effect on feed efficiency. The best feed efficiency was obtained in the 50% protein diet with both energy levels, as 0.88 and 0.82 for 20 kJ g^{-1} diet and 19 kJ g^{-1}diet, respectively.

The highest feed intake was recorded in the 50% protein diets (both high and low energy levels), while the lowest feed intake was registered in the 44 and 41% protein diets with low energy level.

Effects of dietary levels of protein and energy on body composition of Japanese flounder juveniles are given in Table 3. Generally, fish fed different experimental diets did not differ in body composition, however, levels of body lipid in fish increased as dietary energy level increased from 19 to 20 kJ g^{-1} diet in all dietary protein levels.

Mean ammonia-N excretion rates and the ratio of ammonia-N excretion to nitrogen intake of flounder are shown in Table 4. Cumulative ammonia-N excretion in all protein levels at the end of the 5 h period, rose as dietary energy decreased and vice versa. There was a significant interaction of protein and energy levels on ammonia excretion rates of flounder.

The best total digestibility (72.2%) was obtained in flounder fed the diet containing 50% protein-20 kJ g^{-1} diet (Table 5). In all groups, flounder digested protein with high efficiency (89 to 92%), with values slightly increasing as the dietary energy level increased and the α-cellulose content decreased. There was no significant interaction of

Table 2: Relative growth rate (RGR %), daily feed intake (DFI), feed conversion efficiency (FCE) and protein efficiency rate (PER) for Japanese flounder juveniles fed test diets for 45 days

	BW (g)						
Diet	Initial	Final	RGR* (%)	DFI (g)	FCE	PER	Survival (%)
50-20	5.9±0.14^{a}	29.9±0.88^{a}	410.8±3.15^{a}	0.61±0.01	0.88±0.04^{a}	1.90±0.13	100±0.00^{a}
47-20	5.8±0.06^{a}	25.5±1.60^{bce}	337.2±32.21^{bcd}	0.58±0.00	0.75±0.07^{abc}	1.74±0.17	100±0.00^{a}
44-20	5.9±0.00^{a}	24.8±0.45^{be}	320.3±7.16^{bd}	0.59±0.01	0.71±0.01^{abcd}	1.78±0.02	93.75±8.84^{a}
41-20	5.8±0.50^{a}	23.6±0.80^{ef}	306.6±10.26^{d}	0.58±0.01	0.69±0.04^{bcd}	1.80±0.07	93.75±8.84^{a}
50-19	5.9±0.00^{a}	28.3±0.77^{ac}	379.1±13.21^{ac}	0.61±0.04	0.82±0.08^{b}	1.76±0.15	93.75±8.84^{a}
47-19	6.0±0.10^{a}	27.4±3.44^{ab}	360.1±50.06^{abc}	0.60±0.00	0.80±0.13^{abc}	1.80±0.27	93.75±8.84^{a}
44-19	5.9±0.03^{a}	21.2±0.01^{df}	257.8±5.23^{e}	0.45±0.01	0.75±0.02^{abcd}	1.82±0.07	87.50±0.00^{a}
41-19	5.9±0.09^{a}	19.1±0.95^{d}	223.3±14.55^{e}	0.44±0.01	0.66±0.06^{cd}	1.69±0.16	93.75±8.84^{a}

Values (Mean±S.D.) with different superscripts in the same row differ significantly at 5 % level

* RGR(%) = 100 [(final body weight–initial body weight)/initial body weight]; FCE= g weight gain/g feed intake;

PER = (wet weight gain (g) / protein intake (g))

Table 3: Initial and final proximate composition (whole body-% dry basis except for moisture) and gross energy of Japanese flounder juveniles fed the experimental diets. Initial body composition is given in the first row

Diet	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Crude Ash (%)	GE^{a} (kJ g^{-1})
Initial	71.1±0.14	70.3±0.73	6.6±0.56	15.2±0.04	19.58±0.05
50-20	69.7±0.51	65.8±0.04^{ab}	9.3±0.14^{a}	15.6±0.23^{a}	19.68±0.04
47-20	65.6±0.33	64.9±3.80^{a}	10.7±0.11^{b}	14.5±0.57^{b}	20.39±0.23
44-20	71.4±0.18	66.0±2.33^{ab}	9.0±1.19^{a}	15.6±0.08^{ab}	19.58±0.85
41-20	71.0±0.39	68.5±2.50^{ab}	8.8±0.08^{a}	15.4±0.29^{ab}	19.34±0.16
50-19	72.8±0.14	70.3±0.41^{b}	7.5±0.24^{c}	16.1±0.79^{ac}	19.83±0.12
47-19	76.2±0.06	67.3±0.74^{ab}	6.2±0.02^{d}	16.1±0.46^{ac}	18.76±0.12
44-19	72.8±0.13	69.5±1.39^{ab}	6.5±0.43^{cd}	16.8±0.58^{c}	19.21±0.25
41-19	67.7±0.24	66.6±0.16^{ab}	6.2±0.12^{d}	15.3±0.20^{ab}	18.82±0.07

Means with different superscripts in the same row differ significantly at 5% level

