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Effect of dietary lysine on growth, intestinal enzymes activities and antioxidant status of sub-adult grass carp (*Ctenopharyngodon idella*)

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Abstract The dietary lysine requirement of sub-adult grass carp (460 ± 1.5 g) was assessed by feeding diets supplemented with grade levels of lysine (6.6, 8.5, 10.8, 12.9, 15.0 and 16.7 g kg^{-1} diet) for 56 days. The test diets (28 % CP) contained fish meal, casein and gelatin as sources of intact protein, supplemented with crystalline amino acids. Weight gain (WG), feed intake and feed efficiency were significantly improved with increasing levels of lysine up to 12.9 g kg^{-1} diet and thereafter declined ($P < 0.05$). Quadratic regression analysis of WG at 95 % maximum response indicated lysine requirement was 10.9 g kg^{-1} diet. Activities of trypsin, chymotrypsin, lipase, Na^+, K^+-ATPase and alkaline phosphatase in intestine, creatine kinase activity in proximal and mid-intestine responded similar to WG ($P < 0.05$). In addition, lipid and protein oxidation decreased with increasing levels of lysine up to certain values and increased thereafter ($P < 0.05$); the anti-hydroxyl radical capacity, dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase (GST) activities and glutathione content were increased with increasing dietary lysine levels up to certain values in the detected tissues, except for hepatopancreatic GST. Requirement estimated on the basis of malondialdehyde content in intestine and hepatopancreas was 10.6 and 9.53 g lysine kg^{-1} diet, respectively.

Keywords *Ctenopharyngodon idella* · Lysine · Growth · Digestion · Intestinal enzymes activities · Antioxidant enzyme

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Introduction

Grass carp (*Ctenopharyngodon idella*) is one of the most important freshwater fish cultivated in China (Tian et al. 2004). It would be useful to develop nutrients-balanced practical diets for the intensive culture of grass carp (Shireman et al. 1977). Nutrient requirements provide a basis for practical diet formulation. Therefore, it is important to ensure adequate

nutrition for critical nutrients, particularly with regard to essential amino acids requirement.

Lysine is indispensable for all fish species studied (NRC 2011). It has been noted that lysine deficiency caused reduced growth and low feed efficiency (FE) as shown in several cyprinid fish species (Ahmed and Khan 2004; Wang et al. 2005; Zhou et al. 2008). Moreover, it is the most limiting amino acid in many available protein sources in fish feeds, especially plant proteins (Deng et al. 2010). However, to date, dietary lysine requirements have been estimated to be 20.7 g kg^{-1} of the diet (corresponding to 54 g kg^{-1} dietary protein) of 3–15 g grass carp exclusively (Wang et al. 2005). In fish, the requirements of nutrients vary with growth stage. The estimated lysine requirement generally progressively decreased when the live weight of the fish increased in rainbow trout and Atlantic salmon (*salmo salar*) (NRC 2011). Moreover, the portion of the endogenous loss and growth requirement of amino acids vary in different growth stage (Shearer 1995). Thus, it is valuable to determine the lysine requirements of grass carp in sub-adult stage.

The ability of fish to utilize ingested nutrients for growth partially depends on digestive enzymes (trypsin, chymotrypsin, lipase and amylase) and brush-border enzymes (creatine kinase, alkaline phosphatase, γ-glutamyl transpeptidase and Na^+, K^+-ATPase) activities in intestine (Tengjaroenkul et al. 2000) and normal function of digestive organs (Hofer and Uddin 1985). Our previous study showed that diet containing coated lysine improved intestine protein concentration, the activities of intestinal protease, lipase, AKP and Na^+, K^+-ATPase of juvenile Jian carp compared with uncoated lysine on equal basis (Zhou et al. 2007). Lysine promoted the secretion of cholecystokinin (CCK) in larvae gilthead seabream (Naz and Türkmen 2008). Grendell et al. (1984) reported that CCK can regulate the release of trypsin in the gut of rats. However, little study has been focused on the effects of lysine on the digestive and brush-border membrane enzyme activities of grass carp in sub-adult stage.

The function of digestive system is partly dependent on the structural integrity. Fish digestive organs contain large quantities of polyunsaturated fatty acid essential for membrane function which makes it extremely susceptible to oxidation stress (Lushchak et al. 2001; Martínez-Álvarez et al. 2005). To prevent oxidative damage, fish must possess effective antioxidant defence systems. Like all aerobic organisms, antioxidant defence systems in fish include both antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and low-molecular-weight antioxidants, such as glutathione (GSH) (Zhang et al. 2010). However, there is no study that has been conducted to investigate the effects of lysine on antioxidant defence in fish. Harpaz (2005) reported that lysine could convert into carnitine in fish. Carnitine depressed lipid peroxidation and elevated enzymatic antioxidants activities in tissues of juvenile black sea bream (Ma et al. 2008). Lysine can stimulate growth hormone (GH) release in human (Chromiak and Antonio 2002). Bolzán et al. (1995) revealed a positive correlation between serum levels of GH and activity of SOD in mammary tissue of rats. Therefore, it is valuable to investigate the effects of lysine on digestive organ antioxidant ability in fish.

The main purpose of this study was to estimate the dietary lysine requirement of sub-adult grass carp and investigate the possible effects of dietary lysine on intestinal enzyme activities and antioxidant status of digestive organs.

Materials and methods

Experimental design and diets

Formulation of the basal diet is presented in Table 1. Fish meal (Pesquera Lota Protein Ltd., Villagram, Chile), casein (Hulunbeier Sanyuan Milk Co., Ltd., Inner Mongolia, China), gelatin (Rousselot Gelatin Co., Ltd., Guangdong, China) and crystalline amino acids (Shanghai Yimengsi Amino Acid Co., Ltd., Shanghai, China) were used as the main protein sources. Crystalline L-amino acids were used to simulate the amino acid profile similar to that of 280 g kg^{-1} whole chicken egg protein except for lysine (Ahmed and Khan 2004). L-lysine sulphate was supplemented to the basal diet to provide targeted graded concentration of 6.7 (unsupplemented control), 8.7, 10.7, 12.7, 14.7 and 16.7 g lysine kg^{-1} diet. All diets were made isonitrogenous with the addition of appropriate amounts of crystalline glycine. The levels of crude protein and crude lipid in all diets were formulated to be 280.6 and 42.2 g kg^{-1}, respectively, which are considered to be sufficient to support optimal growth of grass carp. The pH of each diet

