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Dietary L-lysine requirement of juvenile Chinese sucker, *Myxocyprinus asiaticus*

Yucong Lin[1], Yuan Gong[2], Yongchao Yuan[1,2], Shiyuan Gong[1,2], Denghang Yu[2], Qiang Li[2] & Zhi Luo[2]

[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Fishery, Huazhong Agricultural University, Wuhan, China

[2]Nutrition Laboratory, College of Fishery, Huazhong Agricultural University, Wuhan, China

Correspondence: Y Yuan and S Gong, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, China. E-mails: yuanyc2007@yahoo.com.cn; gsy@mail.hzau.edu.cn

Abstract

An 8-week feeding trial was conducted to quantify the dietary L-lysine requirement of juvenile Chinese sucker with an initial weight of 1.81 ± 0.04 g reared in indoor flow-through and aerated tanks. Six isonitrogenous and isoenergetic practical diets were formulated to contain graded levels of lysine (1.23%, 1.80%, 2.39%, 2.98%, 3.56% and 4.18% dry matter) at 0.6% increments from dietary ingredients and crystalline L-lysine. Each diet was randomly assigned to triplicate groups of 30 fish each and was fed to apparent satiation by hand three times a day (09:00, 13:00 and 17:00 hours) for 8 weeks. There were significant differences in growth performance and feed utilization among the treatments. Weight gain (WG), specific growth rate and protein efficiency ratio (PER) significantly increased with increasing lysine levels up to 2.39% of diet ($P < 0.05$) and remained nearly the same thereafter ($P > 0.05$). Feed efficiency was the poorest for fish fed the lowest lysine diet ($P < 0.05$) and showed no significant differences when dietary lysine level increased from 2.39% to 4.18%. The N retention (% N intake) significantly increased with dietary lysine level but did not attain a plateau ($P < 0.05$). Survival could not be related to dietary treatments. Whole body protein increased ($P < 0.05$) and whole body lipid decreased ($P < 0.05$) with increasing dietary lysine level. The condition factor and hepatosomatic index were significantly affected by dietary lysine levels, however, viscersomatic index, whole body moisture and ash did not differ significantly among dietary treatments. Broken-line analysis on the basis of WG and PER showed that dietary lysine requirements of juvenile Chinese sucker were 2.43% and 2.40% dry diet (5.52% and 5.45% dietary protein) respectively. Based on the ideal protein approach and the A/E ratios determined from muscle amino acid profile an estimation of the EAA requirements of Chinese sucker juveniles were calculated.

Keywords: juvenile Chinese sucker (*Myxocyprinus asiaticus*), growth performance, lysine requirement, A/E ratio

Introduction

Formulation of balanced and cost-effective diets requires complete knowledge of nutritional requirements of the cultured species. All fish species studied to date have been shown to require 10 indispensable amino acids in their diet for maximum growth (Wilson 1985). Lysine is of particular concern because it is the indispensable amino acid found in the highest concentration in the carcass of many species of fish (Wilson & Cowey 1985; Wilson & Poe 1985; NRC 1993). Moreover, lysine is often the most limiting indispensable amino acid in the ingredients commonly used in formulating feed for fish (Harris 1980; Forster & Ogata 1998; Small & Soares 2000; Tantikitti & Chimsung 2001). It serves along with methionine as a precursor to carnitine that is involved in the transportation of long chain fatty acyl groups into the mitochondria for beta oxidation (Walton, Cowey & Adron 1984). Furthermore, hydroxylysine and hydroxyproline products of lysine and proline hydroxylation, respectively, are the main constituents of collagen (Sandell & Daniel 1988). In addition, quantitative EAA requirements have been mostly established by dose–response studies, based on animal growth or nitrogen retention response to increasing dietary levels of the EAA under study (Shearer 2000).

Chinese sucker, *Myxocyprinus asiaticus*, is an endemic freshwater fish and has been listed as Class II of protected animals in China (Wang & Xie 2004). Furthermore, the species are also an important commercial fish that have been widely cultured in China because of its delicious meat and rapid growth. Traditional culture of this species mainly depends on chopped or minced trash fish and oligochetes worms, *Limnodrilus hoffmeisteri*, which are difficult to store, easy to deteriorate water quality and may result in the spread of diseases (Yuan, Gong, Yang, Lin, Yu & Luo 2010b). It has been reported that Chinese sucker juveniles showed good performance (Zhou, Yang, Wu, Wang, Liu & Chen 1999; Wan, Lai, Liu, Shen, Sun, Chen & Wang 2006) when fed with commercial eel diets (crude protein 45%) which is close to their optimum dietary protein of 46.5% (Zhang, Gong, Yuan, Chu & Yuan 2009). However, intensive culture of Chinese sucker with commercial eel diets has readily led to excess fat deposition in the viscera of the fish, leading to morbidity in some cases (Yuan, Gong, Luo, Yang, Zhang & Chu 2010a). This indicates the necessities of developing cost-effective and environmental friendly diets for the species. Up to now, only a few studies have been conducted on the nutrient requirements of this fish species (Zhang *et al.* 2009; Yuan, Yang, Gong, Luo, Yuan & Chen 2010c; Yuan *et al.* 2010a,b). To our knowledge, no information has been published concerning the lysine requirements of this species. Therefore, the present investigation was undertaken to determine the optimum dietary L-lysine requirement for juvenile Chinese sucker. Based on the determined lysine requirement an estimation of the EAA requirements was calculated according to the ideal protein concept.

Materials and methods

Experimental diets

The amino acid composition of the ingredients used in the experimental diets is presented in Table 1. The basal diet was formulated to contain a combination of fish meal, corn gluten meal, soybean meal as the intact protein sources (Table 2). Experimental diets (Table 2) were formulated to contain 44% crude protein which is slightly lower than the optimum protein requirement (46.5%, Zhang *et al.* 2009) to assure maximum utilization of the limiting amino acid (Wilson 1989). Dietary lysine was

Table 1 Amino acid composition (g 100 g^{-1} dry diet) of dietary ingredients for experimental diets

Amino acids	Supplied by 10% fishmeal	Supplied by 15% soybean meal	Supplied by 40% corn gluten meal	Supplied by CAA	Total	44% whole body protein
EAA						
Arg	0.43	0.45	0.59	1.11	2.58	2.58
His	0.14	0.16	0.36	0.17	0.83	0.83
Ile	0.25	0.28	0.74	0.33	1.60	1.60
Leu	0.49	0.50	3.36	0	3.35	2.91
Lys	0.48	0.42	0.32	Variable	Variable	3.32
Met	0.20	0.06	0.43	0.49	1.18	1.18
Phe	0.26	0.33	1.22	0	1.81	1.70
Thr	0.29	0.25	0.66	0.34	1.54	1.54
Val	0.32	0.29	0.84	0.33	1.78	1.78
NEAA						
Ala	0.40	0.28	1.74	0.38	2.80	2.80
Asp	0.61	0.72	1.18	1.04	3.55	3.55
Cys	0.08	0.08	0.22	0	0.38	0.31
Glu	0.91	1.21	4.47	Variable	Variable	6.19
Gly	0.49	0.27	0.51	2.16	3.43	3.43
Ser	0.33	0.33	1.02	0.50	2.18	2.18
Tyr	0.22	0.20	0.93	0	1.35	1.21

CAA, crystalline amino acids; EAA, essential amino acids; NEAA, non-essential amino acids.