^{a}Gross energy (GE) determined according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} NFE

Table 4: Nitrogen intake, ammonia-N excretion ratio and ratio of ammonia-N excretion to nitrogen intake (Mean±S.D.) in tanks where flounder were fed the experimental diets for the excretion trial

Diet	BW (g)	Nitrogen intake (mg-N/100 g fish)	Ammonia-N excretion (mg-N/100 g fish)	NE/NI (%)
50-20	28.49±7.4	179.9±51.5	4.97±1.54^{a}	2.78±0.54
47-20	25.26±3.4	180.1±26.1	5.88±0.99^{ab}	3.27±0.24
44-20	26.07±4.7	167.3±31.0	4.70±0.99^{a}	2.80±0.17
41-20	24.43±4.2	169.0±37.9	7.22±2.05^{bc}	4.24±0.35
50-19	27.53±9.6	205.7±97.9	5.41±2.82^{ab}	2.60±0.27
47-19	28.90±9.1	172.3±46.9	7.24±2.70^{bc}	4.13±0.89
44-19	18.99±5.6	162.7±35.6	4.96±0.90^{c}	3.07±0.20
41-19	22.13±5.2	202.6±64.6	8.44±2.40^{a}	4.25±0.86

Values within columns sharing the same letter did not differ significantly ($P<0.05$)

NE/NI = (ammonia-N excretion for 5 h/nitrogen intake) x 100

Table 5: Apparent digestibility coefficients of lipid, protein and energy (Mean±S.D.) for juvenile Japanese flounder fed test diets

	Digestibility (%)			
Diet	Lipid	Protein	Energy	Total
50-20	83.0±3.9^{a}	92.0±1.6^{a}	89.7±2.2^{a}	72.2±6.4^{a}
47-20	85.8±6.4^{a}	92.1±3.4^{a}	86.2±7.4^{ab}	69.2±13.8^{a}
44-20	85.8±0.5^{a}	92.3±0.8^{a}	83.4±0.6^{ab}	62.9±1.3^{ab}
41-20	85.5±0.8^{a}	92.4±0.4^{a}	81.3±0.3^{abc}	62.7±2.0^{ab}
50-19	72.6±7.8^{ab}	91.3±0.8^{a}	84.4±4.9^{ab}	67.2±9.3^{ab}
47-19	72.1±8.3^{ab}	90.0±1.4^{a}	80.4±2.0^{abc}	57.7±1.4^{ab}
44-19	67.9±3.7^{ab}	89.0±3.2^{a}	79.8±2.7^{bc}	55.3±5.1^{ab}
41-19	60.2±18.6^{b}	89.4±5.2^{a}	73.6±6.4^{c}	51.0±8.6^{b}

Values with different superscripts are significantly different at 5% level

protein and energy levels on lipid digestibility. Lipid digestibility was significantly affected only by the dietary energy levels with values increasing as the dietary energy level increased and the dietary α-cellulose content decreased.

DISCUSSION

In the present study, growth performance and feed conversion efficiencies of juvenile Japanese flounder tended to improve with increasing dietary protein level. When fish fed diets containing protein levels above the requirements, plateaus or decreases in weight gain are reported in some species[11,12]. In the present study, weight gain of Japanese flounder rose when the dietary protein level increased from 41 to 50% and no plateau was observed, indicating that the protein requirement for maximum growth may be over 50%. Kikuchi *et al.*[2] reported dietary protein requirement for Japanese flounder, *Paralichthys olivaceus*, as a value between 47 and 60%, while Lee *et al.*[4] reported that the proper dietary protein level for the growth of Japanese flounder is 50%.

Cowey *et al.*[13], Caceres-Martinez *et al.*[14] and Helland and Grisdale-Helland[15] reported dietary protein requirement for plaice *Pleuronectes platessa*, turbot *Scophthalmus maximus* and halibut *Hippoglossus hippoglossus* as 50, 69.8 and 51%, respectively. In the present study, the best growth was found in the 50% dietary protein and 15.7% dietary lipid (GE of 20 kJ g^{-1}, DE of 14.8 kJ g^{-1}). Although, Kikuchi *et al.*[3] showed that there were no significant differences in the final body weight and percent weight gain of Japanese flounder among dietary protein levels of 45 and 55%, best growth was reported in fish fed 55% protein and 17.2% lipid (DE of 19.5 kJ g^{-1}). In contrast, the results in the present study show that body weight and percent weight gain improved significantly as dietary protein increased from 44 to 50% in both high and low energy levels.

Japanese flounder showed a better performance (growth and feed efficiency) with high energy level than with low energy level in all dietary protein levels except for the 47% dietary protein. This result, called "protein sparing effect" was also reported by some other workers[16-20], while Caceres-Martinez *et al.*[14] reported negative effects of dietary lipid on growth and feed efficiency for turbot and Lee *et al.*[4,5] reported negative effects of dietary lipid on growth and feed efficiency for Japanese flounder.