Table 1 Composition and nutrients content of the basal diet

Ingredients	g kg^{-1}	Nutrient content[a]	g kg^{-1}
Fish meal	68.0	Crude protein	280.6
Casein	30.0	Crude lipid	42.2
Gelatin	39.9	ω-3	10.0
Amino acid mix[b]	141.6	ω-6	10.0
Lysine premix[c]	50.0	Lysine	6.6
Glycine premix[d]	58.4	Available phosphorus	6.0
Corn starch	81.0		
α-Starch	280.0		
Fish oil	22.8		
Soya bean oil	18.9		
Trace mineral premix[e]	20.0		
Vitamin premix[f]	10.0		
$Ca(H_2PO_4)_2$	22.9		
Choline chloride	6.0		
α-Cellulose	150.0		
Ethoxyquin	0.5		

[a] Crude protein and crude fat were the measured values. Available phosphorus, n-3 and n-6 contents were calculated according to NRC (1993)

[b] Amino acid mix (g kg^{-1}): arginine, 11.80; histidine, 7.23; isoleucine, 11.82; leucine, 18.99; methionine, 7.3; cystine, 0.81; phenylalanine, 12.53; tyrosine, 10.00; threonine, 11.22; tryptophan, 3.27; valine, 14.24; glutamic acid, 32.32

[c] L-lysine sulphate was added to obtain graded level of lysine. Per kilogram of lysine premix composition from diet 1–6 was as follows (g kg^{-1}): L-lysine sulphate 0.00, 78.59, 157.18, 235.77, 314.36, 392.95 g and corn starch 1,000, 921.41, 842.82, 764.23, 685.64, 607.05 g, respectively

[d] Each mixture was made isonitrogenous with the addition of reduced amounts of glycine and compensated with appropriate amounts of corn starch. Per kilogram of glycine premix composition from diet 1–6 was as follows (g kg^{-1}): glycine 990.62, 921.52, 852.41, 783.31, 714.20, 645.10 g and corn starch 9.38, 78.48, 147.59, 216.69, 285.80, 354.90 g, respectively

[e] Per kilogram of mineral premix (g kg^{-1}): $FeSO_4 \cdot H_2O$, 25.00 g; $CuSO_4 \cdot 5H_2O$, 0.60 g; $ZnSO_4 \cdot H_2O$, 4.35 g; $MnSO_4 \cdot H_2O$, 2.04 g; KI, 1.10 g; $NaSeO_3$, 2.50 g; $MgSO_4 \cdot H_2O$, 230.67 g; corn starch 733.74 g. All ingredients were diluted with corn starch to 1 kg

[f] Per kilogram of vitamin premix (g kg^{-1}): retinyl acetate (5,000,00 IU g^{-1}), 0.80 g; cholecalciferol (5,000,00 IU g^{-1}), 0.48 g; DL-α-tocopherol acetate (500 g kg^{-1}), 20.00 g; menadione (230 g kg^{-1}), 0.22 g; thiamine hydrochloride (980 g kg^{-1}), 0.12 g; riboflavin (800 g kg^{-1}), 0.99 g; pyridoxine hydrochloride (980 g kg^{-1}), 0.62 g; cyanocobalamin (10 g kg^{-1}), 0.10 g; niacin (990 g kg^{-1}), 2.58 g; D-biotin (20 g kg^{-1}), 5.00 g; meso-inositol (990 g kg^{-1}), 52.33 g; folic acid (960 g kg^{-1}), 0.52 g; ascorhyl acetate (930 g kg^{-1}), 7.16 g; calcium-D-pantothenate (900 g kg^{-1}), 2.78 g. All ingredients were diluted with corn starch to 1 kg

was adjusted to 7.0 by the addition of 6.0 N NaOH (Deng et al. 2010). The pellets were prepared and stored at −20 °C until use (Lin and Shiau 2007). The lysine concentration in the experimental diets was measured to be 6.6 (unsupplemented control), 8.5, 10.8, 12.9, 15.0 and 16.7 g kg^{-1} diet by using high-performance liquid chromatography (Agilent Technologies, Palo Alto, CA, USA).

Experimental management

The feeding trial was conducted at Experiment Station of Dayi, Sichuan Province, China. Sub-adult grass carp, obtained from a commercial cultivation (Bailong lake, Sichuan, China), were conditioned for 2 weeks. A total of 600 fish with the similar size (mean initial weight 460 ± 1.5 g) were randomly distributed to 30 cages (1.4 × 1.4 × 1.4 m). During the experimental period, fish were reared under natural light conditions, the water temperature and pH were maintained at 25 ± 2 °C and 7.5 ± 0.3, respectively. Rate of water flow was adjusted to keep dissolved oxygen at 7.0 ± 0.5 mg L^{-1}. Ammonia (total ammonia-N) and nitrites were recorded daily (0.10 ± 0.01 and 0.05 ± 0.01 mg L^{-1}, respectively). Each of the six diets was fed to quintuplicate of fish four times daily to satiation for 56 days. Thirty minutes after feeding, uneaten feed was collected through a 1-mm gauze disc of 80 cm diameter equipped at the bottom of each cage. Feeding management was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Animal Nutritional Institute, Sichuan Agricultural University.

Sample collection and analysis

Fish in each cage were weighed and counted at the initiation and termination of the feeding trial. At the end of feeding trial, fifteen fish collected from each cage were anaesthetized in benzocaine bath (50 mg L^{-1}) as described by Bohne et al. (2007) 12 h after the last feeding and sampled for determination of intestine and hepatopancreas protein content, activities of intestinal, hepatopancreatic and muscular enzymes. The muscle, hepatopancreas and intestine were quickly collected and removed, weighed and frozen in liquid nitrogen, then stored at −70 °C until analyzed. All procedures were approved by the

Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University.

Proximate analysis of diets was performed according to the methods of the AOAC (1998). Intestine, hepatopancreas and muscle samples were homogenized in 10 volumes (w v^{-1}) of ice-cold physiological saline solution and centrifuged at 6,000*g* for 20 min at 4 °C; then, the supernatant was collected for enzyme activity analysis. Trypsin and chymotrypsin activities were measured by Hummel (1959), and amylase and lipase activities were determined according to Furne et al. (2005). CK, AKP, Na^+/K^+-ATPase and γ-GT activities in the intestine were determined according to Tanzer and Gilvarg (1959), Bessey et al. (1946), Weng et al. (2002) and Bauermeister et al. (1983), respectively. Intestine and hepatopancreas protein content were measured by the method of Bradford (1976). GOT and GPT activities in hepatopancreas and muscle were determined according to the method of Bergmeyer (1974). The MDA and PC contents were determined by Livingstone et al. (1990) and Armenteros et al. (2009), respectively. Reduced GSH was quantitated by the method of Vardi et al. (2008). The ASA and anti-hydroxyl radical (AHR) capacity were determined as described by Jiang et al. (2009). SOD and GPX activities were assayed by the method described by Vardi et al. (2008). The activities of CAT, GST and glutathione reductase (GR) were assayed by Aebi (1984), Lushchak et al. (2001) and Lora et al. (2004), respectively.