Table 2 Composition and proximate analysis of the experimental diets (% dry matter)

	Diet no.(%)					
Ingredients (%)	Diet 1 (1.23)	Diet 2 (1.80)	Diet 3 (2.39)	Diet 4 (2.98)	Diet 5 (3.56)	Diet 6 (4.18)
Fish meal*	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal†	15.00	15.00	15.00	15.00	15.00	15.00
Corn gluten meal‡	40.00	40.00	40.00	40.00	40.00	40.00
Corn starch	6.70	6.70	6.70	6.70	6.70	6.70
Mixed amino acids	6.85	6.85	6.85	6.85	6.85	6.85
Fish oil	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix§	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix¶	2.00	2.00	2.00	2.00	2.00	2.00
Betaine	0.30	0.30	0.30	0.30	0.30	0.30
Antimold**	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
CMC	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.00	0.60	1.20	1.80	2.40	3.00
Glutamic acid	3.00	2.40	1.80	1.20	0.60	0.00
Proximate analysis (*n* = 3)						
Lysine (% DM)	1.23	1.80	2.39	2.98	3.56	4.18
Crude protein(% DM)	44.4	44.0	44.2	44.5	43.9	43.9
Crude lipid (% DM)	11.8	12.2	12.5	12.4	12.4	12.5
Ash (% DM)	6.3	6.2	6.2	6.1	6.2	6.1

*Gao Long Diary Company, Wuhan, China, Imported from USA.
†Soybean meal obtained from Gao Long Diary Company, Wuhan, China.
‡Corn gluten meal obtained from Xin Wang Diary Company, Wuhan, China.
§Mineral premix(mg or g kg^{-1} diet): NaF, 3 mg; KI, 1.2 mg; $CoCl_2$-$6H_2O$(1%), 70 mg; $CuSO_4$-$5H_2O$, 15 mg; $FeSO_4$-H_2O, 110 mg; $ZnSO_4$-H_2O, 70 mg; $MnSO_4$-H_2O, 80 mg; $MgSO_4$-$7H_2O$, 1600 mg; $Ca(H_2PO_3)_2$-H_2O, 1000 mg; NaCl, 120 mg; Zoelite, 17.45 g.
¶Vitamin premix(mg or g kg^{-1} diet):thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 40 mg; vitamin B_{12}, 0.2 mg; vitamin K_3, 20 mg; inositol, 800 mg; pantothenic acid, 120 mg; niacin acid, 400 mg; folic acid, 40 mg; biotin, 2.40 mg; retinol-acetate, 64 mg; cholecalciferol, 10 mg; alpha-tocopherol, 240 mg; ascorbic acid, 4000 mg; wheat middling, 36.78 g.
**Anitimold: 50% calcium propionic acid and 50% fumaric acid.
CMC, carboxymethycellulose.

quantitatively increased at the expense of glutamic acid. L-Crystalline amino acids mixture was used to simulate the whole body amino acid pattern of Chinese sucker except for lysine. The basal diet contained 1.23% of lysine on a dry diet basis (Table 2). Crystalline L-lysine was added to the basal diet by an increasing trend, 0.0% to 3.0% at 0.6% increments of all six levels, and the analysed data by automatic amino acid analyser were 1.23%, 1.80%, 2.39%, 2.98%, 3.56%, 4.18% respectively. The range of dietary lysine content covered the lysine level (3.32%) in 44% crude protein from the whole body tissue of this species (Tables 2).

The diets were prepared as described by Yuan *et al.* (2010b). Before mixing with the other ingredients, the crystalline-AA mixture was mixed with CMC, to delay crystalline-AA absorption from the digestive tract to some extent. All dry ingredients were finely grounded, weighed and mixed manually for 5 min, and then transferred to a food mixer for another 30 min mixing. Soya lecithin was added to a preweighed premix of fish oil and soybean oil, and mixed until homogenously distributed. The oil mix was then slowly added to the food mixer while mixing was continued. All ingredients were mixed for another 10 min, and then distilled water was added (40%, v/w) to form a dough. Diets were pelleted by passing the dough through an experimental feed mill [F-26(II), South China University of Technology, China] and dried for about 12 h in a ventilated oven at 45°C. After drying, the diets were broken up and sieved into proper pellet sizes (1.0 × 0.75 mm). All diets were sealed in bags and stored at −20°C until used.

Experimental procedure

Five hundred and forty juvenile *M. asiaticus* were obtained from a commercial hatchery (Wuhan, Hubei Province, China) and were reared in 1600-L indoors flow-through circular fibreglass tanks provided with sand-filtered aerated freshwater for 14 days to acclimate to the experimental conditions. During the period of acclimatization, Chinese sucker juveniles were fed the diet containing the lowest lysine formulated in the present experiment. At the beginning of the trial, 30 uniform-sized fish (mean initial weight: 1.81 ± 0.04 g, mean ± SD) in good health and condition were stocked in 18, 200-L indoor flow-through circular fibreglass tanks provided with sand-filtered aerated freshwater. Each diet was assigned to three tanks in a completely randomized design (3 replicates × 6 diets = 18 tanks total). The fish were hand-fed little by little to satiation (09:00, 13:00 and 17:00 hours) to prevent waste of dietary pellets, feed consumption by the fish in each tank was recorded daily. Faecal matter was removed before each morning feeding. Tanks were thoroughly cleaned every 2 weeks when the fish were removed for weighing.

During the experimental period, a 12 h light: 12 h dark photoperiod was maintained, dissolved oxygen content was approximately 6.6 mg L^{-1}; pH 7.5–8.4. Temperature ranged from 26 to31°C. The trial lasted for 8 weeks, during this period, the number and weight of dead fish were recorded. At the end of the trial, the fish were fasted for 24 h and fish in each tank were weighed and counted.

