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Quantitative dietary lysine requirement of juvenile grass carp *Ctenopharyngodon idella*

Sheng Wang[a], Yong-Jian Liu[a,*], Li-Xia Tian[a], Ming-Quan Xie[a,b], Hui-Jun Yang[a], Yong Wang[a], Gui-Ying Liang[a]

[a]*Nutrition Laboratory, Institute of Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, PR China*
[b]*Department of Science and Technology of Guangdong, Guangzhou 510033, PR China*

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Abstract

A 90-day feeding trail was conducted to determine the dietary lysine requirement of juvenile grass carp using six isonitrogenous and isoenergetic diets (38% protein, 14 MJ digestible energy kg^{-1}) with wheat gluten–casein–gelatin as protein resource supplemented with six graded levels of crystalline lysine (from 0.69% to 3.08% of dry diet). Crystalline amino acid mixtures were supplemented to simulate the amino acid pattern found in the whole-body protein of grass carp except for lysine. Each diet was randomly assigned to triplicate groups of 20 fish each (3.15 ± 0.01 g, mean ± S.E.M.) and fish were fed five times daily at 2.5% body weight per day. No mortality or nutritional deficiency signs were observed except for growth depression in fish fed the diet with low content of lysine. Weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio, nutrient retention, proximate body composition, morphometry and hematology were significantly ($P < 0.05$) affected by the dietary lysine concentrations. WG, SGR and FE were significantly higher in fish fed diet containing lysine 2.18% of the diet. Second-degree polynomial regression analysis of the WG and FE data indicated that the minimum recommended dietary lysine requirement for optimal growth of juvenile grass carp was 2.24% of the dry diet, corresponding to 5.89% of dietary protein. While the lysine availability (92.37%) of the protein sources was considered, the optimal lysine requirement of juvenile grass carp was calculated to be 2.07% of the diet, corresponding to 5.44% of the dietary protein. Additionally, the estimated requirements for the other essential amino acids were calculated from *A*/*E* ratios of whole-body amino acid profile based on the lysine requirement determined from the present experiment.

Keywords: Grass carp *Ctenopharyngodon idella*; Requirement; Lysine; *A*/*E* ratio

1. Introduction

Grass carp (*Ctenopharyngodon idella*) represent the second largest aquaculture industry in the

* Corresponding author. Tel.: +86 20 8411 0789; fax: +86 20 8411 0789.
E-mail address: ls59@zsu.edu.cn (Y.-J. Liu).

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world inferior to silver carp *Hypophthalmichthys molitrix*, constituting 14.7% of the world aquaculture production, with an average annual increase of 14% in China (FAO, 1999). In China, grass carp also is one of most popular and important freshwater species for its rapid growth, good meat quality and taste (Lin, 1991). One of the major reasons for the increase in production is the use of pelleted feed, which enables the higher density or net-cage monoculture of this species to be achieved. Feed cost has to keep low for farmers as the low retail price of grass carp in China (FAO, 2001). Nutrient balanced diet with a low cost is of serious importance faced to current position in China.

Ten amino acids have been found to be essential for all fish studied to date, and lysine is of particular concern because it is the essential amino acid found in the highest concentration in the carcass of many species of fish (Wilson and Cowey, 1985; Wilson and Poe, 1985; Kim and Lall, 2000). Lysine is the most limiting amino acid in plant protein meal such as cereal grains, which are important feed stuffs locally available for formulating fish diets (Robinson et al., 1980; Forster and Ogata, 1998; Small and Soares, 2000; Murillo-Gurrea et al., 2001; Tantikitti and Chimsung, 2001). From an economical point of view, it is beneficial to use low-cost protein sources for warmwater fish, which generally are able to more efficiently utilize plant protein sources than certain other fish species. Therefore, it is of high priority to evaluate the dietary lysine requirement in order to formulate a more cost-effective diet, since protein is usually the most expensive feed component.

Except for methionine (Wang et al., unpublished data), there have been no studies with regard to EAA requirements of grass carp. The present research was undertaken to estimate the dietary lysine requirement for the maximum growth and assess the effects of dietary lysine on growth, nutrient retention, body composition, morphometry, digestibility and hematology of this fish. We also estimated the requirements for the other essential amino acids based on the lysine requirement by using *A*/*E* ratios [1000 × (essential amino acid content / total essential amino acid contents plus cystine and tyrosine)].