Calculations and statistical analysis

Data on initial body weight (IBW), final body weight (FBW), feed intake (FI), hepatopancreas weight (HW), intestine weight (IW), intestine length (IL), body length, hepatopancreas and intestine protein were used to calculate percentage weight gain (PWG), feed efficiency (FE), specific growth rate (SGR), protein efficiency ratio (PER), relative gut length (RGL), hepatosomatic index (HSI), intestosomatic index (ISI), hepatopancreas protein content (HPC) and intestine protein content (IPC).

$$\mathrm{PWG} = 100 \times [\text{final weight(g)} - \text{initial weight(g)}] / \text{initial body weight(g)}$$

$$\mathrm{FE} = [\text{weight gain(g)}/\text{feed intake(g)}]$$

$$\mathrm{SGR} = 100 \times [(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{number of feeding days}]$$

$$\mathrm{PER} = \text{weight gain(g)}/\text{protein intake(g)}$$

$$\mathrm{RGL} = 100 \times [\text{intestine length(cm)}/\text{total body length(cm)}]$$

$$\mathrm{HSI} = 100 \times [\text{wet hepatopancreas weight(g)} / \text{wet body weight(g)}]$$

$$\mathrm{ISI} = 100 \times [\text{wet intestine weight(g)}/\text{wet body weight(g)}]$$

$$\mathrm{HPC} = 100 \times [\text{hepatopancreas protein (g)} / \text{wet hepatopancreas weight(g)}]$$

$$\mathrm{IPC} = 100 \times [\text{intestine protein(g)}/\text{wet intestine weight(g)}]$$

Results were presented as mean ± SD. All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test to determine significant differences among treatment groups at the level of $P < 0.05$ through SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The quadratic regression analysis model was used to calculate the dietary lysine requirement based on weight gain and lipid peroxidation at 95 % maximum response. The relationships between dietary lysine and the growth performance, activities of GOT and GPT, activities of digestive and brush-border enzymes and antioxidant indicators were subjected to quadratic regression model, respectively.

Results

Growth

The weight gain, specific growth rate, feed intake, feed efficiency and protein efficiency ratio of grass carp fed different levels of dietary lysine are presented in Table 2. Weight gain and feed intake increased up to a lysine concentration of 12.9 g kg^{-1} diet; thereafter, further increases in lysine intake resulted in decrease in weight gain and feed intake ($P < 0.05$). Specific growth rate, feed efficiency ratio and protein efficiency ratio were improved with increasing dietary lysine levels up to 12.9 g kg^{-1} diet and decreased

Table 2 Initial body weight (IBW, g fish^{-1}), final body weight (FBW, g fish^{-1}), percentage weight gain (PWG), feed intake (FI, g fish^{-1}), specific growth rate (SGR), feed efficiency ratio (FE), protein efficiency ratio (PER), the activities of glutamate–oxaloacetate transaminase (GOT, U g^{-1} tissue) and glutamate–pyruvate transaminase (GPT, U g^{-1} tissue) in muscle and hepatopancreas of grass carp fed diets with graded levels of lysine for 56 days

	Dietary lysine levels (g kg^{-1} diet)					
	6.6	8.5	10.8	12.9	15.0	16.7
IBW	459.4 ± 1.349^{a}	459.6 ± 1.341^{a}	458.8 ± 1.481^{a}	460.4 ± 1.673^{a}	460.2 ± 1.483^{a}	459.2 ± 1.643^{a}
FBW	659 ± 24.3^{a}	739 ± 47.9^{b}	949 ± 9.45^{d}	1,052 ± 57.6^{e}	836 ± 53.2^{c}	816 ± 55.5^{c}
WG	200 ± 23.7^{a}	279 ± 47.2^{b}	491 ± 9.10^{d}	592 ± 57.7^{e}	376 ± 54.5^{c}	357 ± 56.8^{c}
FI	465 ± 32.6^{a}	608 ± 33.6^{b}	968 ± 25.8^{e}	1,094 ± 53.5^{f}	808 ± 48.0^{d}	751 ± 44.7^{c}
SGR	0.64 ± 0.06^{a}	0.85 ± 0.11^{b}	1.30 ± 0.02^{d}	1.47 ± 0.10^{e}	1.06 ± 0.12^{c}	1.02 ± 0.13^{c}
FE	0.428 ± 0.025^{a}	0.457 ± 0.052^{ab}	0.507 ± 0.016^{bc}	0.540 ± 0.027^{c}	0.463 ± 0.041^{ab}	0.474 ± 0.057^{ab}
PER	1.53 ± 0.09^{a}	1.63 ± 0.19^{ab}	1.81 ± 0.06^{bc}	1.92 ± 0.10^{c}	1.65 ± 0.14^{ab}	1.69 ± 0.20^{ab}
Hepatopancreas						
GPT	30.1 ± 1.78^{c}	24.9 ± 1.31^{b}	18.5 ± 0.80^{a}	19.7 ± 0.85^{a}	23.6 ± 1.16^{b}	23.8 ± 1.03^{b}
GOT	16.6 ± 1.44^{b}	18.4 ± 1.17^{c}	18.3 ± 1.62^{c}	18.1 ± 0.88^{bc}	16.7 ± 0.95^{b}	13. 6 ± 0.86^{a}
Muscle						
GPT	1.34 ± 0.15^{a}	4.78 ± 0.43^{c}	7.25 ± 0.23^{d}	7.40 ± 0.27^{d}	10.87 ± 0.75^{e}	3.22 ± 0.25^{b}
GOT	12.1 ± 2.61^{bc}	11.5 ± 1.93^{b}	7.71 ± 1.29^{a}	11.6 ± 1.91^{b}	11.4 ± 2.63^{b}	14.1 ± 1.15^{c}
Regression						
$Y_{PWG} = -226.45 + 53.978x - 2.157x^2$					$R^2 = 0.736$	$P < 0.01$
$Y_{FI} = -1{,}562.9 + 405.12x - 16.051x^2$					$R^2 = 0.837$	$P < 0.01$
$Y_{GOT\ in\ hepatopancreas} = 3.766 + 2.827x - 1.332x^2$					$R^2 = 0.684$	$P < 0.01$

All data were expressed as mean ± SD ($n = 5$). Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

thereafter ($P < 0.05$). Specific growth rate and feed intake showed significant quadratic responses to the increasing levels of dietary lysine. Using weight gain as the response criterion estimated the dietary lysine requirement by the second degree polynomial regression curve. The relationship described by the equation at 95 % of the maximum response was 10.9 g kg^{-1} diet (38.9 g kg^{-1} protein).