Sample collections and analysis

For the experimental diets to simulate the amino acid pattern of the whole fish, 20 juveniles (with 1.81 g mean body weight) were sampled from a farm, cut into pieces, pooled and homogenized for amino acid analysis before the beginning of the trial. Twenty fish at the start and 10 fish per tank at the termination of the feeding trial were sampled and stored frozen at −70°C for subsequent analysis of whole body composition. The feed ingredients, experimental diets and fish samples were analysed in triplicate for dry matter, crude protein, crude lipid and ash using standard methods of AOAC (1995). Samples of diets and fish were dried to a constant weight at 105°C for the determination of dry matter. Crude protein (N × 6.25) was determined using the Kjeldahl method after an acid digestion using an auto-Kjeldahl System (2300 Auto analyzer; Foss Tecator. AB, Hoganas, Sweden). Crude lipid was determined by ether extraction method using Soxhlet. Ash was determined using a muffle furnance at 550°C.

Five fish per tank were individually weighed, and the livers and viscera were weighed for calculation of hepatosomatic index (HSI) and viscerosomatic index (VSI). The body length and weight of fish were used to calculate the condition factor (CF) of fish. Dorsal muscles of fish were sampled, sealed in plastic bags and stored frozen (−70°C) until to evaluate the effect of dietary treatments on the amino acid profile of fish muscle. Muscle tissue used for analysis of amino acid content was freeze-dried at −55°C for 48 h. The amino acid content of the ingredients and experimental diets was determined by using an automatic amino acid analyser (Hitachi L-8800, Tokyo, Japan), after acid hydrolysis using 6N HCl for 22 h at 110°C in glass tubes under vacuum. Tryptophan could not be measured because of its degradation during acid hydrolysis. All chemical analyses (from each tank) were done in duplicate.

Calculations and statistical analysis

A/E ratios were calculated for each EAA as:

$$A/E = [(EAA/\text{total EAA}) \times 1000]$$

And the requirement for EAA other than lysine was estimated as:

$$\text{EAA requirement} = (\text{requirement for lysine} \times \text{specific A/E ratio}) / \text{A/E ratio for lysine}$$

All data were subjected to one-way analysis of variance using SPSS 16.0 for windows (SPSS, Chicago, IL, USA). When overall differences were significant ($P < 0.05$), Tukey's test was used to compare means among individual treatments (Zar 1984). The optimum dietary lysine requirements based on WG, PER were estimated using broken-line model ($Y = a+bX$) (Robbins, Norton & Baker 1979) or second-order polynomial model ($Y = a+bx+cx^2$) (Zeitoun, Ullrey, Magee, Gill & Bergen 1976) and asymptotic model ($Y = u+v(1-e^{wx})$) (Robbins *et al.* 1979). Based on the coefficient of determination (R^2) among the three models, the one giving maximum of R^2 was chosen as the best-fit model.

Results

Growth performance, feed utilization and survival of juvenile Chinese sucker given graded levels of

Table 3 Growth performance of Chinese sucker fed diets with graded levels of lysine for 8 weeks

	Diets no.					
	1 (1.23)	2 (1.80)	3 (2.39)	4 (2.98)	5 (3.56)	6 (4.18)
IBW	1.81 ± 0.04	1.81 ± 0.06	1.81 ± 0.05	1.81 ± 0.03	1.81 ± 0.04	1.81 ± 0.04
FBW	7.57 ± 0.13c	8.60 ± 0.12b	10.58 ± 0.15a	10.61 ± 0.10a	10.50 ± 0.15a	10.42 ± 0.12a
WG	318.86 ± 7.12c	375.87 ± 6.65b	485.66 ± 8.52a	487.32 ± 5.45a	481.23 ± 8.38a	476.99 ± 6.60a
FI	11.33 ± 0.25b	11.48 ± 0.09b	11.90 ± 0.05a	12.06 ± 0.06a	12.07 ± 0.07a	11.98 ± 0.12a
SGR	2.56 ± 0.03c	2.79 ± 0.02b	3.16 ± 0.03a	3.16 ± 0.02a	3.14 ± 0.03a	3.13 ± 0.02a
PER	1.17 ± 0.02c	1.34 ± 0.03b	1.68 ± 0.02a	1.67 ± 0.02a	1.64 ± 0.02a	1.64 ± 0.04a
NR	16.34 ± 0.59d	19.40 ± 0.63c	25.28 ± 0.53b	26.14 ± 0.50ab	26.64 ± 0.43ab	27.54 ± 0.51a
FCR	1.93 ± 0.02a	1.70 ± 0.04b	1.36 ± 0.03c	1.36 ± 0.04c	1.39 ± 0.01c	1.39 ± 0.03c
Survival	95.56 ± 1.92	96.67 ± 3.33	96.67 ± 0.00	98.89 ± 1.92	97.78 ± 1.92	98.89 ± 1.92

Data represents mean ± SD of three replicates and values in the same column with different letters are significantly different determined by Tukey's test ($P < 0.05$); IBW (g fish^{-1}), initial mean body weight; FBW (g fish^{-1}), final mean body weight; WG (weight gain, %) = 100 × (final mean weight − initial mean weight)/initial mean weight; SGR (specific growth rate, % day^{-1}) = 100 × [ln (final mean weight) − ln (initial mean weight)]/days; PER (protein efficiency ratio) = wet weight gain in g/protein intake in dry basis in g; FCR (feed conversion ratio) = dry diet fed in g/wet weight gain in g; FI (g fish^{-1}) = Feed intake; Nitrogen retention (% nitrogen intake) = ((FBW×FBN − IBW×IBN)/(NI)) × 100 where IBW and FBW were initial and final body weights; IBN and FBN were initial and final body nitrogen and NI was nitrogen intake.

