Dietary Lysine Requirement of Juvenile Yellowtail Flounder *Pleuronectes ferrugineus*

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ABSTRACT : The lysine requirements of juvenile yellowtail flounder (*Pleuronectes ferrugineus*) having 19.5 g initial body weight were estimated by feeding six practical-type diets containing graded levels of lysine (1.21 to 2.69% of dry diet). Dietary amino acid profile simulated that of whole body of yellowtail flounder. Most of amino acids in the diets were provided by corn gluten meal, herring meal and gelatin. Protein efficiency ratio (PER) improved significantly until lysine level increased up to 2.1% (4.3% of protein). Same trend was observed in feed:gain ratio (FGR) which maintained constant in fish groups fed diets containing lysine above 2.1%. The highest nitrogen gain (0.34 g/fish) in whole body was found in fish fed 2.1% lysine, though the value was not different from those of fish fed above the level of lysine. Fish fed 2.1% lysine also showed the best nitrogen retention efficiency of 24.6%. The broken-line analysis of protein efficiency ratio and body nitrogen gain against dietary lysine level yielded an estimated lysine requirement of 2.2% (4.5% of protein) and 2.3% (4.7% of protein), respectively. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1777-1781)***

Key Words : *Pleuronectes Ferrugineus*, Lysine Requirement, PER, FGR, Nitrogen Gain

INTRODUCTION

Despite a substantial culture worldwide of flatfish species, the artificial diet formulation has not been completed for the species due to a lack of the information in the nutritional requirements for essential amino acid (EAA). Although Cowey et al. (1970) reported that both plaice and sole required the same EAAs as other vertebrates and salmonids, the quantitative requirements were not reported for any species of flatfish to our knowledge.

Lysine requirement values have been published for channel catfish (Wilson et al., 1977), tilapia (Jackson and Capper, 1982; Santiago and Lovell, 1988), sea bass (Tibaldi and Lanari, 1991), milkfish (Borlongan and Coloso, 1993), red drum (Brown et al., 1988; Craig and Gatlin, 1992), striped bass (Griffin et al., 1992), chum salmon (Akiyama, et al., 1985), cherry salmon (Ogata et al., 1983), Atlantic salmon (Anderson et al., 1993) and yellowtail (Ruchimat et al., 1997). For rainbow trout, the requirement values were reported which varied from 3.7 to 6.2% of dietary protein (Ketola, 1983; Walton et al., 1986; Kim et al., 1992). Under a current aquaculture situation in Atlantic Canada being based on salmonids, yellowtail flounder is considered as an alternative species to diversify the base of aquaculture (Brown and Crim, 1998). The present study was conducted to quantify dietary lysine need as a preliminary step to develop an artificial diet for yellowtail flounder.

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MATERIALS AND METHODS

Diet preparation

The basal diet was formulated to be limiting in lysine while being similar in all other amino acids to those of 45% whole body protein of yellowtail flounder. A fixed amount of the dietary protein (36.8%) was supplied by corn gluten meal, herring meal, gelatin and krill meal and the remaining portion of the protein equivalent was provided by a crystalline amino acid premix (Tables 1 and 2). Fish meal and corn gluten meal were first ground to a fine particle using a Fitz mill (The Fitzpatrick Co., Ill., USA) before mixing with other ingredients. A graded levels of crystalline L-lysine were added to the basal diet by weight for weight replacement of L-glutamic acid in the amino acid mixture. Each amino acid mixture blended with dissolved agar (20 g in 200 g hot water) and 400 g deionized water were added to the mixture of the other ingredients to make each diet of 2 kg. Dietary mixture, fully homogenized using a Hobart mixer, was then passed through a Hobart food grinder to form 2 mm diameter pellet. The spaghetti-like strands were lyophilized using a freeze drier for 3 days.

Feeding trial

Prior to the feeding trial, yellowtail flounder were adapted to the experimental conditions for 3 weeks during which commercial diet was fed for first week and then switched to mixed experimental diets for two weeks. Growth trial was conducted in the NRC experimental station at Sandy cove (Nova Scotia, Canada) at a constant water temperature of 12±1°C for a period of 8 weeks. Yellowtail flounder (*Pleuronectes ferrugineus*) having a mean initial body weight (IBW) of 19.5 g were allotted into