The activities of GOT and GPT in hepatopancreas and muscle

As shown in Table 2, the activities of GOT in muscle and GPT in hepatopancreas decreased with increasing lysine levels up to 10.8 g kg^{-1} diet and thereafter increased ($P < 0.05$), whereas the trend of GOT in hepatopancreas and GPT in muscle were properly opposite and obtained maximum values when lysine levels were 8.5 and 15.0 g kg^{-1}, respectively. Moreover, regression analysis showed that GOT activity in hepatopancreas was quadratic responses to the increasing levels of dietary lysine.

Intestinal digestive and brush-border enzymes activities

As shown in Table 3, trypsin, chymotrypsin, lipase and amylase in intestine, and brush-border membrane enzymes activities in proximal intestine, mid-intestine and distal intestine were significantly affected by dietary lysine level. Trypsin, chymotrypsin and lipase activities in intestine were improved with increasing dietary lysine levels up to 12.9, 8.5 and 8.5 g kg^{-1} diet, respectively, and decreased thereafter ($P < 0.05$). Amylase activities in treatment group containing 12.9 g kg^{-1} or higher lysine were lower than those fed 6.6, 8.5 and 10.8 g lysine kg^{-1} diet ($P < 0.05$). Regression analysis showed that the activities of trypsin and chymotrypsin were quadratic responses to

Table 3 The activities of trypsin (U mg^{-1} prot), chymotrypsin (U g^{-1} prot), lipase (U g^{-1} prot) and amylase (U mg^{-1} prot) in whole intestine, the activities of creatine kinase (CK, μmol of phosphorus released g^{-1} tissue h^{-1}) in whole intestine, Na^+, K^+-ATPase (μmol of phosphorus released g^{-1} tissue h^{-1}), alkaline phosphatase (AKP, mmol of nitrophenol released g^{-1} tissue h^{-1}) and γ-glutamyl transpeptidase (γ-GT, μmol of 5-amino-2-nitrobenzoate released g^{-1} tissue min^{-1}) in proximal intestine (PI), mid-intestine (MI) and distal intestine (DI) of grass carp fed diet with graded level of lysine for 56 days

	Dietary lysine levels (g kg^{-1} diet)					
	6.6	8.5	10.8	12.9	15.0	16.7
Trypsin	269 ± 10.7^{a}	323 ± 21.2^{b}	349 ± 14.6^{c}	358 ± 20.2^{c}	323 ± 29.9^{b}	301 ± 21.3^{b}
Chymotrypsin	19.9 ± 1.11^{b}	35.7 ± 2.93^{e}	34.0 ± 2.58^{e}	29.7 ± 2.36^{d}	26.2 ± 1.55^{c}	16.4 ± 1.09^{a}
Lipase	17.1 ± 2.04^{b}	42.1 ± 4.02^{d}	20. 6 ± 1.84^{c}	19.8 ± 1.53^{c}	16.8 ± 1.99^{b}	13.8 ± 1.37^{a}
Amylase	2.62 ± 0.102^{b}	2.66 ± 0.042^{b}	2.67 ± 0.077^{b}	2.52 ± 0.078^{a}	2.51 ± 0.052^{a}	2.45 ± 0.061^{a}
CK						
PI	2,940 ± 267^{b}	4,482 ± 335^{d}	5,146 ± 359^{e}	3,444 ± 306^{c}	2,551 ± 91.0^{a}	2,487 ± 90.4^{a}
MI	4,357 ± 230^{c}	4,242 ± 390^{c}	4,852 ± 444^{d}	4,557 ± 431 ^{cd}	3,193 ± 216^{b}	2,501 ± 224^{a}
DI	6,052 ± 300^{c}	5,780 ± 238^{c}	6,036 ± 350^{c}	5,243 ± 497^{b}	4,261 ± 404^{a}	4,504 ± 243^{a}
Na^+, K^+-ATPase						
PI	600 ± 56.7^{b}	915 ± 69.9^{d}	838 ± 88.4^{c}	518 ± 54. 9^{a}	515 ± 48.9^{a}	489 ± 27.2^{a}
MI	502 ± 33.6^{b}	664 ± 27.6^{c}	902 ± 95.2^{d}	669 ± 40.1^{c}	416 ± 42.6^{a}	442 ± 52.1^{ab}
DI	590 ± 64.5^{bc}	666 ± 71.8^{c}	794 ± 79.9^{d}	658 ± 61.2^{c}	525 ± 43.7^{ab}	474 ± 56.4^{a}
AKP						
PI	137.3 ± 10.87^{a}	137.2 ± 4.66^{a}	133.8 ± 12.69^{a}	139.4 ± 11.04^{a}	177.9 ± 15.13^{b}	165.3 ± 11.07^{b}
MI	65.7 ± 6.62^{a}	102 ± 9.41^{b}	121 ± 7.88^{c}	101 ± 7.89^{b}	107 ± 6.45^{b}	70.7 ± 7.15^{a}
DI	20.1 ± 1.96^{a}	20.0 ± 1.06^{a}	23.3 ± 2.31^{b}	35.5 ± 3.60^{c}	35.6 ± 3.70^{c}	19.2 ± 1.65^{a}
γ-GT						
PI	3,126 ± 205^{a}	3,265 ± 137^{a}	3,203 ± 190^{a}	3,235 ± 235^{a}	3,190 ± 233^{a}	3,077 ± 315^{a}
MI	3,323 ± 288^{a}	3,202 ± 187^{a}	3,438 ± 214^{a}	3,255 ± 75.5^{a}	3,395 ± 246^{a}	3,407 ± 229^{a}
DI	1,089 ± 122^{a}	1,011 ± 71.1^{a}	1,030 ± 51.5^{a}	981 ± 90.4^{a}	984 ± 83.8^{a}	1,013 ± 71.3^{a}
Regressions						
$Y_{\text{trypsin}} = -51.073 + 67.177x - 2.776x^2$					$R^2 = 0.695$	$P < 0.01$
$Y_{\text{chymotrypsin}} = -39.237 + 13.323x - 0.601x^2$					$R^2 = 0.797$	$P < 0.01$
$Y_{\text{CK MI}} = -256.12 + 1{,}005.3x - 50.55x^2$					$R^2 = 0.804$	$P < 0.01$
$Y_{\text{AKP MI}} = -132.90 + 42.464x - 1.805x^2$					$R^2 = 0.758$	$P < 0.01$

All data were expressed as mean ± SD ($n = 5$). Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

the increasing levels of dietary lysine. Alkaline phosphatase activities in proximal intestine, mid-intestine and distal intestine were improved with increasing dietary lysine levels up to 15.0, 10.8 and 15.0 g kg^{-1} diet, respectively ($P < 0.05$). Na^+/K^+-ATPase activities also increased significantly in response to the increasing lysine levels up to 8.5, 10.8 and 10.8 g kg^{-1} diet in proximal intestine, mid-intestine and distal intestine, respectively, and then, it decreased ($P < 0.05$). Creatine kinase (CK) activities in proximal intestine and mid-intestine followed the same pattern as the Na^+/K^+-ATPase activity, while the CK activity in distal intestine decreased with increasing dietary lysine level up to 15.0 g kg^{-1} diet ($P < 0.05$) and plateaued ($P > 0.05$). Quadratic regression analysis showed that creatine kinase and alkaline phosphatase activities in mid-intestine increased with increasing levels of dietary lysine. However, γ-glutamyl transpeptidase activities in proximal intestine, mid-intestine and distal intestine were not affected by dietary lysine concentration ($P > 0.05$).