Lee *et al.*[4] reported that the best growth of Japanese flounder was obtained from fish fed the 50% protein diet with low energy of 300 kcal g^{-1}, whereas in the present sudy the best weight gain was obtained with 50% dietary protein but high energy level of 480 kcal/100 g (20 kJ g^{-1}). Lee *et al.*[5] also suggested that juvenile Japanese flounder grew better on the low energy diet (303 kcal/100 g) than on the high energy diet (411 kcal/100 g) at 48% protein level when the fish was fed to satiety twice daily. However, in the present study, fish was fed to satiety twice daily, but juvenile Japanese flounder grew better on the high energy diet (20 kJ g^{-1} = 480 kcal/100 g) than on the low energy diet (19 kJg^{-1} = 450 kcal/100 g). The low energy level (19 kJ g^{-1}) tested in the present study was even higher than the energy level what Lee *et al.*[4,5] called as high energy level (411 kcal/100 g). On the other hand, Lee *et al.*[6] reported that a diet containing 45% protein with 7% lipid, dextrin (31% NFE) and 4.7 kcal of gross energy per gram of diet might be recommended for optimum growth and efficient protein utilization by young flounder growing between 23 and 110 g bodyweight. The differences between the present and previous studies may be due to different sizes of fish or other factors like differences of laboratory, handling, genetic variation etc. Furthermore Lee *et al.*[4] used white fish meal as a single protein source, whereas brown fish meal was used in the present study. Yigit *et al.*[21] suggested a better utilization of white fish meal than brown fish meal in turbot diets. This also might have an effect on the differences in results of the present and the previous studies.

Increasing dietary protein level up to 50% at 20 kJ g^{-1} diet, improved the performance of flounder. Therefore, the proper P/E ratio for flounder was found as 24 mg protein/kJ in the present study. This is similar to the value (28 mg protein/kJ) previously reported by Kikuchi *et al.*[3] for Japanese flounder. The P/E ratio reported in the present study is lower than those reported by Bromley[22] (31-40 mg protein/kJ) and Yigit *et al.*[21] (35 mg protein/kJ) for the best growth in turbot, but higher than those reported in arctic charr *Salvelinus alpinus* (19 mg protein/kJ), cod *Gadus morhua* (17 mg protein/kJ) and rainbow trout *Oncorhynchus mykiss* (22 mg protein/kJ) by Jobling and Wandsvik[23], Jobling *et al.*[24] and Yigit *et al.*[20], respectively. It may be that the higher P/E ratios optimize growth in flatfish because of their limited ability to utilize dietary fat[21] and flounder may expend less energy on voluntary activity as compared to pelagic fish species in the water column.

Bromley[22] and Kaushik *et al.* [25]found that high dietary energy level increased body lipid as well as growth in turbot *Scophthalmus maximus* and rainbow trout *Salmo gairdneri*, respectively. In contrast, Peres and Oliva-Teles[26] reported that high dietary energy level increased body lipid without improvement of growth performance in juvenile European sea bass *Dicentrarchus labrax*. In the present study, levels of body lipid as well as growth in fish increased as dietary energy level increased from 19 kJ g^{-1} to 20 kJ g^{-1} diet, indicating that flounder can utilize dietary lipid levels up to 16% as energy source for good growth.

Oku and Ogata[27] reported that replacing dietary protein with the lipid has no serious effects on weight gain and protein efficiency rates (PER) of Japanese flounder. On the other hand, Sato[28] showed that the growth of juvenile fish mostly depends on the dietary protein level regardless of the lipid content and PER is not affected by the dietary protein and lipid levels. Different from these studies, PER of juvenile Japanese flounder was improved significantly by increasing dietary lipid in the 50% crude protein diet in this study. This finding is similar to that of Kikuchi *et al.*[3] where feed efficiencies and PER improved with increasing dietary lipid in the groups with 55% crude protein, however the lipid inclusion did not apparently improve these parameters in the 45% protein diets. These findings are quite similar to those in the present study, where PER of fish improved by increasing dietary lipid in the 50% crude protein diet, but not in the 44% crude protein diet. Differences in PER between studies may be considered to be originated from fish size and duration of feeding experiment.

In the present study the total digestibility was almost 10% lower than lipid digestibility and almost 20% lower than protein and energy digestibility, which may have been due to the dietary α-cellulose content.

The total ammonia-N excretion rates recorded over a period of 5 h after feeding with 50 and 44% dietary protein with high energy level and 41% dietary protein with low energy level, were similar to those reported by Kikuchi[29] in Japanese flounder, *Paralichthys olivaceus*. The cumulative rates of ammonia-N excretion 5 h after feeding tended to decrease with increasing dietary energy level in all protein levels. Hence, the protein efficiency showed an improvement in all dietary protein levels with increasing dietary energy from 19 kJ g^{-1} to 20 kJ g^{-1} diet.

The results in this study suggest that less protein was catabolized and more nitrogen accumulated in the body of fish with increasing dietary energy up to 20 kJ g^{-1} diet, indicating that dietary protein was not used as an energy source but for growth of fish. It can be concluded that the proper dietary protein and energy levels for the growth of juvenile Japanese flounder are 50% and 20 kJ g^{-1} diet, respectively, under the experimental conditions employed in this study.

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