Table 1
Formulation and proximate composition of the experimental diets (as fed basis)

Diet code	Test diets (g kg^{-1} dry diet)					
	1	2	3	4	5	6
Dietary lysine level	7.1	12.0	16.9	21.8	26.7	31.6
Ingredients						
Casein[a]	35.3	35.3	35.3	35.3	35.3	35.3
Gelatin[a]	53.0	53.0	53.0	53.0	53.0	53.0
Wheat gluten[b]	216.2	216.2	216.2	216.2	216.2	216.2
Amino acid mixture[c]	115.5	115.5	115.5	115.5	115.5	115.5
Lysine[c]	0.0	4.9	9.8	14.7	19.6	24.5
Aspartic acid/glycine (1:1)[c]	24.5	19.6	14.7	9.8	4.9	0.0
Fish oil/corn oil (1:3)[d]	60.0	60.0	60.0	60.0	60.0	60.0
Cellulose	112.0	112.0	112.0	112.0	112.0	112.0
Vitamin mix[e]	20.0	20.0	20.0	20.0	20.0	20.0
Mineral mix[f]	80.0	80.0	80.0	80.0	80.0	80.0
Others[g]	283.5	283.5	283.5	283.5	283.5	283.5
Composition as analysis (g kg^{-1} dry diet)						
Moisture	84.2	89.3	89.5	85.5	80.0	82.7
Crude protein	379.0	383.8	377.2	382.7	376.9	383.5
Crude lipid	51.7	52.4	50.6	49.9	48.7	51.4
Ash	89.4	88.8	86.7	85.8	85.4	81.2
Lysine	6.9	11.5	17.3	21.4	26.0	30.8
Lysine (g kg^{-1} dry protein)	18.2	30.0	45.9	55.9	69.0	80.3
GE (MJ/kg)	17.6	17.9	17.8	17.7	17.7	17.8
DE (MJ/kg)[h]	13.8	14.3	14.3	14.2	14.1	14.1

[a] Imported from Australia. Casein (Crude protein: 85%), gelatin (CP: 95%).

[b] Crude protein: 74% Guangzhou Chengyi Company Ltd, Guangzhou, China.

[c] See Table 2; supplied as L-form; Shanghai Cangda Amino acid Company Ltd, Shanghai, China.

[d] Imported from New Zealand.

[e] Vitamin: (mg kg^{-1} of diet): thiamin HNO_3, 50; riboflavin, 50; vitamin A, 25,000 IU; vitamin E, 400; vitamin D3, 24,000 IU menadione, 40; pyridoxine HCl, 40; cyanocobalamin, 0.1; biotin, 6; calcium pantothenate, 100; folic acid, 15; niacin, 200; inositol, 2000.

[f] Mineral mix: (g kg^{-1} of diet): calcium biphosphate, 9.8; calcium lactate, 37.9; sodium chloride, 2.6; potassium sulfate, 13.1; potassium chloride, 5.3; ferrous sulfate, 0.9; ferric citrate, 3.1; magnesium sulfate, 3.5; zinc sulfate, 0.04; manganese sulfate, 0.03; cupric sulfate, 0.02; cobalt chloride, 0.03; potassium iodide, 0.002; cellulose, 4.2 (Huang and Liu, 1989).

[g] Others (g kg^{-1} diet): Dextrine, 200; Ascorbyl-2-monophosphate, 10; Choline chloride (50%), 8; betaine, 4; Carboxymethy cellulose (CMC), 40; κ-Carrageenan, 25; Y_2O_3, 0.1.

[h] DE (digestible energy) was calculated from gross energy analysed and digestibility of energy measured (see Table 5).

2. Materials and methods

2.1. Experimental diet and diet preparation

Experimental diets (Table 1) were formulated to contain 38% crude protein which is slightly lower than the optimum protein requirement (41%, Dabrowski, 1977) to assure maximum utilization of the limiting amino acid (Wilson, 1989). The diets contained an analysis digestible energy level of $14 MJ \cdot kg^{-1}$ calculated from gross energy and the measured digestibility of energy. The basal diet (diet 1) contained the minimum level of lysine, 0.69% of the diet, from wheat gluten, casein and gelatin. The basal diet was supplemented with crystalline L-amino acids to simulate the amino acid pattern found in 38% crude protein from grass carp whole-body protein except for lysine (Table 2). Incremental levels of L-lysine were added to the basal diet ranging from 0.69% to 3.08% of the dry diet. Cellulose and the glycine/aspartic acid mixture were adjusted to maintain all diets isonitrogenous and isoenergetic (see Table 1).

Diet preparation was as described by Millamena et al. (1998) and Alam et al. (2002). Briefly, the crystalline amino acid (CAA) mixtures were pre-coated with 1.5 g cooked carboxymethylcellulose (CMC) in water at 50 °C. Then CMC-bound CAA mixture and the other even mixed dry ingredients were added to the casein–gelatin paste with water. To improve the water stability of the diets, 2.5 g κ-carrageenan per 100 g dry diets was gelatinized at 85 °C in a water bath to form a homogenous gel and added to re-coat the mixture. The pH of the diets was adjusted to 7.0–7.5 with 6 N sodium hydroxide (Wilson et al., 1977). The pellets were obtained (1.2 mm in diameter) using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, PR China) and air dried to a moisture content of less than 10%. The noodle-like diets were ground, sieved and stored in plastic bags at −20 °C.