Table 4 Intestinal length (IL, cm $fish^{-1}$), relative gut length (RGL), intestinal weight (IW, g $fish^{-1}$), intestosomatic index (ISI), intestinal protein content (IPC), hepatopancreatic weight (HW, g $fish^{-1}$), hepatosomatic index (HSI) and hepatopancreatic protein content (HPC) of grass carp fed diets with graded levels of lysine for 56 days

	Dietary lysine levels (g kg^{-1} diet)					
	6.6	8.5	10.8	12.9	15.0	16.7
Intestine						
IL	59.4 ± 5.06^{a}	60.3 ± 5.71^{a}	60.7 ± 5.63^{a}	65.3 ± 5.84^{b}	61.4 ± 6.00^{ab}	60.6 ± 4.45^{a}
RGL	161 ± 13.0^{b}	154 ± 15.4^{ab}	151 ± 14.5^{ab}	149 ± 13.5^{a}	$1{,}534 \pm 13.9^{ab}$	158 ± 12.8^{ab}
IW	10.353 ± 1.55^{ab}	9.506 ± 1.04^{a}	10.506 ± 1.09^{ab}	13.287 ± 1.81^{c}	11.126 ± 1.23^{ab}	11.133 ± 1.90^{ab}
ISI	1.70 ± 0.23^{d}	1.38 ± 0.17^{abc}	1.35 ± 0.14^{ab}	1.29 ± 0.13^{a}	1.44 ± 0.18^{bc}	1.51 ± 0.25^{c}
IPC	7.55 ± 0.44^{a}	7.76 ± 0.70^{ab}	8.08 ± 0.73^{ab}	8.58 ± 0.92^{b}	8.46 ± 0.65^{ab}	8.01 ± 0.81^{ab}
Hepatopancreas						
HW	14.5 ± 4.21^{a}	14.6 ± 2.79^{a}	17.1 ± 3.26^{ab}	22.2 ± 4.77^{c}	20.0 ± 3.50^{bc}	18.3 ± 5.67^{b}
HSI	2.36 ± 0.597^{ab}	2.11 ± 0.354^{a}	2.20 ± 0.407^{ab}	2.15 ± 0.411^{a}	2.58 ± 0.360^{b}	2.47 ± 0.714^{ab}
HPC	7.54 ± 0.51^{a}	9.16 ± 0.88^{b}	10.9 ± 0.96^{c}	9.27 ± 0.60^{b}	9.87 ± 0.90^{b}	9.83 ± 0.69^{b}
Regressions						
$Y_{\text{MDA in intestine}} = 8.669 - 1.063x + 0.044x^2$					$R^2 = 0.824$	$P < 0.01$
$Y_{\text{MDA in hepatopancreas}} = 4.878 - 0.668x + 0.030x^2$					$R^2 = 0.863$	$P < 0.01$
$Y_{\text{PC in intestine}} = 10.143 - 0.836x + 0.025x^2$					$R^2 = 0.787$	$P < 0.01$
$Y_{\text{AHR in hepatopancreas}} = 20.923 + 28.607x - 0.931x^2$					$R^2 = 0.870$	$P < 0.01$
$Y_{\text{GR in hepatopancreas}} = -105.80 + 22.982x - 0.914x^2$					$R^2 = 0.865$	$P < 0.01$
$Y_{\text{GSH in intestine}} = -6.895 + 1.620x - 0.067x^2$					$R^2 = 0.826$	$P < 0.01$

All data were expressed as mean ± SD ($n = 5$). Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

Intestine and hepatopancreas growth and development

The effects of lysine on intestine and hepatopancreas growth are presented in Table 4. Intestinal length, intestinal weight, hepatopancreatic weight and hepatopancreatic protein content of grass carp were significantly improved with increasing dietary lysine levels up to 12.9, 12.9, 12.9 and 10.8 g kg^{-1} diet, respectively, and decreased thereafter ($P < 0.05$). Relative gut length and intestosomatic index decreased in response to the increasing levels up to 12.9 g lysine kg^{-1} diet.

Antioxidant indicators in hepatopancreas and intestine

Malondialdehyde and protein carbonyl contents, anti-superoxide anion and anti-hydroxyl radical capacities, antioxidant enzymes activities and glutathione content in intestine and hepatopancreas of grass carp fed graded levels of lysine are displayed in Table 5. Quadratic regression analysis showed that malondialdehyde and protein carbonyl contents decreased with increasing levels of dietary lysine. Malondialdehyde content in intestine and hepatopancreas decreased with increasing dietary lysine levels up to 12.9 g kg^{-1} diet, and then, it increased ($P < 0.05$). A similar trend was observed in protein carbonyl content in intestine and hepatopancreas. Based on malondialdehyde content in intestine and hepatopancreas, the optimum lysine requirement was estimated to be 10.6 and 9.5 g kg^{-1} diet. The anti-hydroxyl radical capacity in intestine and hepatopancreas gradually increased with increasing dietary lysine levels up to 10.8 and 12.9 g kg^{-1} diet and decreased thereafter ($P < 0.05$). In hepatopancreas, the anti-hydroxyl radical capacity showed a quadratic response to increasing dietary lysine level. The anti-superoxide anion capacity in hepatopancreas followed a similar trend of

Table 5 Malondialdehyde content (MDA, nmol mg^{-1} protein), protein carbonyl content (PC, nmol mg^{-1} protein), anti-superoxide anion (ASA, U g^{-1} protein) and anti-hydroxyl radical (AHR, U mg^{-1} protein) capacities, superoxide dismutase (SOD, U mg^{-1} protein), catalase (CAT, U mg^{-1} protein), glutathione-S-transferase (GST, U mg^{-1} protein), glutathione peroxidase (GPX, U mg^{-1} protein), glutathione reductase (GR, U g^{-1} protein) activities and glutathione (GSH, mg g^{-1} protein) content in intestine and hepatopancreas of grass carp fed diet with graded level of lysine for 56 days