L-lysine for 8 weeks are shown in Table 3. Fish promptly accepted the experimental diets from the beginning and maintained normal behaviour throughout the experiment period. Mean survival rates were generally high in all treatments, ranging from 96% to 99% and was unaffected by the dietary lysine level ($P > 0.05$). There was no evidence of any external pathological signs in fish given diets that contained low levels of lysine. Feed intake (FI) was the lowest for fish fed with 1.23% lysine of diet and showed no significant difference as the dietary lysine increased from 2.39% to 4.18% ($P > 0.05$). Weight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) increased significantly when dietary lysine was increased from 1.23% to 2.39% ($P < 0.05$), but remained relatively constant from 2.39% to 4.18% dietary lysine ($P > 0.05$). Nitrogen retention (% N intake) did not attain a plateau, increasing with the increase in dietary lysine level therefore not allowing an estimation of lysine requirement for maximum N retention. To choose the best-fit model for describing the relationship between WG (PER) and dietary lysine level, we compared the broken-line model with non-linear model (second-order polynomial model and asymptotic model) based on the values of R^2. The R^2 values for WG were 0.9637, 0.9344 and 0.9018 for broken-line, second-order polynomial and asymptotic models respectively. Similarly, for PER, these values were 0.9593, 0.9141 and 0.8842 for broken-line, second-order polynomial and asymptotic models respectively. Based on the calculated R^2, the broken-line model with the best fit in the two cases was chosen. Broken-line regression analysis showed that, based on WG and PER, the optimum requirement of dietary lysine for growth of Chinese sucker was 2.43% and 2.40% of dry diet (5.52% and 5.45% of protein) respectively (Figs 1 and 2). The FCR decreased significantly with increasing dietary lysine from 1.23% to 2.39% and then showed no significant difference as the lysine level increases from 2.39% to 4.18%, while the highest FCR was observed for fish fed with the diet containing 1.23% lysine.

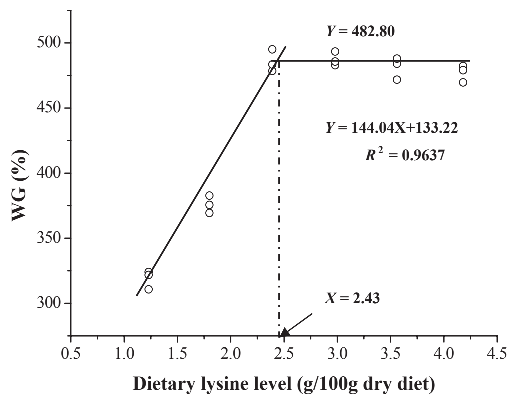

Figure 1 Optimal dietary lysine requirement of juvenile Chinese sucker based on broken-line model of Weight gain (WG) versus dietary lysine level.

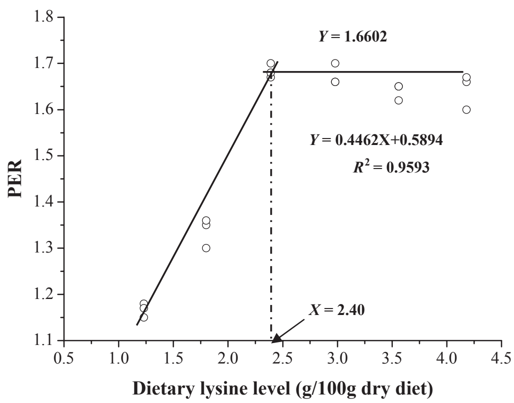

Figure 2 Optimal dietary lysine requirement of juvenile Chinese sucker based on broken-line model of Protein efficiency ratio (PER) versus dietary lysine level.

Table 4 Condition factor (CF), viscersomatic index (VSI) and hepatomatic index (HSI) of the Chinese sucker fed different levels of dietary lysine

Lysine level (%)	HSI (%)	VSI (%)	CF (%)
Diet 1 (1.23)	1.54 ± 0.07b	7.79 ± 0.08	2.17 ± 0.04b
Diet 2 (1.80)	1.72 ± 0.15ab	7.96 ± 0.71	2.24 ± 0.06ab
Diet 3 (2.39)	1.75 ± 0.06ab	7.96 ± 0.13	2.32 ± 0.03a
Diet 4 (2.98)	1.76 ± 0.05ab	7.73 ± 0.34	2.31 ± 0.03a
Diet 5 (3.56)	1.85 ± 0.10a	7.81 ± 0.73	2.32 ± 0.05a
Diet 6 (4.18)	1.78 ± 0.09ab	7.54 ± 0.63	2.30 ± 0.04a

Data represent mean ± SD of three replicates. Values in the same column with different letters are significantly different determined by Tukey's test ($P < 0.05$). Hepatosomatic index (HSI, %) = 100 × (liver weight, g)/(whole body weight, g).
Viscersomatic index (VSI, %) = 100 × (viscera weight, g)/(whole body weight, g).
Condition factor (CF, %) = 100 × (body weight, g)/(body length, cm)^3.

The CF and HSI were significantly affected by dietary lysine levels (Table 4); condition factor was the lowest for fish fed with the lowest lysine diet ($P < 0.05$), but remained nearly the same for other treatments ($P > 0.05$) (Table 4). The VSI showed no significant differences among the treatments ($P > 0.05$). The HSI of 3.56% treatments was significantly higher than that of fish fed with the lowest lysine diet ($P < 0.05$). Whole body moisture and ash were not affected by dietary lysine level, but protein content in whole body ranged from 14.04% to 15.76% was positively correlated with dietary lysine level ($P < 0.05$), while lipid content in whole body was negatively correlated with it. The EAA contents of muscle increased significantly with increasing dietary lysine level ($P < 0.05$) from 1.23% to 2.39%, and then showed no significant difference from 2.39% to 4.18%, the same trend was seen with lysine contents of muscle (Tables 5 and 6). The estimated requirements for the other essential amino acid were calculated from A/E ratio data based on the lysine requirement (mean value of two optimum dietary lysine requirements determined by WG and PER) calculated in the present study (Table 7).

Discussion

In the current study, supplementing lysine to the diets significantly improved growth performance of Chinese sucker, which indicates that lysine is essential for the growth of Chinese sucker and the crystalline lysine can also be used by this species. Broken-line regression analysis was employed to establish the relationship between WG (PER) and dietary lysine. Based on WG and PER, the optimum requirements of dietary lysine for Chinese sucker were 2.43% and 2.40% of dry diet (5.52% and 5.45% of dietary protein) respectively. However, when taking the leaching of the crystalline amino acids and digestibility of the dietary ingredients into consideration, the actual lysine requirement of the fish maybe a little lower than the estimated value. In the present study, feeds were

Table 5 Final whole body composition of Chinese sucker fed six diets with graded levels of lysine for 8 weeks

Lysine level (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Moisture (%)
Diet 1 (1.23)	14.04 ± 0.36c	5.80 ± 0.21a	2.89 ± 0.09	75.32 ± 1.01
Diet 2 (1.80)	14.33 ± 0.16bc	5.38 ± 0.05b	2.96 ± 0.09	76.30 ± 0.41
Diet 3 (2.39)	15.02 ± 0.57ab	5.24 ± 0.11b	2.87 ± 0.06	75.28 ± 1.09
Diet 4 (2.98)	15.13 ± 0.24ab	5.10 ± 0.14bc	2.89 ± 0.12	75.94 ± 0.47
Diet 5 (3.56)	15.40 ± 0.23a	4.91 ± 0.04cd	2.82 ± 0.21	76.83 ± 0.56
Diet 6 (4.18)	15.76 ± 0.30a	4.74 ± 0.06d	2.73 ± 0.20	76.49 ± 0.45

Values are means ± SD of three replicates. Values within the same column sharing a common letter are not significantly different determined by Tukey's test ($P > 0.05$).