Table 1. Composition of the basal diet

Ingredient	g/kg
Corn gluten meal[1]	330.0
Herring meal[2]	80.0
Gelatin[3]	50.0
Krill meal[5]	20.0
Amino acid mixture[3,4]	82.1
Agar[6]	10.0
Corn starch (pregelatinized)[7]	87.9
Cellulose (Celufil)[3]	58.0
Dextrin[3]	80.0
Mineral premix[8]	30.0
Vitamin premix[9]	20.0
Choline HCl[3]	2.0
Fish oil (herring)[10]	150.0

[1] Corey Feed Mills, Fredericton, NB. [2] Sea Life Fisheries Inc., Canada
[3] US Biochemical, Cleveland, OH. [4] See Table 2.
[5] Special Marine Products Ltd., West Vancouver, Canada.
[6] Spectrum Diagnostics, Glenwood, IL.
[7] National Starch and Chemical Co., USA.
[8] Mineral added to supply the following (per kg diet): manganous sulfate (32.5% Mn), 123.1 mg; ferrous sulfate (20.1% Fe), 248.7 mg; copper sulfate (25.4% Cu), 39.5 mg; zinc sulfate (22.7% Zn), 330.4 mg; cobalt chloride (24.8% Co), 20.2 mg; potassium iodide (76.4% I), 6.5 mg; sodium selenite (45.6% Se), 2.2 mg; sodium fluoride (45.2% F), 10.0 mg; calcium phosphate, monobasic (15.9% Ca, 24.6% P), 14,228.0 mg; potassium phosphate, dibasic (22.8% P, 28.7% K), 6,579.0 mg.
[9] Vitamin added to supply the following (per kg diet): vitamin A, 8,000 IU; vitamin D_3, 4,000 IU; vitamin E, 300 IU vitamin K_3, 40 mg; thiamine HCl, 50 mg; riboflavin, 70 mg; D-Ca pantothenate, 200 mg; biotin, 1.5 mg; folic acid, 20 mg; vitamin B_{12}, 0.2 mg; niacin, 300mg; pyridoxine HCl, 20 mg; ascorbic acid, 500 mg; l-ascorbyl-2-polyhosphate (Stay C, 15%), 250 mg; inositol, 400 mg; BHT, 15 mg; BHA, 15 mg.
[10] Stabilized with 0.06% ethoxyquin. Commeau Seafood, Saulnierville, NS.

groups of 14 fish each into 18 quadrilateral plastic tanks. The filtered and UV-treated water (salinity, 30‰) were supplied to each tank at a flow rate of 0.8 l/min in an indoor flow-through system. Water levels in each tank maintained at 35 l (holding capacity: 45 l) to ensure the renewal rate of once every hour. At the start and every 4 weeks of the experiment, fish were bulk-weighed to estimate feed utilization. Fish were held on a 12 h dark: 12 h light photoperiod and the light intensity at the surface of the water ranged between 40 and 60 lux. Dissolved oxygen and its saturation levels were checked every morning which averaged 11 mg/L and 100%, respectively. For weighing and counting, fish were fasted for the day before the beginning and for the day after the end of the experiment, respectively. Fish were fed by hand twice (9:00 and 15:00) for weekday and once a day for weekend at a 2% of body weight.

Analytical methods

Chemical composition of the experimental diets and carcass was determined following procedures (AOAC, 1984): dry matter by drying in an oven at 110°C for 24 h; crude protein (N×6.25) by the Kjeldahl method after an acid digestion; crude fat after ether extraction by the Soxhlet method; crude ash by incineration in a muffle furnace at 550°C for 24 h, Ca by a wet ash method and titration with KMnO4 and P by the vanado-molybdate method. Amino acid in feed ingredients and whole body of fish was analyzed after acidic hydrolysis in 6 N HCl for 24 h at 110°C in glass tube under nitrogen. Cystine was determined after performic acid oxidation before the acidic hydrolysis as described by Gehrke et al. (1985). After hydrolysis, the hydrolysate was dried under vaccum, dissolved in sodium citrate, pH 2.2, and filtered through a Millipore membrane

Table 2. Composition of the amino acid mixture (g/100 g diet)

Amino acids	Dietary supply	Provided by crystalline amino acids	Total	45% protein from yellowtail flounder
Lysine	1.17	-	1.17	3.40
Histidine	0.71	0.27	0.98	0.98
Arginine	1.73	0.96	2.69	2.69
Aspartic acid	2.60	1.50	4.10	3.95
Threonine	1.25	0.54	1.79	1.79
Serine	1.20	0.67	1.87	1.87
Glutamic acid	6.96	1.50	8.46	5.81
Proline	3.12	-	3.12	1.78
Glycine	2.58	1.50	4.08	3.31
Alanine	3.37	-	3.39	2.55
Cystine	0.50	-	0.50	0.45
Valine	1.65	0.42	2.07	2.07
Methionine	0.95	-	0.95	0.90
Isoleucine	1.32	0.35	1.67	1.67
Leucine	4.46	-	4.46	3.02
Tyrosine	1.47	-	1.47	0.99
Phenylalanine	1.79	-	1.79	1.62
Tryptophan	0.23	0.28	0.51	0.51
Taurine	-	0.22	0.22	0.25