2.2. Experimental procedures

Grass carp (*C. idella*) juveniles from our facilities were used in this experiment and their initial weights were 3.15 ± 0.01 g (mean ± S.E.M., $n = 18$). Before the experiment, the fish were acclimated to the experimental conditions for 2 weeks and fed a diet contain-

Table 2
Amino acid composition (g per 100 g dry diet) of experimental diets for lysine requirement study

Amino acids[1]	Amount in casein (3 g)	Amount in gelatin (5 g)	Amount in wheat gluten (16 g)	Crystalline amino acids	Amount in 38 g whole-body protein
Essential amino acids					
Arginine	0.12	0.43	0.51	1.01	2.08
Histidine	0.08	0.03	0.31	0.68	1.12
Isoleucine	0.15	0.09	0.85	0.61	1.70
Leucine	0.29	0.20	1.12	1.48	3.08
Lysine	0.24	0.22	0.25	variable	3.53
Methionine	0.08	0.04	0.22	0.84	1.19
Phenylalanine	0.15	0.13	0.54	0.88	1.70
Threonine	0.13	0.21	0.50	0.67	1.50
Tryptophan	0.03[2]	0.00[2]	0.14[3]	0.26	0.43[4]
Valine	0.18	0.14	0.61	1.01	1.95
Non-essential amino acids					
Aspartic acid	0.22	0.30	0.53	2.67	3.73
Glutamic acid	0.60	0.57	5.76	0.00	5.56
Serine	0.17	0.20	0.85	0.00	1.03
Proline	0.18	0.41	1.68	0.31	2.58
Glycine	0.07	1.27	0.59	0.98	2.90
Alanine	0.11	0.51	0.42	1.35	2.39
Tyrosine	0.18	0.03	0.55	0.34	1.11
Cystine	0.01	0.01	0.29	0.10	0.40

[1] Supplied as L-form; [2] calculated from NRC (1993); [3] calculated from Storebakken et al. (2000); [4] calculated from Lin (1991).

ing 35% protein and 6% lipid to satiation five times per day. Twenty healthy fish were randomly distributed to each of 18 experimental fiberglass tanks (98 L × 48 W × 42 H cm, water volume of 200 l) connected to a recirculation system. Water exchange in each aquarium was maintained at 10 l min^{-1}. The water was oxygenated, passed through artificial sponge (3 cm thickness), coral-sand (25 cm thickness) and active-carbon filter (25 cm thickness) to remove chlorine. During the trial period, the diurnal cycle was 12-h light/12-h dark. Water quality parameters monitored weekly were as follows (mean ± S.E.M.): temperature, 28.7 ± 2.5 °C; dissolved oxygen, 7.6 ± 0.29 mg l^{-1}; total ammonia-nitrogen, 0.093 ± 0.005 mg l^{-1}; pH, 8.0 ± 0.07, respectively.

The fish were fed for 90 days with a daily ration of 2.5% of body weight divided into five meals day^{-1} which is a level near satiation. Feces were collected daily during the last 2 weeks as described by Zhou et al. (2004). Feces $tank^{-1}$ was dried at 105 °C and stored at −70 °C for determination of digestibility with Y_2O_3 as indicator. Leaching loss of crystalline amino acid from diets was measured after immersion into distilled water for 2, 5 and 10 min as described by López-Alvardo et al. (1994) and Alam et al. (2004).

2.3. Sampling and analytical methods

At the beginning of the feeding trial, 18 juveniles were randomly sampled from the initial fish and killed for analyses of whole-body composition. At the end of the 90-day experiment, 12 fish from each tank were randomly collected for proximate analysis, 4 for analysis of whole-body composition and 8 were anaesthetized with tricaine methane sulphonate (MS222) (50 mg l^{-1}) for blood collection and to obtain weights of individual whole body, viscera, liver and mesenteric fat. White muscle from both sides of the fillets without skin and liver were dissected and frozen immediately in liquid nitrogen and stored at −70 °C until used. Refer to Ruchimat et al. (1997), 6 h after last feeding, blood was drawn from the sinus of 8 anaesthetized fish with heparinized syringes, and the plasma separated by centrifugation and stored at −70 °C until analyzed.

Diets and fish samples (including white muscle and liver) were analyzed in duplicate for proximate composition. Moisture, crude protein, crude lipid and ash were determined using standard methods (AOAC, 1984). Moisture was determined by drying in an oven at 105 °C for 24 h; crude protein (N × 6.25) was analyzed by the Kjeldahl method after acid digestion (1030-Auto-analyzer, Tecator, Höganäs, Sweden); crude fat was determined by the ether-extraction method by Soxtec System HT (Soxtec System HT6, Tecator, Sweden); crude ash by incineration in a muffle furnace at 550 °C for 24 h. The amino acid compositions of all samples including ingredients, diets and feces were analyzed following acid hydrolysis using an automatic amino acid analyser (Hitachi 835-50, Japan) with a column (Hitachi custom ion exchange resin no. 2619) by a professional laboratory. In brief, performic acid oxidation was performed prior to hydrolysis to oxidize cystine and methionine to cysteic acid and methionine sulfone. Then sodium metabisulfite was added to decompose surplus performic acid. Subsequently, amino acid were liberated from protein by hydrolysis with 6 N HCl. Hydrolysed samples were diluted with sodium citrate buffer, pH was adjusted to 2.2, and individual amino acid components were separated by ion exchange chromatography at 570 nm. Tryptophan was not determined. The concentrations of dietary and fecal Y_2O_3 were determined by inductively coupled plasma atomic emission spectrophotometer (ICP; model: IRIS Advantage (HR), Thermo Jarrel Ash Corporation, Boston, U.S.A) after perchloric acid digestion (Bollin et al., 1952). The concentrations of plasma protein, cholesterol, triacylglycerol and glucose were determined using an automatic blood analyser (Hitachi 7170A, Japan) from a clinical laboratory.