Table 6 The essential amino acid profile in the muscle of Chinese sucker fed diets with graded levels of 8 weeks (g per 16 g N)

	Dietary lysine levels					
EAA	Diet 1 (1.23)	Diet 2 (1.80)	Diet 3 (2.39)	Diet 4 (2.98)	Diet 5 (3.56)	Diet 6 (4.18)
Arg	4.25 ± 0.03c	4.27 ± 0.04c	4.56 ± 0.08a	4.53 ± 0.05a	4.53 ± 0.08ab	4.37 ± 0.05bc
His	1.62 ± 0.03b	1.63 ± 0.02bc	1.73 ± 0.01ab	1.78 ± 0.04a	1.77 ± 0.05a	1.77 ± 0.05a
Ile	2.95 ± 0.02	2.95 ± 0.03	3.00 ± 0.04	3.04 ± 0.05	3.05 ± 0.05	2.98 ± 0.04
Leu	4.95 ± 0.02	4.97 ± 0.05	4.95 ± 0.03	4.97 ± 0.04	4.95 ± 0.02	4.97 ± 0.03
Lys	7.19 ± 0.07b	7.19 ± 0.05b	7.46 ± 0.05a	7.50 ± 0.03a	7.53 ± 0.04a	7.51 ± 0.03a
Met	2.32 ± 0.04c	2.43 ± 0.05ab	2.43 ± 0.02ab	2.42 ± 0.02b	2.51 ± 0.03a	2.46 ± 0.02ab
Phe	1.37 ± 0.02	1.38 ± 0.02	1.40 ± 0.02	1.40 ± 0.03	1.39 ± 0.02	1.40 ± 0.03
Thr	3.37 ± 0.02b	3.41 ± 0.02b	3.62 ± 0.03a	3.59 ± 0.03a	3.61 ± 0.04a	3.63 ± 0.04a
Val	3.49 ± 0.03b	3.52 ± 0.02b	3.76 ± 0.02a	3.78 ± 0.06a	3.80 ± 0.02a	3.76 ± 0.04a
ΣEAA	31.10 ± 0.14c	31.72 ± 0.16b	32.91 ± 0.11a	33.11 ± 0.20a	33.14 ± 0.11a	32.85 ± 0.13a

Values are means ± SD of three replicates. Values within the same column sharing a common letter are not significantly different determined by Tukey's test ($P > 0.05$).

Table 7 Estimated dietary amino acid requirements of juvenile Chinese sucker based on lysine requirement (mean value of two optimum dietary lysine requirements determined by WG, PER) and muscle amino acid profile

	EAA composition* (g 16 g^{-1} N)	A/E ratio†	EAA requirements (% of dietary protein)‡
Arg	4.50	134.21	3.29
Lys	7.50	223.68	5.49
His	1.76	52.49	1.29
Ile	3.02	90.07	2.21
Leu	4.96	147.93	3.63
Val	3.77	112.44	2.76
Met+cys	2.96	88.28	2.16
Phe+Tyr	1.45	43.24	1.06
Thr	3.61	107.66	2.64
Trp	nd	nd	nd

*Averaged muscle EAA composition of groups fed lysine sufficient diets.

†A/E ratio = specific EAA content × 1000/total EAA.

‡EAA requirements except for lysine are calculated as follows: requirement = lysine requirement from this study × (A/E ratio/223.68).

offered by hand slowly and carefully, utmost care was taken to avoid feed waste and to assure that all the feed supplied was consumed. Results of the current study were similar to values of 5.44% for grass carp (Wang, Liu, Tian, Xie, Yang, Wang & Liang 2005), and lower than that for Indian major carp (5.75%, Ahmed & Khan 2004), or Japanese seabass (5.80 ± 0.12%; 6.07 ± 0.37%; 6.05 ± 0.30%, Mai, Zhang, Ai, Duan, Zhang, Li, Wan & Liufu 2006), but is higher than that for striped bass (4.90%, Small & Soares 2000); gilthead seabream (5.04%, Marcouli, Alexis, Andriopoulou & Iliopoulou-Georgudaki 2006); cobia (5.30%, Zhou, Wu, Chi & Yang 2007) or pacu (4.4–4.7%, Bicudo, Sado & Cyrino 2009). The variations in the estimated lysine requirements for various fish species, are probably due to the difference in species, dietary protein sources, dietary energy content, digestibility (Forster & Ogata 1998; De Silva, Gunasekera & Gooley 2000), feeding practices and rearing conditions (Ahmed & Khan 2004; Mai *et al.* 2006), furthermore, environmental condition, mathematical model and response criteria can also affect the value estimated for lysine requirement (Montes-Girao & Fracalossi 2006; Zhou *et al.* 2007; Zhang, Ai, Mai, Tan, Li & Zhang 2008). Moreover, the broken-line model are considered to generally underestimate requirements when compared with non-linear models (Baker 1986), and the non-linear models have been indicated as the most appropriate approach to describe the responses from a biological background (Shearer 2000). However, comparing the coefficient of estimation (R^2) of broken-line model with non-linear models (second-order polynomial model and asymptotic model), which indicate that the broken-line model is most suitable one to describe the relationship between dietary lysine level and WG. The same reason is also applicable to PER.

Studies have indicated that fish utilized crystalline amino acids (CAAs) less efficiently than protein-bound forms (Yamada, Simpson, Tanaka & Katayama 1981; Zarate & Lovell 1997) because

CAAs were absorbed from the digestive tract faster than protein-bound amino acids (Yamada *et al.* 1981; Ronnestad, Conceicao, Aragao & Dinis 2000). In other words, CAAs had a shorter residence time in the digestive tract compared with protein-bound amino acids. The disproportionate absorption rates of CAAs and protein-bound amino acids could reduce the efficiency of protein synthesis (Zarate & Lovell 1997; Ambardekar & Reigh 2007), causing an imbalance in amino acid profile in the tissues and diverting amino acids into catabolic rather than anabolic processes (Cowey & Sargent 1979). In addition, the imbalance in the dietary amino acid profile can also lead to the eutrophication of receiving water by AA-catabolites excretion (Cheng, Hardy & Usry 2003; Conceição, Grasdalen & Rønnestad 2003). We have observed that the supplementation of coated lysine and methionine was effective in improving the nutritive value of FSBM-based diets deficient in methionine and lysine for juvenile Chinese sucker in our recent study (Yuan, Gong, Yang, Lin, Yu & Luo 2011). More feeding frequency increased efficiency of CAAs for carp and channel catfish (Yamada *et al.* 1981; Zarate, Lovell & Payne 1999). It also has been reported that European sea bass can utilize large amounts of CAAs when included in diets containing intact proteins and when the feeding frequency was high (Tibaldi & Lanari 1991). In the current study, feeding frequency of three times per day had been taken, which might improve utilization of CAAs to some extent (Yu *et al.* 2011).