	Dietary lysine levels (g kg^{-1} diet)					
	6.6	8.5	10.8	12.9	15.0	16.7
Intestine						
MDA	3.63 ± 0.21^{d}	2.68 ± 0.17^{b}	2.25 ± 0.13^{a}	2.18 ± 0.18^{a}	2.75 ± 0.23^{bc}	2.95 ± 0.25^{c}
PC	5.58 ± 0.554^{e}	5.07 ± 0.428^{d}	4.10 ± 0.363^{c}	3.81 ± 0.217^{bc}	2.69 ± 0.212^{a}	3.60 ± 0.267^{b}
ASA	184 ± 14.0^{a}	201 ± 19.4^{a}	207 ± 19.3^{a}	$205 \pm 14.\ 6^{a}$	202 ± 15.6^{a}	204 ± 19.4^{a}
AHR	133 ± 9.94^{a}	165 ± 14.5^{b}	205 ± 13.6^{c}	154 ± 9.81^{b}	155 ± 12.9^{b}	151 ± 10.0^{b}
SOD	24.9 ± 2.34^{a}	26.9 ± 2.94^{a}	28.0 ± 2.68^{a}	25.8 ± 1.16^{a}	25.5 ± 2.62^{a}	$25.\ 9 \pm 2.44^{a}$
CAT	23.18 ± 2.06^{c}	30.57 ± 2.39^{d}	34.69 ± 1.91^{e}	17.44 ± 1.49^{b}	10.35 ± 0.92^{a}	10.45 ± 0.82^{a}
GST	16.10 ± 1.27^{b}	27.63 ± 2.40^{c}	16.61 ± 1.65^{b}	15.94 ± 1.43^{b}	15.20 ± 1.28^{b}	9.56 ± 0.67^{a}
GPX	160 ± 11.6^{b}	177 ± 16.5^{c}	152 ± 15.7^{b}	112 ± 12.1^{a}	111 ± 11.5^{a}	113 ± 8.13^{a}
GR	22.1 ± 1.74^{a}	22.0 ± 1.42^{a}	47.7 ± 2.48^{b}	45.6 ± 3.34^{b}	23.1 ± 1.87^{a}	23.4 ± 1.41^{a}
GSH	0.82 ± 0.087^{a}	1.98 ± 0.114^{c}	3.37 ± 0.197^{f}	2.45 ± 0.119^{e}	2.29 ± 0.121^{d}	1.71 ± 0.110^{b}
Hepatopancreas						
MDA	1.818 ± 0.163^{b}	1.363 ± 0.098^{a}	1.270 ± 0.075^{a}	1.248 ± 0.103^{a}	1.823 ± 0.162^{b}	2.179 ± 0.153^{c}
PC	3.84 ± 0.35^{c}	3.86 ± 0.37^{c}	2.11 ± 0.26^{a}	2.94 ± 0.28^{b}	2.89 ± 0.25^{b}	3.51 ± 0.27^{c}
ASA	296 ± 25.0^{ab}	295 ± 29.1^{ab}	290 ± 25.7^{a}	324 ± 17.3^{b}	264 ± 23.2^{a}	269 ± 26.0^{a}
AHR	167 ± 15.0^{a}	201 ± 8.79^{b}	217 ± 10.1^{c}	239 ± 6.57^{d}	237 ± 9.89^{d}	241 ± 10.2^{d}
SOD	97.3 ± 7.60^{bc}	102 ± 3.01^{c}	96.3 ± 5.57^{abc}	99.3 ± 5.56^{c}	90.0 ± 5.68^{a}	90.9 ± 3.48^{ab}
CAT	17.4 ± 1.21^{a}	17.6 ± 0.78^{ab}	17.0 ± 1.17^{a}	19.2 ± 1.35^{b}	18.5 ± 1.29^{ab}	18.0 ± 1.51^{ab}
GST	46.5 ± 3.21^{c}	47.4 ± 4.65^{c}	39.2 ± 1.87^{a}	40.0 ± 3.74^{ab}	43.7 ± 3.48^{bc}	45.3 ± 4.12^{c}
GPX	870 ± 90.4^{a}	955 ± 37.1^{b}	983 ± 46.5^{b}	971 ± 59.0^{b}	822 ± 44.2^{a}	823 ± 41.3^{a}
GR	4.32 ± 0.43^{a}	24.3 ± 1.43^{b}	40.9 ± 3.63^{e}	36.4 ± 3.00^{d}	27.4 ± 1.82^{c}	27.2 ± 1.63^{c}
GSH	8.30 ± 0.51^{a}	9.95 ± 0.73^{bc}	9.84 ± 0.56^{b}	10.6 ± 0.78^{c}	8.84 ± 0.58^{a}	8.75 ± 0.55^{a}

All data were expressed as mean ± SD ($n = 5$). Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

the anti-hydroxyl radical capacity ($P < 0.05$). The activity of catalase in intestine and hepatopancreas increased with increasing dietary lysine levels up to 10.8 and 12.9 g kg^{-1} diet ($P < 0.05$). Meanwhile, activities of intestinal and hepatopancreatic glutathione peroxidase and glutathione reductase increased with the increasing dietary lysine levels up to 8.5, 10.8, 10.8 and 10.8 g kg^{-1} diet and then decreased ($P < 0.05$). Glutathione content in intestine and hepatopancreas showed a similar trend as glutathione reductase activities. Regression analysis showed that glutathione reductase activities in hepatopancreas and glutathione content in intestine were quadratic responses to increasing dietary lysine levels. Intestinal glutathione-S-transferase activities were improved with increasing dietary lysine levels up to 8.5 g kg^{-1} and decreased thereafter ($P < 0.05$). However, the activity of glutathione-S-transferase in hepatopancreas showed the pattern opposite to that in intestines. Superoxide dismutase activities of fish fed diet containing lysine from 6.6 to 12.9 g kg^{-1} were not significant from each other and decreased with dietary lysine levels up to 15.0 g kg^{-1} ($P < 0.05$). Dietary lysine level had no significant effect on intestinal anti-superoxide anion capacity and superoxide dismutase activity ($P > 0.05$; Fig. 1).