Table 3. Lysine and proximate composition (g/100 g diet, DM) of the experimental diets[1]

Diet	Lysine		C. protein	C. lipid	C. ash	Ca	P
	% diet	% protein					
Basal	1.21	2.44	49.60	16.49	3.73	1.07	0.96
+0.3%	1.53	3.10	49.31	16.28	3.69	1.06	0.97
+0.6%	1.84	3.75	49.07	16.44	3.75	1.06	0.94
+0.9%	2.13	4.32	49.30	16.63	3.65	1.05	0.97
+1.2%	2.41	4.90	49.22	16.62	3.72	1.02	0.93
+1.5%	2.69	5.47	49.22	16.54	3.64	1.05	0.93

[1] Values are means of two determinations.

Table 4. Growth, feed utilization and nitrogen retention of fish fed different levels of lysine for 8 weeks[1]

Lysine (%) in diet	Final weight (g/fish)	PER[2]	FGR[3]	N gain[4] (g/fish)	NRE (%)[5]
1.2	25.0±1.74^{c}	0.71±0.13^{b}	3.05±0.55^{a}	0.12±0.00^{c}	10.0±1.39^{b}
1.5	26.4±1.80^{c}	0.88±0.05^{b}	2.32±0.14^{ab}	0.14±0.01^{bc}	11.7±1.23^{b}
1.8	27.6±1.55^{bc}	0.94±0.09^{b}	2.20±0.19^{b}	0.17±0.01^{b}	12.8±0.90^{b}
2.1	33.7±1.65^{a}	1.61±0.05^{a}	1.26±0.04^{c}	0.34±0.02^{a}	24.6±1.02^{a}
2.4	32.9±1.59^{ab}	1.53±0.10^{a}	1.33±0.09^{c}	0.34±0.02^{a}	24.5±1.36^{a}
2.7	32.3±1.66^{ab}	1.52±0.12^{a}	1.36±0.10^{c}	0.32±0.02^{a}	24.0±1.78^{a}

[1] Values (means±SE of three replicate groups) in the same row not sharing a common superscript letter are significantly different ($p<0.05$).
[2] Protein efficiency ratio=weight gain/protein intake. [3] Feed : gain ratio=feed intake. DM/wet weight gain.
[4] Nitrogen gain=(% N×final body wt.)-(% N×initial body wt.). [5] Nitrogen retention efficiency=N gain/N intake×100.

filter (0.45 μm). Amino acids were separated by an ion exchange chromatography (Beckman 6.300, California). Tryptophan was determined by the colorimetric method of Basha and Roberts (1977) after alkaline hydrolysis.

Statistical analyses were performed by using analysis of variance and a multiple range test at the 5% probability level of Duncan (1955). Estimations of the dietary lysine requirement were made from non-linear regression of the values of protein efficiency ratio (PER) and body nitrogen gain against dietary lysine level using one-slope break-point analysis (Robbins et al., 1979). The model employed was: $Y=L-U(R-X_L)$. Where Y is the value of the parameter, L is the maximum value of the parameter, U is slope, X_L is the level of lysine in diet (% of diet) and R is the requirement value.

RESULTS AND DISCUSSION

Experimental diets were similar in proximate composition except lysine content, which increased from 1.21% (2.4% of protein) for basal to 2.69% (5.5% of protein) for the 1.5% lysine added diet (Table 3). Dietary lysine level had a significant effect on final weight, which increased with the lysine level until the lysine requirement was met. Thereafter, further increases in lysine level resulted in no significant increase in final weight (Table 4). Protein efficiency ratio (PER) also improved significantly until lysine level increased up to 2.1% (4.3% of protein). Same trend was observed in feed:gain ratio (FGR) which maintained constant in fish groups fed diets containing lysine above 2.1%. The highest nitrogen gain (0.34 g/fish) in whole body was found in fish fed 2.1% lysine, though the value was not different from those of fish fed above the level of lysine. Fish fed 2.1% lysine also showed the best nitrogen retention efficiency of 24.6%. After 4 weeks on experimental diet, significant differences were observed between treatments. At 8 weeks, it was apparent that weight gains of fish fed diets containing 1.2, 1.5 and 1.8% lysine were depressed compared to those of the other groups. No mortality was observed in all fish groups until the end of the feeding trial, though all fish died during last three days of the ninth week due to furunculosis caused by *Aeromonas* spp. This sudden mortality resulted in failure in observing both further growth and a sign of nutritional deficiency. The broken-line regression (Robbins et al., 1979) of protein efficiency ratio and body nitrogen gain against dietary lysine level yielded an estimated lysine requirement of 2.2% ($Y=1.53-0.89(R-X_L)$, $R=2.2\pm0.24$) and 2.3% ($Y=0.33-0.22(R-X_L)$, $R=2.3\pm0.25$), respectively.

