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Apparent Amino Acid and Energy Digestibilities of Common Feed Ingredients for Flounder *Paralichthys olivaceus*

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Flounder were fed a reference diet and test diets containing various feed ingredients: mackerel fish meal, meat meal, soybean meal, wheat flour, wheat gluten, corn gluten meal and brewer's yeast. Apparent digestibility was determined using a reference diet with 0.5% chromic oxide indicator and test diets contained 70% reference diet and 30% of the feed ingredient being evaluated. Apparent digestibility coefficients for amino acid and energy in the reference and test diets were determined, and digestibility coefficients for the test ingredients were calculated based on differences in the digestibility of test diets relative to the reference diet. The fish averaging 300 g were held in 2000 L tanks at a density of 20 fish per tank. Feces were collected from three replicate groups of fish using a fecal collection column attached to fish rearing tank. Apparent total amino acids digestibilities (90-95%) of mackerel fish meal, soybean meal, wheat gluten, corn gluten meal and brewer's yeast were higher than those of meat meal and wheat flour ($P<0.05$). Apparent energy digestibilities (86-98%) of mackerel fish meal, meat meal, soybean meal, wheat gluten and corn gluten meal were significantly higher ($P<0.05$) than those of wheat flour and brewer's yeast. These results provide useful information about nutrient and energy utilization for flounder.

Keywords: Digestibility, Flounder (*Paralichthys olivaceus*), Dietary protein source

Introduction

The flounder, *Paralichthys olivaceus* is one of the most important marine fish in Korea for aquaculture, and its culture production has been steadily increased since the late 1980's. Commercial flounder aquaculture is becoming global in scale, and culture techniques are continuously being improved and it is a fast growing species compared with many species of marine fish. Several studies on flounder nutrition and feeding have revealed its protein requirement (Kikuchi et al., 1992; Lee et al., 2000a, 2002), utilization of protein, lipid and carbohydrate ingredients (Kikuchi, 1999; Sato and Kikuchi, 1997; Kim et al., 2000; Lee et al., 2003) and the feeding frequency (Lee et al., 1999, 2000b).

The impact of feed on growth and feed utilization may be mainly affected by the kind of ingredient used in diets. Dietary protein sources are the most important ingredients affecting growth performance of fish and feed cost. Fish meals are in high demand as the protein sources for many formulated diets. Fish meal is well recognized as the best dietary protein source for most marine carnivorous fish which require high dietary protein levels compared to omnivorous or herbivorous fish. However, the increasing price and potentially unstable supply of fish meal in the market could be limiting factors for marine fish culture. The search for fish meal substitutes and alternative dietary protein sources is an international research priority that could be of considerable economic advantage.

The determination of nutrient digestibility is the first step in evaluating the potential of an ingredient for use in the fish feed. Information on digestibility coefficients of feed ingredients is particularly needed for fish to improve the accuracy of diet formulation and allow appropriate substitution of feedstuffs in least-cost formulation. Moreover determining the digestibility of nutrients in a feed is important not only to enable formulation of diets that maximize the growth of cultured fish by providing appropriate amounts of available nutrients, but also to limit the wastes produced by the fish.

Limited reports are available for the apparent digestibility coefficients of various feed ingredients in marine carnivores (Lee, 2002) including flounder. The present study, therefore, was conducted to determine apparent digestibility coefficients for total amino acid and energy in various proteins: mackerel fish meal, meat meal, soybean meal, wheat flour, wheat gluten, corn gluten meal, and brewer's yeast in diets

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for flounder using a fecal collector attached to fish rearing tank.

Materials and Methods

Diet preparation

The reference diet (Table 1) was formulated to satisfy the nutrient requirements of flounder using mackerel fish meal (produced by a steam dry method, Chile) and squid liver oil as protein and lipid sources, respectively. Chromic oxide (Cr_2O_3) was used as an inert marker at a concentration of 0.5% in diets. Experimental diets were formulated to mixed differently in the protein sources used apparent digestibility trial.