Discussion

The present study indicates that fish fed deficiency dietary lysine showed significant lower weight gain,

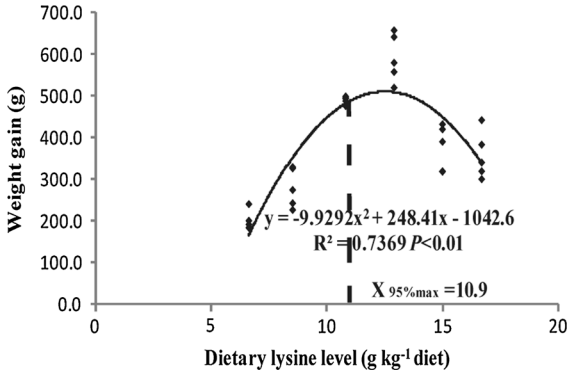

Fig. 1 Quadratic regression analysis of weight gain (WG) for sub-adult grass carp (*Ctenopharyngodon idella*) fed diets containing graded levels of lysine for 56 days

FI, FE and SGR compared to the optimum dietary lysine level, which agreed with the reports in juvenile grass carp (Wang et al. 2005) and rainbow trout (Walton et al. 1984). Maximum weight gain, FI, FE and SGR occurred in grass carp fed diets containing 12.9 g lysine kg^{-1}, after which it decreased as the lysine level increased, which confirmed the results from other reports (Ahmed and Khan 2004; Wang et al. 2005).

Dose–response experiments with increasing supply of amino acid are accepted in principle as a method for determining dietary amino acid requirements (Cowey 1995). In the present study, on subjecting the weight gain to quadratic regression analysis at 95 % maximum response, the dietary lysine requirements of 500–1,000 g grass carp were 10.9 g kg^{-1} diet (38.9 g kg^{-1} protein), which are similar to those in other fish species, such as freshwater catfish (*mystus nemurus*) (3.5 g/100 g of CP; Tantikitti and Chimsung 2001), rainbow trout (*oncorhynchus mykiss*) (3.7 g/100 g of CP; Kim et al. 1992) and Atlantic salmon (*salmo salar*) (4.0 g/100 g of CP; Anderson et al. 1993). Furthermore, the lysine requirements were lower than that of 3–15 g grass carp, 20.7 g kg^{-1} diet (corresponding to 54 g kg^{-1} dietary protein) reported by Wang et al. (2005). The low requirements of lysine in sub-adult grass carp may be partly related to protein concentration, feed conversion ratio (FCR) and growth rate. Hua (2012) concluded that dietary protein content has a significant effect on lysine requirement through meta-analysis of thirty-four fish species. Moreover, the efficiency of utilization of the amino acid decreases progressively with increasing protein level in fish (Hafedh 1999). In our study, the FCR of sub-adult grass carp is lower than that in juvenile grass carp (Fig. 2).

Glutamate–oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) are two important amino acid metabolic enzymes of fish (D'apollonia and Anderson 1980). The present results showed that the activities of GOT in muscle and GPT in hepatic were the highest in lysine-unsupplemented group, suggesting that lysine deficiency may improve transamination. However, GOT activity in hepatic and GPT activity in muscle increased with the increase in dietary lysine level. The reason for these interesting results may attribute to the special metabolism of lysine in vivo. Studies have showed that lysine was not

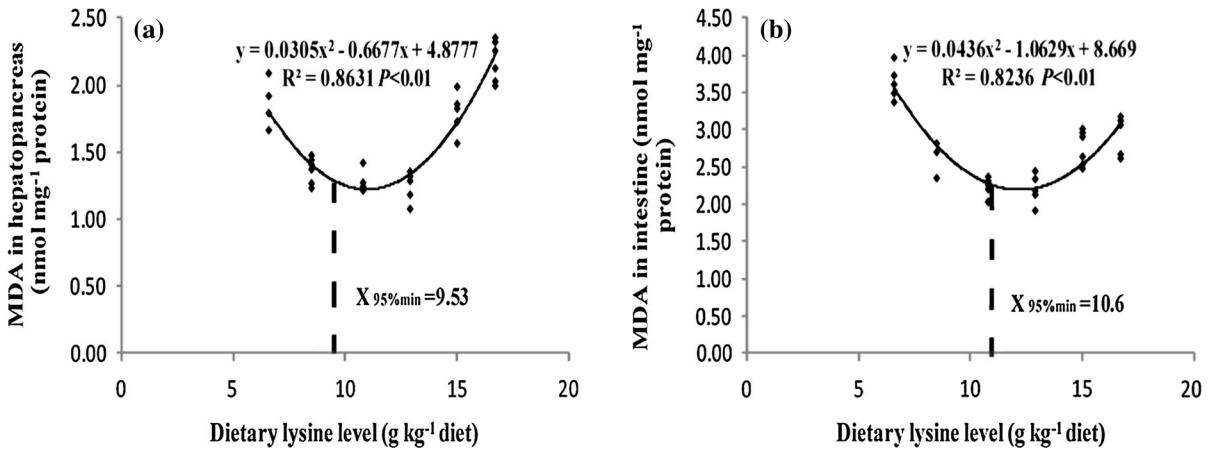

Fig. 2 Quadratic regression analysis of malondialdehyde content (MDA) in hepatopancreas (**a**) and intestine (**b**) for sub-adult grass carp (*Ctenopharyngodon idella*) fed diets containing graded levels of lysine for 56 days

involved in transamination directly in goldfish tissues (van Waarde 1981). The discrepancy of patterns of GOT and GPT may be related to the tissue differences as described for other fish species (Salvatore et al. 1965; van Waarde 1981). Further research is needed to clarify this aspect.

In our study, the activities of trypsin, chymotrypsin and lipase in intestine were improved with increasing lysine levels up to certain values, suggesting lysine supplementation improved the activities of digestive enzymes of sub-adult grass carp. The benefit effects of dietary lysine on digestive enzymes activities may be explained by several hypotheses. Firstly, lysine may stimulate synthesis of digestive enzymes in pancreas of fish. Grendell et al. (1984) indicated that lysine increased total trypsinogen output in pancreas of rat. Secondly, lysine may affect the secretion and release of digestive enzyme in fish. In larvae gilthead seabream, lysine served as stimulant to release trypsinogen in pancreas (Naz and Türkmen 2008). Grendell et al. (1984) reported that cholecystokinin (CCK) can regulate the release of trypsin in the gut of rats. Lysine promoted CCK secretion in larvae gilthead seabream (Naz and Türkmen 2008). Interestingly, in our study, amylase activity decreased with increasing dietary lysine level. These may attribute that lysine inhibited secretion of thyroid hormone, which can increase the activity of amylase in fish. Carew et al. (2005) reported that lysine deficiency caused an elevation in plasma triiodothyronine levels of chickens, which was an important thyroid hormone. Thyroid increased the amylase activities in the pancreas of infant rats (Takeuchi et al. 1977). To our knowledge, nutrients absorption and transport in fish intestine is related to gut brush-border membrane enzymes activities, such as Na^+/K^+-ATPase, AKP, CK and γ-GT (Tengjaroenkul et al. 2000; Weng et al. 2002). In our studies, dietary lysine supplementation significantly increased Na^+/K^+-ATPase and AKP activities in PI, MI and DI, and CK activities in PI and MI of sub-adult grass carp, suggesting dietary lysine improved absorptive function of sub-adult grass carp. However, the effects of lysine on the activities of brush-border membrane enzymes have not been studied in fish. A recent study from our laboratory reported that coated lysine increased intestinal AKP activity in PI and MI compared with uncoated lysine on equal basis of Jian carp (Zhou et al. 2007). Interestingly, CK was decreased with increasing dietary lysine levels in DI. Studies showed that nutrients digestion and absorption is mainly on proximal of intestine (Tengjaroenkul et al. 2000). In our study, γ-GT in PI, MI and DI was not affected by dietary lysine levels. In rats, protein-energy malnutrition did not alter the mRNA levels of γ-GT in the ileum (Jonas et al. 2000). However, the underlying mechanism by which lysine influences intestinal digestive enzymes and brush-border enzymes activities needs further investigation in fish.