2.4. Statistical analysis

All data are presented as means ± S.E.M. and subjected to one-way analysis of variance (ANOVA) to test the effects of experimental diets using the software of the SPSS for Windows (ver11.0, U.A.S.). Duncan's new multiple range test was used to resolve the differences among treatment means (Duncan, 1955). Statistical significance was examined at $P<0.05$ unless otherwise noted. Second-degree polynomial regression analysis ($Y=a+bX+cX^2$) (Zeitoun et al., 1976) or broken-line model ($Y=a+bX$) (Robbins et al., 1979) was

employed to estimate the optimum lysine requirement. The apparent digestibility coefficients (ADCs) of the dry diet, protein, lipid, energy and apparent availability coefficient (AAC) of lysine were calculated by the formula of Cho et al. (1974).

3. Results

3.1. Growth performance and nutrition retention

Chemical analysis of the diets (Table 1) showed that the desired lysine levels of 0.71–3.16% on a dry matter basis were achieved. Fish readily accepted the experimental diets and there was no mortality during the 90-day feeding trial.

Growth performances of grass carp juveniles presented in Table 3 were significantly ($P<0.05$) affected by the dietary lysine concentrations. The weight gain (WG), specific growth rate (SGR) and feed efficiency (FE) of the test fish can be easily divided into two groups according to the statistical significance. WG of fish increased with increasing levels of lysine up to 2.18% of diet and peaked at 332.9%, beyond which it showed a declining tendency. SGR of grass carp followed the same pattern as WG and achieved the maximum value at 2.18% dietary lysine level, which was not significantly different from values for the 1.69% to 3.16% lysine levels. The most efficient FE was observed in groups fed 2.18% lysine diet Fish fed the diets exceeding 2.18% lysine level did not show any improvement in protein efficiency ratio whereas fish fed the diets with lower lysine level reduced the efficiency of protein utilization. Nitrogen retention (NR) showed a similar response to the growth parameters. No diet related mortality or deficiency signs were observed in this study other than reduced growth.

Between the two regression models used to describe the relationship between weight gain and dietary, the second-degree polynomial model had a better fit ($R=0.9633$) than the broken-line model ($R=0.7661$). When second-degree polynomial regression analysis was used, based on data of weight gain (as shown in Fig. 1) and feed efficiency for estimating the requirement of dietary lysine, the regression equations were as follows:

$$WG = -0.6076LY^2 + 27.9708LY - 0.5736, \quad r = 0.9633;$$

$$FE = -0.09283LY^2 + 4.1642LY + 9.8503, \quad r = 0.9910.$$

From above, the optimal dietary requirement of lysine for juvenile grass carp was estimated to be

Table 3
Effect of dietary lysine levels on growth performance of juvenile grass carp fed experimental diets for 90 days[1]

Diet code	1	2	3	4	5	6
Lysine (%)	0.71	1.2	1.69	2.18	2.67	3.16
FBW (g)[2]	8.60 ± 0.33^{d}	10.11 ± 0.32^{c}	13.12 ± 0.37^{ab}	13.51 ± 0.44^{a}	12.57 ± 0.35^{ab}	12.17 ± 0.36^{b}
WG (%)[3]	172.22 ± 11.02^{d}	218.40 ± 11.11^{c}	315.95 ± 12.39^{ab}	332.93 ± 10.62^{a}	295.86 ± 11.10^{ab}	290.65 ± 12.68^{b}
SGR[4]	1.11 ± 0.05^{c}	1.28 ± 0.04^{b}	1.58 ± 0.03^{a}	1.63 ± 0.03^{a}	1.53 ± 0.03^{a}	1.51 ± 0.04^{a}
FE (%)[5]	34.82 ± 1.34^{d}	43.78 ± 1.04^{c}	55.36 ± 0.32^{a}	57.01 ± 1.78^{a}	54.25 ± 0.89^{ab}	50.38 ± 2.52^{b}
PER[6]	0.92 ± 0.04^{d}	1.14 ± 0.03^{c}	1.47 ± 0.01^{a}	1.49 ± 0.05^{a}	1.44 ± 0.02^{a}	1.31 ± 0.07^{b}
NR (%)[7]	11.55 ± 0.48^{d}	15.35 ± 0.43^{c}	20.98 ± 0.32^{a}	21.49 ± 0.51^{a}	20.81 ± 0.28^{a}	18.62 ± 0.43^{b}
LR (%)[8]	54.46 ± 0.79^{d}	67.82 ± 1.84^{c}	88.90 ± 1.65^{ab}	95.17 ± 1.49^{a}	91.39 ± 3.49^{ab}	83.53 ± 3.89^{b}