Lysine deficiency causes loss of appetite (showed in Table 3) and reduced growth performance, which is also shown in Japanese seabass (Mai *et al.* 2006), grouper (Luo, Liu, Mai, Tian, Tan, Yang, Liang & Liu 2006), cobia (Zhou *et al.* 2007), yellow croaker (Zhang *et al.* 2008). In the present study, fish fed with deficiency dietary lysine showed significant lower WG, SGR and PER compared with the optimum dietary lysine level. Increasing lysine in the diet improved the growth performance and feed utilization, which confirmed results from other reports (Walton *et al.* 1984; Rodehutscord, Borchert, Gregus, Pack & Pfeffer 2000; Wang *et al.* 2005). However, further increase in the dietary lysine level did not improve growth performance and feed utilization compared with the optimum dietary lysine level or even depressed growth and gave a lower efficiency of feed utilization (Walton *et al.* 1984; Murthy & Varghese 1997; Ahmed & Khan 2004). In the current study, the fish fed with higher levels of (diet 5 and 6) showed slightly lower SGR, PER and higher FCR compared with the optimum dietary lysine level (diet 3 and 4), which is similar to the reports for rainbow trout (Cheng *et al.* 2003), grass carp (Wang *et al.* 2005), Japanese seabass (Mai *et al.* 2006) and yellow croaker (Zhang *et al.* 2008). These discrepancies are possibly related to species, ingredient quality and diet composition. The nitrogen retention (% N intake) significantly increased with increasing lysine level but a plateau was not attained even at the higher lysine levels. Therefore, it is impossible to estimate the lysine requirement for maximum nitrogen retention. This indicates that higher dietary lysine levels may be required for maximum nitrogen gain than for weight gain, as also reported in rainbow trout (Encarnação, de Lange, Rodehutscord, Hoehler, Bureau & Bureau 2004) and turbot (Peres & Oliva-Teles 2008).

In the current study, fish fed with high lysine diets showed significant increase in whole body protein when compared with that of fish fed with low lysine diets, while the highest whole body lipid content was noted with fish fed with lowest dietary lysine, which was in agreement with other reports where lysine supplementation resulted in increased body protein and decreased body lipid (Zarate & Lovell 1997; Mai *et al.* 2006; Zhang *et al.* 2008). In contrary, Tantikitti & Chimsung (2001) found higher lipid content in freshwater catfish (*Mystus nemurus*) fed diets containing higher concentrations of free amino acids and suggested that amino acids in excess would be catabolized and the carbon skeletons used in lipid synthesis and deposited in tissue. There is a significant decrease in both whole body protein and muscle-lysine content in fish fed diets deficient in lysine at the end of the present trial. This partially agrees to what was observed by some authors (Rodehutscord *et al.* 2000; Encarnação *et al.* 2004; Marcouli *et al.* 2006), probably due to dietary restriction of one EAA led to an increase in oxidation of other essential and non-essential amino acids present at normal levels in the diets (Ronnestad *et al.* 2000; Ozorio, Booms, Huisman & Verreth 2002). In the present study, dietary lysine levels had a significant effect on CF and HSI of juvenile Chinese sucker (Table 4), which was partially in agreement with other studies. However, it has been reported that grouper fed with low lysine diets tended to have lower HSI than those fed high lysine diets (Luo *et al.* 2006).

In the current study, supplementary lysine did not only affect growth performance but also the lysine concentration in fish muscle. Similar results have been reported in rainbow trout (Rodehutscord *et al.* 2000), Japanese sea bass (Mai *et al.* 2006) and large yellow croaker (Zhang *et al.* 2008). The results suggest that the difference in the total lysine in fish muscle fed different diets is probably because of the difference in free lysine in the amino acid pool of the muscle. It has been reported that the level of certain indispensable amino acids in fish muscle could be used as an index of dietary amino acid status in fish nutrition studies (Mai *et al.* 2006; Zhang *et al.* 2008). In the present study, increasing lysine level from 3.56% to 4.18%, the total arginine in the muscle of Chinese sucker showed a slight decline tendency probably due to the results of the negative effects (lysine-arginine interaction) of excessive amount of free lysine. However, dietary lysine-arginine antagonism in fish is still in controversy. Kaushik and Fauconneau (1984) reported lysine-arginine antagonism occurred at some metabolic processes and excess lysine inhibited arginine catabolism and urea excretion in rainbow trout. In contrast, some authors indicated that no dietary lysine-arginine antagonism existed in channel catfish (Robinson, Wilson & Poe 1981), rainbow trout (Kim, Kayes & Amunderson 1992), hybrid striped bass (Grrifin, Wilson & Brown 1994) because there were no significant differences in growth performance and feed efficiency among the treatments.

Estimation of EAA requirements according to the ideal protein concept has been widely used in fish (Forster & Ogata 1998; Small & Soares 2000; Wang *et al.* 2005; Peres & Oliva-Teles 2008). However, such approach cannot provide the precise information on the actual EAA requirements, as it ignores maintenance requirement and is dependent on the determination of only one EAA requirement (usually lysine) (Wilson 2002). Although this method is not perfect, it is still useful to estimate the other EAA requirements 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 acids (Wilson 2002; Peres & Oliva-Teles 2008). In the present study, muscle amino acid profile has been used to estimate the other EAAs of Chinese sucker. It is feasible to use muscle amino acid profile or whole body amino acid profile to estimate the other EAAs, the method has been demonstrated by several investigators (Ogata, Arai & Nose 1983; Wilson & Poe 1985; Borlongan & Coloso 1993; Mambrini & Kaushik 1995; Bicudo *et al.* 2009; Abimorad, Favero, Squassoni & Carneiro 2010).

In summary, results of this trial indicated that dietary lysine requirement of juvenile Chinese sucker is not much different from that of other animals. Based on broken-line regression analysis of the WG and PER, an inclusion of 2.43% and 2.40% of dry diet (corresponding to 5.52% and 5.45% of dietary protein) are recommended for optimum growth of juvenile Chinese sucker. The estimated requirements for the other EAA 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 Chinese sucker.