Table 1. Ingredients and chemical composition of the reference diet

	%
Ingredients	
Mackerel fish meal[1]	56.0
Wheat flour	29.0
Alpha-potato starch	5.0
Squid liver oil	6.0
Vitamin premix[2]	1.5
Mineral premix[3]	2.0
Chromic oxide	0.5
Proximate analysis in dry matter	
Crude protein	46.5
Crude lipid	10.7
Ash	9.7
Carbohydrate[4]	33.1
Gross energy (kcal 100 g^{-1})	480
Essential amino acids in protein	
Arg	7.9
His	4.4
Ile	4.8
Leu	11.1
Lys	9.2
Met	2.8
Phe	6.2
Tyr	3.5
Thr	6.0
Val	6.9

[1]Produced by steam dry method, Chile.
[2]Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): L-ascorbic acid, 121.2; DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.0031.
[3]Mineral premix contained the following ingredients (g/kg premix): $MgSO_47H_2O$, 80.0; $NaH_2PO_42H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_47H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_36H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4H_2O$, ; $CoCl_26H_2O$, 1.0.
[4]100-(crude protein+crude lipid+ash).

Seven test diets for apparent amino acid and energy digestibilities of protein ingredients were formulated using 70% reference diet and 30% of each of the test ingredients (mackerel fish meal, meat meal, soybean meal, wheat flour, wheat gluten, corn gluten meal, and brewer's yeast) on an air-dry basis. Proximate and amino acid composition of the test ingredients are shown in Table 2. Experimental diets were mechanically mixed with water (300-400 g kg^{-1} diet mix), pelleted diets were dried overnight at room temperature, and stored at -25°C until used.

Experimental animals and culture conditions

Flounder (*Paralichthys olivaceus*) obtained from private hatchery (Gang-won, Korea) were acclimated to the laboratory conditions and fed commercial feed for 1 month before the beginning of feeding trials. Afterwards, the fish were randomly distributed into 2000 L cylindrical fiberglass tanks (containing 1000 L of water each) of a flow-through aquarium system (Fig. 1) and hand-fed the reference diet to visual satiety once a day for 1 month. After conditioning period, average weight of 300 g fish per tank were selected and redistributed into the tanks with 20 fish per tank. Each rearing tank was supplied with filtered seawater with a continuous flow rate of approximately 5 L min^{-1} and aeration was supplied to each tank. The water temperature and specific gravity was 17.8±2.3°C (mean±SD) and 1.024±0.0013 during the four weeks. Photoperiod followed the natural conditions during the experimental period.

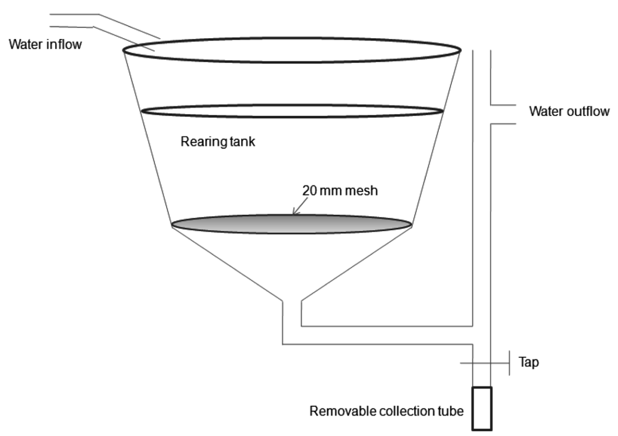

Fig. 1. Digestibility tank system.

Table 2. Proximate and amino acids composition of the ingredients used to test diets