[1] Means ± S.E.M. of three replicates and values within the same row with different superscripts are significantly different ($P<0.05$). Initial body weight of fish was 3.15 ± 0.01 g (mean ± S.E.M., $n=18$).
[2] FBW: final body weight (g).
[3] WG: weight gain = 100 × (final body weight − initial body weight)/initial body weight) (g).
[4] SGR: special growth ratio = 100 × (ln final wt. − ln initial wt.)/90 days.
[5] FE: feed efficiency = 100 × weight gain (g)/dry feed (g).
[6] PER: protein efficiency ratio = weight gain (g)/protein fed (g).
[7] NR: nitrogen retention = 100 × retained nitrogen (g)/nitrogen fed (g).
[8] LR: lipid retention = 100 × retained lipid (g)/lipid fed (g).

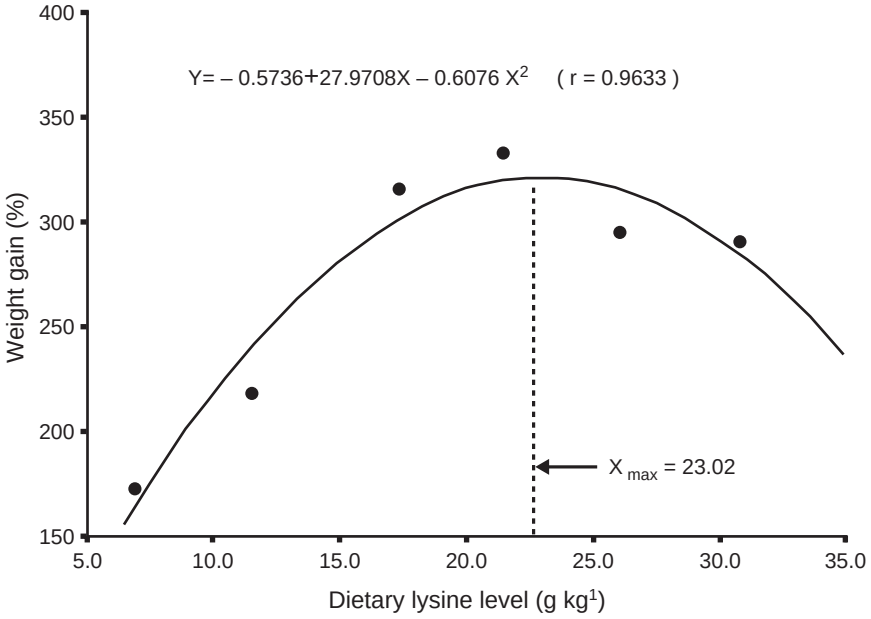

Fig. 1. Optimal dietary lysine requirement of grass carp juveniles based on the second-order polynomial regression model of weight gain versus dietary lysine level.

2.30% and 2.24% of the dry diet, corresponding to 6.05% and 5.89% of dietary protein.

3.2. Whole body, white muscle, liver composition and morphometry index

The proximate compositions of whole body, white muscle and liver of the grass carp fed the diets containing graded lysine level are shown in Table 4. Minimum body moisture and maximum body protein content was observed in fish fed 2.18% dietary lysine, which was not significantly different to those of fish fed diets 3, 5 and 6. Moisture and ash content of whole body decreased with increasing lysine levels up to 2.18%. Body lipid content showed the opposite trend with moisture content though without statistical difference among all the treatments.

Most of the fish had similar moisture and protein content of white muscle irrespective of dietary treatment except for the fish fed the diet 6. The fish fed diet containing 2.18% lysine had the lower liver moisture and higher liver lipid content than other treatments, which had significant difference with that of the fish fed with 0.71% lysine ($P<0.05$).

CF, HSI, MFI and VSI of juvenile grass carp fed graded levels of lysine are presented in Table 4. HSI and MFI were significantly affected by dietary lysine levels ($P<0.05$). HSI had a trend of increase from 2.2% to 2.6% as the dietary lysine level increased from 0.71% to 3.16% ($P<0.05$). With the exception of fish fed 0.71% lysine level MFI had no significant difference among treatments, but it also has the similar trend with HSI. Dietary lysine concentrations had no significant effect on CF and VSI.

3.3. ADCs of nutrients or energy and leaching ratio of test diets

Apparent digestibilities of dry matter, protein, lipid and energy and apparent availability of lysine provided in Table 5 were not significantly affected by the dietary lysine levels among treatments. The availability (92.37% on average) of lysine tended to increase with the dietary lysine level, but was not significant different among treatments. Leaching of total amino acid from diets (% of initial) after 2, 5 and 10 min immersion (means ± S.E.M., $n=6$) was 6.1%, 9.7% and 20.8%, respectively.