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References

Abimorad E.G., Favero G.C., Squassoni G.H. & Carneiro D. (2010) Dietary digestible lysine requirement and essential amino acid to lysine ratio for pacu *Piaractus mesopotamicus*. *Aquaculture Nutrition* **16**, 370–377.

Ahmed I. & Khan M.A. (2004) Dietary lysine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton). *Aquaculture* **235**, 499–511.

Ambardekar A.A. & Reigh R.C. (2007) Sources and utilization of amino acids in channel catfish diets: a review. *North American Journal of Aquaculture* **69**, 174–179.

Association of Official Analytical Chemists (AOAC) (1995) *Official Methods of Analysis of Official Analytical Chemists International* (16th edn). Association of Official Analytical Chemists, Arlington, VA.

Baker D.H. (1986) Problems and pitfalls in animal experiments designed to establish dietary requirements for essential nutrient. *Journal of Nutrition* **116**, 2339–2349.

Bicudo A.J.A., Sado R.Y. & Cyrino J.E.P. (2009) Dietary lysine requirement of juvenile pacu *Piaractus mesopotamicus* (Holmberg, 1887). *Aquaculture* **297**, 151–156.

Borlongan I.G. & Coloso R.M. (1993) Requirements of juvenile milkfish(*Chanos chanos* Forsskal) for essential amino acids. *Journal of Nutrition* **123**, 125–132.

Cheng Z.J., Hardy R.W. & Usry J.L. (2003) Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. *Aquaculture* **215**, 255–265.

Conceição L.E.C., Grasdalen H. & Rønnestad I. (2003) Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. *Aquaculture* **227**, 221–232.

Cowey C.B. & Sargent J.R. (1979) Nutrition. In: *Fish Physiology, Vol. VIII. Bioenergetics and Growth*, (ed. by W.S. Hoar, D.J. Randall & J.R. Brett), pp. l–69. Academic Press, New York.

De Silva S.S., Gunasekera R.M. & Gooley G. (2000) Digestibility and amino acid availability of three protein-rich ingredient-incorporated diets by Murray cod *Maccullochella peelii* peelii (Mitchell) and the Australian short-fin eel *Anguilla australis* Richardson. *Aquaculture Research* **31**, 195–205.

Encarnação P., de Lange C., Rodehutscord M., Hoehler D., Bureau W. & Bureau D.P. (2004) Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. *Aquaculture* **235**, 569–586.

Forster I. & Ogata H.Y. (1998) Lysine requirement of juvenile Japanese flounder *Paralichthys olivaceus* and juvenile red sea bream Pagrus major. *Aquaculture* **161**, 131–142.

Grrifin M.E., Wilson K.A. & Brown P.B. (1994) Dietary arginine requirement of juvenile hybrid striped bass. *Journal of Nutrition* **124**, 888–893.

Harris L.E. (1980) Diet stuffs. In: *Fish Diet Technology* (ed. by T.V.R. Pillay), pp. 111–168. UNDP/FAO, Rome, Italy.

Kaushik S.J. & Fauconneau B. (1984) Effects of lysine administration on plasma arginine and on some nitrogenous catabolites in rainbow trout. *Comparative Biochemistry and Physiology* **79A**, 459–462.

Kim K.L., Kayes T.B. & Amunderson C.H. (1992) Requirements for lysine and arginine by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **106**, 333–344.

Luo Z., Liu Y.J., Mai K.S., Tian L.X., Tan X.Y., Yang H.J., Liang G.Y. & Liu D.H. (2006) Quantitative L-lysine requirement of juvenile grouper *Epinephelus coioides*. *Aquaculture Nutrition* **12**, 165–172.

Mai K.S., Zhang L., Ai Q.H., Duan Q.Y., Zhang C.X., Li H.T., Wan J.L. & Liufu Z.G. (2006) Dietary lysine requirement of juvenile Japanese seabass *Lateolabrax japonicus*. *Aquaculture* **258**, 535–542.

Mambrini M. & Kaushik S.J. (1995) Indispensable amino acid requirements of fish: correspondence between quantitative data and amino acid profiles of tissue proteins. *Journal of Applied Ichthyolgy* **11**, 240–247.

Marcouli P.A., Alexis M.N., Andriopoulou A. & Iliopoulou-Georgudaki J. (2006) Dietary lysine requirement of juvenile gilthead seabream *Sparus aurata* L. *Aquaculture Nutrition* **12**, 25–33.

Montes-Girao P.J. & Fracalossi D.M. (2006) Dietary lysine requirement as basis to estimate the essential dietary amino acid profile to jundiá, Rhamdia quelen. *Journal of World Aquaculture Society* **37**, 388–396.

Murthy H.S. & Varghese T.J. (1997) Dietary requirements of juveniles of the Indian major carp, Labeo rohita, for the essential amino acid lysine. *Israeli Journal of Aquaculture (Bamidgeh)* **49**, 19–24.

NRC (National Research Council) (1993) *Nutrient Requirement of Fish*. National Academy Press, Washington, DC, USA.

Ogata H., Arai S. & Nose T. (1983) Growth response of cherry salmon *Oncorhynchus masou* and amago salmon *O. rhodurus* fry fed purified casein diets supplemented with amino acids. *Nippon Suisan Gakkaishi* **49**, 1381–1385.

Ozorio R.O.A., Booms G.H.R., Huisman E.A. & Verreth J. A.J. (2002) Changes in amino acid composition in the tissues of African catfish (*Clarias gariepinus*) as a consequence of dietary L-cartinine supplements. *Jouranl of Applied Ichthyolgy* **18**, 140–147.

Peres H. & Oliva-Teles A. (2008) Lysine requirement and efficiency of lysine utilization in turbot (*Scophthalmus maximus*) juveniles. *Aquaculture* **275**, 283–290.

Robbins K.R., Norton H.W. & Baker D.H. (1979) Estimation of nutrient requirements from growth data. *Journal of Nutrition* **109**, 1710–1714.

Robinson E.H., Wilson R.P. & Poe W.E. (1981) Arginine requirement and apparent absence of a lysine-arginine antagonist in fingerling channel catfish. *Journal of Nutrition* **111**, 46–52.

Rodehutscord M., Borchert F., Gregus Z., Pack M. & Pfeffer E. (2000) Availability and utilization of free lysine in rainbow trout (*Oncorhynchus mykiss*) 1. Effect of dietary crude protein level. *Aquaculture* **187**, 163–176.