	Test ingredients						
	MFM[1]	MM[2]	SM[3]	WF[4]	WG[5]	CGM[6]	Yeast[7]
Proximate analysis (% in dry matter)							
Crude protein	75.3	74.4	51.8	11.7	79.3	74.3	48.7
Crude lipid	7.9	10.8	1.4	1.0	1.9	1.9	2.0
Ash	15.7	13.2	7.2	1.0	4.9	8.6	9.1
Carbohydrate[8]	1.2	1.6	39.6	86.3	13.8	15.3	40.2
Gross energy (kcal 100 g^{-1})	437	478	423	369	477	535	435
Essential amino acids (% in protein)							
Arg	6.7	5.7	6.7	6.5	6.3	7.2	6.2
His	4.0	3.2	2.7	4.1	3.8	4.0	3.6
Ile	4.0	4.0	3.9	3.9	3.9	4.2	4.0
Leu	8.2	10.1	9.3	8.6	10.3	8.3	9.2
Lys	8.4	6.1	7.2	7.1	7.1	9.1	7.4
Met	3.0	1.8	2.5	1.6	0.7	0.5	1.6
Phe	4.6	4.8	4.8	4.8	5.2	4.7	5.1
Tyr	2.7	2.8	2.7	2.8	2.8	2.6	2.8
Thr	5.1	4.7	4.9	4.7	4.8	5.3	4.9
Val	5.6	5.7	5.3	5.6	5.8	5.7	5.5

[1]Mackerel fish meal was produced by steam dry method.
[2]Meat meal.
[3]Soybean meal (dehulled, solvent extracted).
[4]Wheat flour.
[5]Wheat gluten.
[6]Corn gluten meal.
[7]Brewer's yeast.
[8]100-(crude protein+crude lipid+ash).

Fecal collection techniques

Three replicate groups of fish were fed by hand the reference and test diets (70% reference + 30% ingredient) to apparent satiation once daily at 09.00 h for 4 weeks. Fish were carefully fed by the same person during the feeding trial. The rearing tank and collection column were cleaned at 11.00 h to remove uneaten feed and fecal residues. The next day, feces were collected from the fecal collection columns at 08.00 h prior to the morning feeding. Feces collected from the setting columns were immediately filtered with filter papers (Whatman #1) for 60 min at 4ºC and stored at -80ºC for chemical analyses. Fecal samples from each tank were pooled at the end of experiment.

Analytical methods

Diets and fecal samples were freeze-dried and finely ground using a grinder. Crude protein was determined by Kjeldahl method using an Auto Kjeldahl system (Buchi B-324/435/412, Switzerland). Crude lipid was determined by the ether-extraction method. Moisture was determined by oven drying at 105ºC for 24 h. Ash was determined by muffle furnace at 550ºC for 4 h. Carbohydrate was calculated by difference. Gross energy contents were analyzed using an adiabatic bomb calorimeter (Parr 1356, USA). Amino acids were analyzed using an automatic amino acid analyzer (Hitachi L-8800, Japan). Chromic oxide was determined by the method of Furukawa and Tsukahara (1966). All chemical analyses were done in duplicate or triplicate.

Apparent digestibility coefficients (ADC) for nutrient and energy of the diets were calculated as follows:

ADC of nutrients or energy (%)

$$=\left(1-\frac{\text{dietary } Cr_2O_3}{\text{fecal } Cr_2O_3}\times\frac{\text{fecal nutrient or energy}}{\text{diretary nutrient or energy}}\right)\times 100.$$

ADC for amino acids and energy of protein ingredients in the test diets were determined by the method of Cho et al. (1982) based upon the 70:30 ratio of reference diet mixture and test ingredient in each of the test diets:

ADC of test ingredient (%) = 100/30 × (ADC in test diet – 0.7 ADC in reference diet).

Statistical analysis

The date were subjected to one-way ANOVA, followed by Duncan's multiple range test (Duncan, 1955) at a significance level of $P<0.05$. The data are presented as mean±S.E. of three replicate groups. All statistical analyses were carried out using the SPSS version 10.0 (SPSS Inc., Michigan Avenue, Chicago, Illinois, USA).

Results and Discussion

Apparent amino acid and energy digestibility coefficients of test ingredients for flounder are shown in Table 3. Apparent digestibilities of total amino acid (67-95%) and energy (52-98%) were affected by test ingredients ($P<0.05$). Apparent total amino acid digestibilities (90-95%) of mackerel fish meal, soybean meal, wheat gluten, corn gluten meal and brewer's yeast were higher than those (67-84%) of meat meal and wheat flour ($P<0.05$). Total amino acid digestibility of wheat flour was significantly the lowest among the ingredients tested. Apparent energy digestibilities (86-98%) of mackerel fish meal, meat meal, soybean meal, wheat gluten and corn gluten meal were significantly higher ($P<0.05$) than those (52-65%) of wheat flour and brewer's yeast.