Table 4
Body composition and morphometry index of grass carp fed experimental diets with graded lysine levels for 90 days

Diet code	1	2	3	4	5	6
Lysine (%)	0.71	1.2	1.69	2.18	2.67	3.16
Composition (%)[1]						
Whole body						
Moisture	76.20 ± 0.22^{a}	75.77 ± 0.22^{ab}	74.76 ± 0.52^{c}	74.62 ± 0.28^{c}	75.02 ± 0.26^{bc}	75.01 ± 0.14^{bc}
Protein	12.80 ± 0.14^{c}	13.37 ± 0.24^{bc}	14.02 ± 0.10^{a}	14.14 ± 0.08^{a}	14.13 ± 0.14^{a}	13.91 ± 0.30^{ab}
Lipid	7.45 ± 0.13^{a}	7.57 ± 0.22^{a}	7.95 ± 0.30^{a}	7.88 ± 0.23^{a}	7.73 ± 0.15^{a}	7.90 ± 0.14^{a}
Ash	2.79 ± 0.11^{a}	2.74 ± 0.08^{a}	2.62 ± 0.07^{ab}	2.49 ± 0.04^{ab}	2.53 ± 0.04^{ab}	2.46 ± 0.02^{b}
Muscle						
Moisture	79.87 ± 0.31^{a}	79.85 ± 0.19^{a}	79.72 ± 0.11^{a}	79.74 ± 0.16^{a}	79.77 ± 0.09^{a}	79.47 ± 0.07^{a}
Protein	17.76 ± 0.30^{ab}	17.62 ± 0.22^{b}	17.55 ± 0.11^{b}	17.49 ± 0.08^{b}	17.78 ± 0.20^{ab}	18.28 ± 0.16^{a}
Lipid	0.95 ± 0.03^{b}	1.04 ± 0.06^{b}	1.30 ± 0.06^{a}	1.28 ± 0.02^{a}	1.25 ± 0.10^{a}	1.26 ± 0.04^{a}
Liver						
Moisture	62.34 ± 0.54^{a}	61.11 ± 0.32^{ab}	59.43 ± 1.39^{ab}	58.55 ± 1.43^{b}	59.07 ± 0.52^{ab}	59.34 ± 1.14^{ab}
Protein	13.42 ± 0.33^{a}	12.97 ± 0.24^{a}	13.00 ± 0.24^{a}	13.01 ± 0.13^{a}	13.06 ± 0.20^{a}	12.89 ± 0.04^{a}
Lipid	13.25 ± 0.42^{b}	14.57 ± 0.68^{ab}	17.82 ± 2.21^{ab}	18.48 ± 1.90^{a}	17.51 ± 0.90^{ab}	17.61 ± 1.55^{ab}
Morphometry[2]						
CF[3]	2.04 ± 0.02^{a}	2.06 ± 0.03^{a}	2.06 ± 0.02^{a}	2.06 ± 0.03^{a}	2.07 ± 0.03^{a}	2.08 ± 0.02^{a}
VSI[4]	10.33 ± 0.38^{a}	10.00 ± 0.24^{a}	10.34 ± 0.20^{a}	10.45 ± 0.24^{a}	10.10 ± 0.26^{a}	10.27 ± 0.17^{a}
HSI[5]	2.20 ± 0.08^{c}	2.36 ± 0.07^{bc}	2.47 ± 0.06^{ab}	2.47 ± 0.09^{ab}	2.48 ± 0.06^{ab}	2.60 ± 0.06^{a}
MFI[6]	2.22 ± 0.12^{b}	2.41 ± 0.10^{ab}	2.58 ± 0.08^{a}	2.66 ± 0.11^{a}	2.60 ± 0.11^{a}	2.76 ± 0.14^{a}

[1] Means ± S.E.M. of three replicates and values within the same row with different superscripts are significantly different ($P<0.05$).
[2] Means ± S.E.M. of 24 replicates.
[3] CF: condition factor = 100 × body weight (g)/body length $(cm)^3$.
[4] VSI: viscerasomatic index = 100 × viscerosomatic weight (g)/body weight (g).
[5] HSI: hepatopancreasomatic index = 100 × liver weight (g)/body weight (g).
[6] MFI: mesenteric fat index = 100 × mesenteric fat weight (g)/body weight (g).

3.4. Plasma biochemical analysis

The contents of total protein, cholesterol, triacylglycerol and glucose in plasma are provided in Table 6. In general, each metabolite tended to follow the same pattern as weigh gain, which increased with increasing dietary lysine level up to 2.18% of the diet. After that, total protein reached a plateau, cholesterol and triacylglycerol showed a slight decline whereas glucose continued to increase.