The benefit effects of digestive and brush-border enzymes were probably due to the normal function of digestive organ (Tengjaroenkul et al. 2000). In the present study, the IL, IW, HW and HPC increased with increasing dietary lysine concentrations up to certain values, which agreed with the report in juvenile Jian carp (Zhou et al. 2007). These may attribute that lysine promotes protein synthesis and tissue renew in the digestive organ of fish. Studies showed that dietary lysine was directly utilized by the intestine for protein synthesis in pig (Stoll et al. 1998). Hevrøy et al. (2007) reported lysine upregulated hepatic insulin-like growth factor-1 (IGF-1) mRNA level in Atlantic salmon, which is a major anabolic agent responsible for tissue growth in animals (Sukhanov et al. 2007). However, fish fed optimum lysine showed the lowest ISI. The development of digestive organs is rapid in early stage (Ribeiro et al. 1999), but muscle growth is particularly important for growing fish to reach large size (Dwyer et al. 1993). The decreased ISI may be that the skeletal muscle ratio to digestive organs weight was high, and protein accumulation in skeletal muscle was rapid in sub-adult fish.

Many studies have confirmed that oxidative stress caused the change of cellularity in digestive organ of rats (Vardi et al. 2008). Therefore, we investigated the effects of lysine on antioxidant response in hepatopancreas and intestine. Our study showed that MDA and PC contents decreased with increasing dietary lysine levels up to certain values in hepatopancreas and intestine, suggesting that lipid peroxidation and protein oxidation in these organs were decreased by lysine. Based on MDA content data, the dietary lysine requirements of sub-adult grass carp were estimated to be 9.53–10.6 g kg^{-1} diet, which was slightly lower than the lysine requirement based on weight gain. The oxidative damage of lipid and protein was induced mainly by the superoxide anions and the hydroperoxyl radical (Valko et al. 2007). In the present study, with the increase in dietary lysine levels up to certain

values, the scavenging abilities against the superoxide anion (ASA) and the hydroxyl radical (AHR) in hepatopancreas and AHR ability in intestine were improved, whereas intestine ASA showed no alterations. In vertebrates, lysine provides the carbon backbone of carnitine biosynthesis (Harpaz 2005), carnitine prevents the accumulation of end products of lipid peroxidation and has an effective superoxide anion radical scavenging, hydrogen peroxide scavenging and ferrous ions chelating abilities in vitro (Gülçin 2006). In Atlantic salmon, lysine upregulated hepatic and muscle IGF mRNA level (Hevrøy et al. 2007). IGF can suppress superoxide levels in mice aortae (Sukhanov et al. 2007). Nevertheless, the mechanism about the effects of lysine on free radical scavenging abilities in fish needs further investigation.

Free radical scavenging capacities substantially contribute to their enzymatic and non-enzymatic antioxidant defence systems (Valko et al. 2007). Non-enzymatic antioxidants are represented by GSH (Lora et al. 2004). Our results clearly showed GSH content in hepatopancreas and intestine increased by lysine levels up to certain values. Correlated analysis showed that ASA was positive to GSH in intestine ($r = +0.843$ $P < 0.05$) and hepatopancreas ($r = +0.698$ $P = 0.123$), and AHR was positive to GSH in intestine ($r = +0.899$ $P < 0.05$), suggesting lysine elevated fish non-enzymatic defence. Firstly, lysine may promote GSH synthesis in fish. In rainbow trout liver, lysine is catabolized via the saccharopine pathway in which glutamate is produced (Higgins et al. 2005). Stoll et al. (1998) reported that glutamate can convert into GSH in the intestine of piglets. Secondly, lysine may promote the antioxidant properties of GSH through maintaining the intracellular GSH/oxidized glutathione (GSSG) ratio in fish. In goldfish, GR catalyze the reduction of the GSSG to GSH (Lushchak et al. 2001). In our studies, GR activity was increased by lysine in hepatopancreas and intestine. Correlation analysis showed that GSH was positively related to GR in hepatopancreas ($r = +0.723$ $P = 0.104$) and intestines ($r = +0.766$ $P = 0.076$). Additionally, our study showed that the activities of GPx and CAT in hepatopancreas and intestine, as well as GST activity in intestine, increased by dietary lysine, suggesting lysine can increase enzymatic antioxidant capacity in fish. However, no information is reported about the effects of dietary lysine on antioxidant enzymes activities in fish. The elevation of GPx and GST activities may be related to the increased GSH content. In goldfish, GSH is a substrate for GPx and GST during the enzymatic detoxification (Lushchak et al. 2001). Pandey et al. (2001) reported that the upregulation of GSH levels was concomitant with the increase in GPx and GST activities in Bloch. Correlation analysis showed that GPx was positively related to GSH in hepatopancreas ($r = +0.848$ $P < 0.05$). In our study, hepatopancreatic GST activity was the highest in lysine-unsupplemented diet. The possible explanation may be that the increase in GST activity in hepatopancreas provides protection against lipid peroxidation (LPO) damage during lysine deficiency group in fish. In a word, the influence of lysine on antioxidant defence in fish may be also achieved by this way, which warrants further study.

In conclusion, the present work indicated that dietary lysine improved growth and enhanced intestinal and hepatopancreatic enzyme activities of sub-adult grass carp. Meanwhile, lysine could promote the antioxidant defence in fish intestine and hepatopancreas by increasing enzymatic antioxidant capacity and GSH content, thus protecting the structure and function of these organs. On the basis of this study, the lysine requirement of sub-adult grass carp (500–1,000 g) based on weight gain was determined to be 10.9 g kg^{-1} diet by the quadratic regression. Requirement estimated on the basis of malondialdehyde content in intestine and hepatopancreas was 10.6 and 9.53 g lysine kg^{-1} diet, respectively.

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