Using highly digestible ingredients is especially important in high density culture conditions where accumulations of undigested food can foul the water and increase the chance of fish disease and mortality (Smith, 1995). The nutrients digestibility will vary depending on the composition of ingredients used. It has been reported that carnivorous fish tend to utilize the energy in animal products better than that in plant products (Cho et al., 1982; Bergot and Breque, 1983; Sullivan and Reigh, 1995). The ability of fish to utilize plant starch as an energy source differs among fish species and appears to be rather limited in carnivorous fish than in herbivorous or omnivorous fish (Cowey and Walton, 1989). However, in this study, energy digestibilities of plant protein sources such as soybean meal, wheat gluten and corn gluten meal were high despite of their carbohydrate contents (14-40%). These results indicate that flounder may has good ability to utilize starch as energy. This hypothesis is supported by previous studies for this species (Lee et al., 2003; Lee and Kim, 2005) which showed that carbohydrate rather than lipid as a non-protein energy source in diet was effective to increase dietary energy level. The utilization of dietary carbohydrate by fish appears to be related to their metabolic systems adapted to the different aquatic environment and dietary

Table 3. Apparent digestibility coefficients (%) for amino acid and energy of the test ingredients in flounder[1]

	Ingredients[2]						
	MFM	MM	SM	WF	WG	CGM	Yeast
Amino acids							
Arg	95±2.2c	82±1.7b	95±0.1c	74±1.2a	84±1.8b	97±0.8c	94±1.6c
His	95±1.9c	87±2.6b	95±0.4c	71±1.9a	93±1.8c	96±0.9c	95±0.3c
Ile	92±2.0c	83±3.6b	93±0.1c	62±0.8a	95±0.1c	95±1.0c	92±0.2c
Leu	94±2.1c	94±3.2b	93±0.1c	65±1.2a	96±0.1c	94±1.9c	93±0.3c
Lys	92±2.1c	87±2.3b	94±0.7cd	62±0.7a	95±0.1cd	97±0.5d	95±0.3cd
Met	87±4.2c	64±2.4b	65±2.1b	38±3.2a	96±1.8d	92±3.7cd	96±0.4d
Phe	91±1.8cd	80±2.9b	85±4.7bc	23±0.6a	92±0.2cd	93±0.2d	87±0.2cd
Tyr	94±2.1bc	85±2.7a	93±0.1bc	88±2.8ab	96±0.2c	94±1.7bc	94±0.2bc
Thr	92±2.3c	79±3.4b	92±0.5c	24±0.1a	93±0.1c	94±1.0c	89±0.5c
Val	92±2.3c	80±2.6b	92±0.7c	33±1.4a	93±0.4c	93±1.0c	90±0.8c
Ser	93±2.0c	83±2.3b	91±0.6c	33±1.7a	93±0.1c	94±1.3c	85±2.2b
Glu	94±1.6c	85±2.5b	92±0.7c	32±0.7a	94±0.2c	94±1.4c	90±1.6c
Gly	95±1.9c	80±3.7b	94±0.9c	64±1.2a	95±0.2c	96±1.08c	94±0.1c
Ala	94±2.0c	84±2.6b	80±4.4b	65±1.2a	96±0.1c	95±1.6c	93±0.5c
Asp	89±5.5d	77±1.1c	66±2.9b	25±0.7a	93±0.2d	94±0.9d	92±0.2d
Total AA	90±2.8c	84±2.5b	93±0.1c	67±2.5a	94±0.1c	95±1.3c	92±0.1c
Energy	92±4.9c	94±6.1c	98±0.7c	52±4.1a	90±3.3c	86±2.3c	65±2.4b

[1]Values (mean±SE of three replicate groups) in each row with a different letter are significantly different ($P<0.05$).
[2]See Table 2.
[3]100-(crude protein+crude lipid+crude ash).

carbohydrate level and complexity (Bergot, 1979; Walton and Cowey, 1982).