Quantitative lysine requirements for fish were determined by growth, nitrogen balance and/or plasma free amino acid studies as conducted for chick (Ohno and Tasaki, 1971), rat (Rama et al., 1959) and pig (Mitchell et al., 1968). The values (% dietary protein) were 4.0% for milkfish (Borlongan and Coloso, 1993), 4.3% for sea bass (Tibaldi and Lanari, 1991), 5.1% for channel catfish (Wilson et al., 1977; Robinson et al., 1980), 4.4% for red drum (Craig and Gatlin, 1992), 3.7 to 6.1% for rainbow trout (Ketola, 1983; Walton et al., 1986; Kim et al., 1992), 4.0% for striped bass (Griffin et al., 1992), 5.1% for tilapia (Santiago and Lovell, 1988), 3.98% for Atlantic salmon (Anderson et al., 1993), and 4.13% for yellowtail (Ruchimat et al., 1997).

The requirement value estimated using broken-line analysis, which was 4.5% and 4.7% of dietary protein for PER and body nitrogen gain, respectively, from the present study corresponded well to that for Japanese flounder (Forster and Ogata, 1998), though type and feed ingredients of the experimental diet used, and initial weight of fish were different from each other. Whole body amino acid patterns of yellowtail flounder, Japanese flounder and Atlantic halibut suggested that the amino acid requirements of the flatfish may not be greatly different (Kim and Lall, 2000). Recently, Alam et al. (2002) reported that brown fish meal (sardine) protein was the best as a reference amino acid pattern in the diet of juvenile Japanese flounder. The ideal protein concept (Cole, 1978) has been widely employed in feed formulation for swine and poultry (Baker, 2000). Similarly, determined lysine value and the A/E ratios of the amino acids of whole body were used to predict other amino acid requirements of fish (Wilson, 1991; Moon and Gatlin, 1991; Brown, 1995; Forster and Ogata, 1998). Because lysine is, however, not the first-limiting amino acid in either natural food or a formulated diet for carnivorous fish, it may be necessary to carefully evaluate and apply these findings to extrapolate the requirement data for a practical use (Kim and Lall, 2000).

Nitrogen retention is considered to be the most accurate and sensitive parameter to estimate dietary amino acid requirements, since weight gain may result from an increase in both protein and fat levels of whole body (Cowey, 1992; Rodehutscord et al., 1995). Jakson and Capper (1982) found that nitrogen retention efficiency (NRE) increased from 25% to 35% when lysine level increased from 1.42 to 1.62% in diet. Tibaldi and Lanari (1991) observed the NRE of 25% when fingerling sea bass fed diet containing 2.15% lysine, which was similar to the value obtained in present study with dietary lysine level of 2.13% (4.3% of protein). Somewhat lower NRE (22.6%) than our value was found in yellowtail fed diet containing 1.85% lysine, above which the value remained nearly the same (Ruchimat et al., 1997). However, the higher values of 35.7 and 42.3% were also reported for rainbow trout fed diets containing 1.2 and 1.4% lysine, respectively (Kim et al., 1992). Such a variation in NRE value might be explained from difference in dietary protein level besides different species. Kim et al. (1992) used the basal diet containing 35% protein, while diet in the present study contained 45% protein. On the other hand, the basal fish groups showed a loss of appetite in a period as short as 3 d, resulting in apparent wastage. Although dietary pH could affect feed intake (Nose et al., 1974; Wilson et al., 1977), other groups of fish accepted well their diets. Therefore, it seemed that dietary pH did not cause such feed wastage from the basal groups.

In summary, lysine requirement of juvenile yellowtail flounder was determined to be 2.2% (4.5% of protein) to 2.3% (4.7% of protein) of dry diet.

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