Ronnestad I., Conceicao L.E.C., Aragao C. & Dinis T.M. (2000) Free amino acids are abosorbed faster and assimilated more efficiently than protein in postlarval Senegal sole (*solea senegalensis*). *Journal of Nutrition* **130**, 2809–2812.

Sandell L.J. & Daniel J.C. (1988) Effect of ascorbic acid on RNA levels in short term chondrocyte cultures. *Connect of Tissue Research* **17**, 11–22.

Shearer K.D. (2000) Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. *Aquaculture Nutriton* **6**, 91–102.

Small B.C. & Soares J.H. Jr (2000) Quantitative dietary lysine requirement of juvenile striped bass *Morone saxatilis*. *Aquaculture Nutrition* **6**, 207–212.

Tantikitti C. & Chimsung N. (2001) Dietary lysine requirement of freshwater catfish (*Mystus nemurus* Cuv. & Val.). *Aquaculture Research* **32**, 135–141.

Tibaldi E. & Lanari D. (1991) Optimal dietary lysine levels for growth and protein utilization of fingerling sea bass (*Dicentrarchus labrax* L.) fed semipurified diets. *Aquaculture* **95**, 297–304.

Walton M.J., Cowey C.B. & Adron J.W. (1984) The effect of dietary lysine levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). *British Journal of Nutrition* **52**, 115–122.

Wan Q., Lai N.Y., Liu Y.B., Shen B.P., Sun W.X., Chen Z. H. & Wang X. (2006) Study on intensive cultivation of *Myxocyprinus asiaticus* fingerling fed with mixed feed. *Journal of Anhui Agriculture Science* **18**, 4605–4606 (In Chinese).

Wang S. & Xie Y. (2004) *China Speicies Red List, Vol. 1*. Higher Education Press, Beijing, China, pp. 692 [In Chinese].

Wang S., Liu Y.J., Tian L.X., Xie M.Q., Yang H.J., Wang Y. & Liang G.Y. (2005) Quantitative dietary lysine requirement of juvenile grass carp *Ctenopharyngodon idella*. *Aquaculture* **249**, 419–429.

Wilson R.P. (1985) Amino acid and protein requirements of fish. In: *Nutrition and Feeding in Fish* (ed. by C.B. Cowey, A.M. Mackie & J.G. Bell), pp. 1–15. Academic Press, London.

Wilson R.P. (1989) Amino acids and proteins. In: *Fish Nutrition*, (2nd edn) (ed. by J.E. Halver), pp. 111–151. Academic Press, New York.

Wilson R.P. (2002) Amino acids and proteins. In: *Fish Nutrition*, (3rd edn) (ed. by J.E. Halver & R.W. Hardy), pp. 144–175. Academic Press, San Diego, CA.

Wilson R.P. & Cowey C.B. (1985) Amino acid composition of whole body tissue of rainbow trout and Atlantic salmon. *Aquaculture* **48**, 373–376.

Wilson R.P. & Poe W.E. (1985) Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus*. *Comparative Biochemistry and Physiology* **80B**, 385–388.

Yamada S., Simpson K.L.,Tanaka Y. & Katayama T. (1981) Plasma amino acid changes in rainbow trout (Salmo gairdneri) force-fed casein and a corresponding amino acid mixture. *Bulletin of the Japanese Society of Scientific Fisheries* **47**, 1035–1040.

Yu D.H., Gong S.Y., Yuan Y.C., Luo Z., Lin Y.C. & Li Q. (2011) Effect of partial replacement of fish meal with soybean meal and feeding frequency on growth, feed utilization and body composition of juvenile Chinese sucker, *Myxocyprinus asiaticus* (Bleeker). *Aquaculture Research*, 1–7, doi: 10.1111j.1365-2109.2011.03043.x.

Yuan Y.C., Gong S.Y., Luo Z., Yang H.J., Zhang G.B. & Chu Z.J. (2010a) Effects of dietary protein to energy ratios on growth and body composition of juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture Nutrition* **16**, 205–212.

Yuan Y.C., Gong S.Y., Yang H.J., Lin Y.C., Yu D.H. & Luo Z. (2010b) Apparent digestibility of selected feed ingredients for Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture* **306**, 238–243.

Yuan Y.C., Yang H.J., Gong S.Y., Luo Z., Yuan H.W. & Chen X.K. (2010c) Effects of feeding levels on growth performance, feed utilization, body composition and apparent digestibility coefficients of nutrients for juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture Research* **41**, 1030–1042.

Yuan Y.C., Gong S.Y., Yang H.J., Lin Y.C., Yu D.H. & Luo Z. (2011) Effects of supplementation of crystalline or coated lysine and/or methionine on growth performance and feed utilizaition of the Chinese sucker, *Myxocyprinus asiaticus*. *Aquaculture* **316**, 31–36.

Zar J.H. (1984) *Biostatistical Analysis*, pp. 293–305. Prentice-Hall, Englewood Cliffs, NJ.

Zarate D.D. & Lovell R.T. (1997) Free lysine (L-lysine. HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (Ictalurus punctatus). *Aquaculture* **258**, 551–557.

Zarate D.D., Lovell R.T. & Payne M. (1999) Effects of feeding frequency and rate of stomach evacuation on utilization of dietary free and protein-bound lysine for growth by channel catfish *Ictalurus punctatus*. *Aquaculture Nutrition* **5**, 17–22.

Zeitoun I.H., Ullrey D.E., Magee W.T., Gill J.L. & Bergen W.G. (1976) Quantifying nutrient requirements of fish. *Journal of the Fisheries Research Board of Canada* **33**, 167–172.

Zhang C.X., Ai Q.H., Mai K.S., Tan B.P., Li H.T. & Zhang L. (2008) Dietary lysine requirement of large yellow croaker, *Pseudosciaena crocea* R. *Aquaculture* **283**, 123–127.

Zhang G.B., Gong S.Y., Yuan Y.C., Chu Z.J. & Yuan H. W. (2009) Dietary protein requirement for juvenile Chinese sucker(*Myxocyprinus asiaticus*). *Jouranl of Applied Ichthyolgy* **25**, 715–718.

Zhou J.G., Yang D.G., Wu G.X., Wang Z.L., Liu L.H. & Chen J.S. (1999) Development of Chinese sucker (*Myxocyprinus asiaticus*) larval and juvenile and techniques for fry and fingerlings rearing. *Journal of Huazhong Agriculture University* **3**, 263–267 (In Chinese).

Zhou Q.C., Wu Z.H., Chi S.Y. & Yang Q.H. (2007) Dietary lysine requirement of juvenile cobia (*Rachycentron canadum*). *Aquaculture* **273**, 634–640.