Table 5
Apparent digestibility of nutrients or energy of juvenile grass carp fed graded lysine level diets for 90 days[a]

Diet no.	Lysine level (%)	Dry matter (%)[b]	Protein (%)[c]	Lipid (%)[c]	Lysine (%)[c]	Energy (%)[c]
1	0.71	66.77 ± 1.93	89.38 ± 1.49	92.49 ± 0.79	90.34 ± 1.29	78.23 ± 1.53
2	1.20	67.92 ± 3.25	92.43 ± 0.74	92.03 ± 1.35	91.67 ± 0.93	79.82 ± 2.03
3	1.69	68.61 ± 0.32	92.24 ± 0.88	92.81 ± 0.49	92.04 ± 0.98	80.14 ± 1.07
4	2.18	69.98 ± 4.33	92.79 ± 1.77	92.45 ± 1.19	93.01 ± 1.83	80.37 ± 2.53
5	2.67	67.27 ± 1.19	90.36 ± 2.11	92.68 ± 1.34	93.49 ± 2.34	79.46 ± 1.88
6	3.16	66.72 ± 0.74	91.88 ± 0.83	92.06 ± 0.96	93.65 ± 2.27	79.19 ± 0.97

[a] Means ± S.E.M. of three replicates and values within the same column have no significant difference ($P>0.05$).
[b] ADC of dry matter (%) = 100 × [1 − (dietary Y_2O_3)/fecal Y_2O_3].
[c] ADCs of nutrients or energy (%) = 100 × [1 − (F/D × DY/FY)], where F is the percent of nutrients or energy in feces, D is the percent of nutrients or energy in diet, DY is the percent of Y_2O_3 in diet, and FY is the percent of Y_2O_3 in feces.

Table 6
Biochemical compositions of plasma from juvenile grass carp fed graded lysine level diets for 90 days

Diet no.	Lysine level (%)	Total protein (g/l)	Cholestorol (mmol/l)	Triacyglycerol (mmol/l)	Glucose (mmol/l)
1	0.71	37.67 ± 1.20^{b}	6.54 ± 0.04^{b}	4.15 ± 0.12^{c}	6.37 ± 0.15^{b}
2	1.20	38.67 ± 1.20^{b}	7.05 ± 0.64^{b}	4.30 ± 0.16^{bc}	6.80 ± 0.17^{ab}
3	1.69	40.33 ± 2.18^{ab}	8.84 ± 0.55^{a}	4.91 ± 0.41^{abc}	7.10 ± 0.46^{ab}
4	2.18	44.67 ± 0.88^{a}	9.86 ± 0.41^{a}	5.56 ± 0.30^{a}	7.05 ± 0.35^{ab}
5	2.67	43.00 ± 2.52^{ab}	9.73 ± 0.85^{a}	4.68 ± 0.29^{abc}	7.57 ± 0.19^{a}
6	3.16	44.33 ± 1.45^{a}	9.00 ± 0.34^{a}	5.16 ± 0.34^{ab}	7.63 ± 0.27^{a}

Means ± S.E.M. of three replicates and values within the same column with different superscripts are significantly different ($P<0.05$).

3.5. Estimated dietary amino acid requirements based on A/E ratio

The estimated requirements for the other essential amino acid were calculated from *A*/*E* ratio data based on the lysine requirement determined in the current experiment (Table 7).

4. Discussion

The growth performance measured as weight gain, SGR and FE increased linearly with increasing level of dietary lysine up to 2.18% of the diet, after which it decreased as the lysine level increased. Since the weight gain response of fish in the current study was curvilinear, a second-order polynomial regression-analysis method resulted in the lowest error term for estimating the requirement appeared to give a more precise empirical figure.

Table 7
Estimated dietary amino acid requirements of juvenile grass carp based on lysine requirement and whole-body amino acid profile

EAAs	Estimated requirements (% of dietary protein)[a]	*A*/*E* ratios[b]
Lys	5.89	179.3
Arg	3.47	105.6
His	1.87	56.9
Ile	2.84	86.3
Leu	5.14	156.4
Met+Cys	2.65	80.8
Phe+Tyr	4.52	137.6
Thr	2.50	76.2
Trp	0.72	21.8
Val	3.25	99.0

[a] Estimated requirements for amino acid except for lysine are calculated as follows: requirement = lysine requirement from this study × (*A*/*E* ratio / 179.3).

[b] *A*/*E* ratio = 1000 × (essential amino acid content / total essential amino acid contents plus cystine and tyrosine).

The optimal dietary lysine requirement for juvenile grass carp was estimated to be 2.24% of the dry diet, corresponding to 5.89% of protein. This value is similar to values reported for certain other species, such as common carp (5.70%, Nose, 1979), red drum (5.7%, Brown et al., 1988), rohu (5.88%, Khan and Jafri, 1993; 5.68%, Murthy and Varghese, 1997), African catfish (5.7%, Fagbenro et al., 1998), *C. mrigalared* (5.75%, Ahmed and Khan, 2004), and lower than that for catla (6.20%, Ravi and Devaraj, 1991) and Atlantic salmon (6.1%, Rollin, 1999). Such results further supported that carps seemed to have higher lysine requirement than most other fishes with a lysine requirement of 4–5% of protein (Wilson, 2002).