In this study, energy digestibilities of wheat flour and brewer's yeast were significantly lower than those of other ingredients, and this trend may be due to high carbohydrate contents (40-86%) in these ingredients. High dietary carbohydrate levels can reduce the utilization of other nutrients (Walker, 1975; Shah et al., 1982; Hilton et al., 1983; Anderson et al., 1984). The relatively low amino acid digestibility of wheat flour could be explained partially by high contents of carbohydrate in the ingredient. The non-protein energy sources in diets may influence growth and protein utilization of fish (Lee and Kim, 2005). Hamre et al. (2003) suggested that Atlantic halibut has low tolerance to dietary carbohydrate, whereas dietary lipid can be varied over a wide range without affecting growth performance. It is important to provide an adequate level and ratio of protein, lipid and carbohydrate in diets in order to reduce catabolism of protein for energy. Plant products containing the proper ratio of protein and carbohydrate should be selected carefully to maximize the availability of carbohydrate energy in flounder diets.

The protein quality of dietary ingredients is dependent on the essential amino acid composition and availability of ingredients and the leading factor affecting fish performance. Deficiency of an essential amino acid leads to poor utilization of the dietary protein and consequently decreases growth and feed efficiency. Thus amino acid digestibility is the very important measure of its availability by fish. Since essential amino acids requirements and their availabilities of different protein sources have not been reported for flounder, use of amino acid availability data obtained from this study should allow for economical feed formulation and low metabolic fecal nitrogen excretion in the rearing water (Ogino and Chen, 1973; Wilson et al., 1981).

Amino acid from fish meal in this study was well digested and the value is similar to that for rockfish (Lee, 2002). Generally, fish meal protein is well digested by carnivorous fish (McGoogan and Reigh, 1996; Sugiura et al., 1998). McGoogan and Reigh (1996) reported that high protein digestibility for red drum might be related to high protein content and low carbohydrate of ingredients. The amino acids of soybean meal, wheat gluten, and corn gluten meal were well digested in this study although these ingredients have relative high contents of carbohydrate. Amino acids digestibilities of soybean meal in this study were high compared to those for rockfish, red drum and hybrid striped bass (Sullivan and Reigh, 1995; Gaylord and Gatlin III, 1996; Lee, 2002). This indicates that each of these plant protein sources can be used efficiently as a partial protein source for flounder diets. Increased usage of plant-protein supplements in flounder diets will reduce feed cost and assist in reducing dependence on fish meal as the primary protein source in fish diets (Rumsey, 1993).

Soybean meal is one of important protein source as a partial substitute for fish meal in fish diets. The successful use of soybean meal as a protein source has been reported for flounder (Deng et al., 2006), but soybean meal may contain antinutritional factors such as trypsin inhibitor and phytate. The higher soybean meal utilization of flounder showed in Kikuchi (1999)'s study agree to this study showing that amino acid and energy digestibilities of soybean meal were high in flounder. However other results showed that protein utilization of soybean meal in flounder were variable (Kim et al., 2000; Choi et al., 2004; Deng et al., 2006). These different utilizations of soybean meal maybe due to processing conditions, nutrient composition of formulated diet, fish rearing conditions, etc (Bransden and Carter, 1999).

The amino acids digestibilities of brewer's yeast were relatively high in this study. Brewer's yeast is frequently used as a supplementary ingredient in aquaculture because it contains essential nutrients and may also contain unknown growth factors, attractants, etc. However, the use of brewer's yeast as a feed ingredient for animals is generally poor due to yeast cell walls being composed of complex heteropolysaccharides in a structured carbohydrate-protein complex such as mannoprotein and glucan (Johnson et al., 1980; Farkas, 1985).

In summary, mackerel fish meal, soybean meal, wheat gluten and corn gluten meal showed the high amino acid and energy digestibilities among ingredients tested. Apparent amino acid and energy digestibilities of wheat flour were lower than those of other ingredients, probably due to the high carbohydrate content. These data provide more precise information concerning nutrient and energy utilization of flounder and will allow ingredient substitutions in practical feed based on levels of available nutrients.

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