SGR of juvenile grass carp in the present experiment ranged from 1.11 to 1.63, which was comparatively lower than that reported by Dabrowski (1977) for the same species. Reduced SGR is often observed in fish fed amino acid test diets (Wilson, 2002). The relative poor growth rate may be the main reason for the low feed efficiency. Crystalline amino acids are absorbed from the digestive tract faster than protein-bound amino acids, which may lead to the poorer utilization of free amino acid than that from intact protein (Yamada et al., 1981; Murai et al., 1982; Ronnestad et al., 2000). The disproportionate absorption rates may cause an imbalance in the amino acid profile in the tissue and diverting amino acids into catabolic rather than anabolic processes, as has been suggested by Yamada et al. (1981). Pre-coating the amino acids with carboxymethylcelloluse (CMC) and further binding the diets with both CMC and κ-carrageenan not only likely reduced the leaching of lysine

(Alam et al., 2004), but also may have helped to delay the passage time and absorption of amino acids. The results of the digestibility data of the experimental diets and the availability of lysine confirmed that the amino acids provided in the experimental diets were mostly absorbed. Diets were fed in the current study in small frequent portions, which may have improved the utilization of crystalline amino acids. European sea bass can utilize large amounts of free amino acids when included in diets containing intact proteins and when the feeding frequency was high (Tibaldi and Lanari, 1991).

All the protein digestibility values were higher than 89.38% and averaging 91.53%, indicating that all the nitrogen sources of experimental diets were well utilized by the juvenile grass carp in the present study. When the lysine availability of the protein sources used in the present study were considered, the optimal lysine requirement of juvenile grass carp was calculated to be (2.24×0.9237) 2.07% of the diet, corresponding to 5.44% of the dietary protein.

On average, the diets fed at 0.5% body weight per feeding was consumed in less than 5 min by the fish. The leaching rate of 9.7%, which was similar to the value reported by Alam et al. (2004), showed that at least 90% or more of the dietary total amino acid should have been consumed up by the fish. This observation indicates that the leaching loss of lysine could be considered to be negligible and the relative poor growth was not due to leaching. When taking leaching of crystalline amino acids into consideration, the actual lysine requirement maybe a little lower than the estimated value.

Increasing the lysine level up to the requirement increased protein efficiency ratio and nitrogen retention. This observation can be attributed to an increase of net protein synthesis with the increase in dietary lysine level and remain unchanged thereafter (Ruchimat et al., 1997). Changes in the proximate composition data also tended to support an optimum nutritional status which corresponded to the optimum dietary lysine level. The amino acids in excess from the disproportionate absorption rates or the part of lysine beyond optimal level would be no longer used for increasing weight gain and transformed into body protein or protein synthesis, but was deaminated to catabolism and provided the carbon skeletons used in lipid synthesis and deposited as tissue fat, mainly at mesentery, which may be confirmed by the higher body lipid content and MFI in the fish fed diets containing high concentrations of free lysine, as also reported in freshwater catfish (Chatzifotis et al., 1996; Tantikitti and Chimsung, 2001).

To our knowledge, there is little information on the effects of dietary lysine on plasma total protein, cholesterol, triacylglycerol and glucose in teleosts except for yellowtail (Ruchimat et al., 1997). The decrease in whole-body fat content along with the decrease in plasma triacylglycerol concentrations suggests lipid mobilization in those groups exhibiting very poor growth (Regost et al., 1999), which was observed in the present investigation. Tissue cholesterol concentrations are known to vary depending on the nutritional status of fish (Kaushik et al., 1995; Regost et al., 1999). Compared with data from grass carp, plasma cholesterol concentrations were comparable to that of rainbow trout (Kaushik et al., 1995), but higher in yellowtail (Ruchimat et al., 1997).

A high correlation between muscle or whole-body amino acid profile and amino acid patterns for fish has been demonstrated by several investigators (Arai, 1981; Cowey and Tacon, 1983; Ogata et al., 1983; Wilson and Poe, 1985; Borlongan and Coloso, 1993; Mambrini and Kaushik, 1995). A/E ratios have been used as a method of estimating the requirements of all essential amino acids when only one is known by relating the A/E ratio of each EAA to that of the A/E ratio of the known amino acid times the requirement value for the known amino acid (Moon and Gatlin, 1991; Akiyama et al., 1997; Wilson, 2002). Such useful technique has been applied to other species, such as *Clarias hybrid* (Unprasert, 1994), striped bass (Brown, 1995; Small and Soares, 1998), Japanese flounder and red sea bream (Forster and Ogata, 1998). In the present study, the value (2.65%) of methionine from the estimated requirement relative to A/E ratios was only slightly lower than that based on a growth study (Wang et al., unpublished data).

In conclusion, results of the present investigation indicate that the lysine requirement of grass carp is similar to that of other carp species and little higher than certain other species. Based on value from best growth group and second-degree polynomial regression analysis of the WG and FE data, 2.24% of the

dry diet, corresponding to 5.89% of dietary protein is recommended for optimum growth of juvenile grass carp. When the lysine availability (92.37%) of the protein sources was considered, the optimal lysine requirement of juvenile grass carp was calculated to be 2.07% of the diet, corresponding to 5.44% of the dietary protein. If leaching of crystalline amino acids is taken into consideration, the actual lysine requirement might be a little lower than the estimated value. The estimated requirements for the other essential amino acids calculated from *A*/*E* ratios based on the lysine requirement will provide useful information to formulate more cost-effective and amino acid balanced diets for the grass carp.

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