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Original Research Article

Effect of dietary *L*-glutamate levels on growth, digestive and absorptive capability, and intestinal physical barrier function in Jian carp (*Cyprinus carpio* var. Jian)

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ABSTRACT

The present study explored effects of *L*-glutamate (Glu) levels on growth, digestive and absorptive capability, and intestinal physical barrier functions of Jian carp (*Cyprinus carpio*). A total of 600 Jian carp (126.40 ± 0.21 g) were randomly distributed into 5 groups with 3 replicates each, fed diets containing graded levels of Glu (53.4 [control], 57.2, 60.6, 68.4, and 83.4 g/kg) for 63 d. Results showed compared with control diet, feed intake and percent weight gain (PWG) in fish fed 83.4 g of Glu/kg diet were increased and feed conversion ratio in fish fed 68.4 g of Glu/kg diet was decreased ($P < 0.05$). Similarly, body crude protein and lipid contents in fish fed 68.4 g of Glu/kg diet were higher ($P < 0.05$). The activities of trypsin and chymotrypsin in the hepatopancreas and intestine, and amylase, alkaline phosphatase (AKP), Na^+, K^+-ATPase (NKA), and creatine kinase (CK) in intestine were higher in fish fed 68.4 g of Glu/kg diet ($P < 0.05$). Dietary Glu (57.2 to 83.4 g/kg diet) decreased malondialdehyde (MDA) and protein carbonyl (PCO) contents in the intestine ($P < 0.05$). The activities of catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) in the hepatopancreas and intestine were higher in fish fed 60.6 and 68.4 g of Glu/kg diets ($P < 0.05$). Intestinal the glutathione reductase (GR) activity and glutathione (GSH) content in fish fed 60.6, 68.4, and 83.4 g of Glu/kg diet were increased ($P < 0.05$). The GPx1a, GST, and nuclear factor erythroid 2-related factor 2 (Nrf2) mRNA expressions in the intestine were up-regulated in fish fed 60.6 and 68.4 g of Glu/kg diet ($P < 0.05$). The zonula occludens protein-1 (ZO-1), occludin1, and claudin3 mRNA expressions were also up-regulated in fish fed 83.4 g of Glu/kg diet ($P < 0.05$). Fish fed 68.4 g of Glu/kg diet had higher levels of claudin 2, claudin7, and protein kinase C (PKC) mRNA ($P < 0.05$). These results indicated that Glu improved fish growth, digestive and absorptive ability, and intestinal physical barrier functions. Based on the quadratic regression analysis of PWG, and MDA of the hepatopancreas and intestine, the optimal dietary Glu levels were estimated to be 81.97, 71.06, and 71.36 g/kg diet, respectively.

1. Introduction

L-glutamate (Glu) is regarded as one of dispensable amino acids in fish (NRC, 2011). Recently, growing evidences show that some dispensable amino acids and their metabolites are important regulators of key metabolic pathways, which are necessary for maintenance, growth, feed intake, nutrient utilization, immunity, as well as resistance to environmental stressors and pathogenic organisms in various fishes (Wu et al., 2012, 2011; Xie et al., 2016,

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2014). Beyond the role of protein synthesis, previous studies also demonstrated Glu has an important role in fish growth, feed utilization, and intestinal health (Caballero-Solares et al., 2015; Chika et al., 2016; Zhao et al., 2015). Dietary supplementation with 8 g of Glu/kg diet improves growth, protein and lipid utilization in grass carp (*Ctenopharyngodon Idella*) (Zhao et al., 2015), with 40 g of Glu/kg diet promotes protein and lipid utilization in gilthead seabream *Sparus aurata* (Caballero-Solares et al., 2015), with 20 g of Glu/kg diet increases growth in rainbow trout *Oncorhynchus mykiss* (Chika et al., 2016). The other study shows that dietary supplementation with 15 g of Glu/kg diet has no effect on growth and feed utilization in Atlantic salmon *Salmo salar* (Larsson et al., 2014). These results showed that dietary Glu level can affect the piscine growth and feed utilization rate, but different fish species might have different optimum levels. According to above studies, based on the better growth performance, dietary Glu supplementation level varies from 8 to 40 g/kg diet in different fish species (Caballero-Solares et al., 2015; Chika et al., 2016; Zhao et al., 2015).

Fish growth depends on the nutrient digestion and absorption, which is governed by the activities of enzymes including digestive and absorptive enzymes (Deng et al., 2010; Hakim et al., 2009). To our knowledge, there is only one report regarding the effects of dietary Glu on the digestive and absorptive capacity of fish, which showed that diet supplementation with 8 g of Glu/kg diet improved the intestinal digestive enzymes activities in grass carp, as well as the activities of intestinal brush-border enzymes, including alkaline phosphatase (AKP), creatine kinase (CK), Na^+, K^+-ATPase (NKA), and γ-glutamyl transpeptidase (γ-GT) (Zhao et al., 2015). Meanwhile, Glu is one of the most abundant amino acids, which is a truly functional amino acid (Brosnan and Brosnan, 2013). Nevertheless, despite its importance in fish nutrition, few studies have so far endeavored to determine the optimum level of dietary Glu for fish growth, digestive and absorptive function. Therefore, the 5 isoenergetic diets were formulated to supplement with 0 (53.4), 4 (57.2), 8 (60.6), 16 (68.4), 32 (83.4) g/kg Glu to evaluate the effect of dietary Glu on growth performance and digestive and absorptive capability in Jian carp.

Fish intestinal physical barrier function is closely related to the integrity of cellular structure (Zhang et al., 2002). At the same time, the structural integrity of fish intestine is the guarantee of its normal digestion and absorption. Intestinal antioxidant capacity plays a key role in maintaining piscine intestinal structural integrity and function (Jiang et al., 2015a,b,c). To prevent oxidative damage, fish have developed non-enzymatic and enzymatic antioxidant defense systems (Martínez-álvarez et al., 2005). Glutathione (GSH) is an important non-enzymatic antioxidant compound of fish (Hong et al., 2015). The Glu is the preferred source for mucosal GSH synthesis in the intestine (Reeds et al., 1997). The pathway for the synthesis of GSH in the enterocyte cytosol is probably limited by Glu availability (Blachier et al., 2009). As other aerobic organisms, fish developed diverse antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR), and glutathione peroxidase (GPx) (Zhang et al., 2004). The previous studies showed that dietary supplementation with 8 g of Glu/kg diet improved intestinal antioxidant capacity in grass carp (Zhao et al., 2015) and culture medium pre-supplementation with Glu enhanced antioxidant capacity and regulated antioxidant-related signaling molecule expression in fish enterocytes (Jiang et al., 2015a,b,c). These results suggested Glu plays an important role in fish intestines. Therefore, it is worthy to investigate the optimum level of dietary Glu on effect of intestinal antioxidant capacity and antioxidant-related signaling molecule expression in fish.

In addition to the intestinal antioxidant capacity, the structure integrity also plays a vital role in absorption capacity of intestines (Ballard et al., 1995). The intercellular structural integrity in the intestine depends largely on tight junction (TJ) proteins (such as zonula occludens protein-1 [ZO-1], the transmembrane protein occludin, members of the claudin family, and others) (Gonzalez-Mariscal et al., 2008). Intestinal mucosal absorption ability may be modified by modulating claudin expression specificity (Matsuhisa et al., 2012). The Ca^{2+} and Mg^{2+} absorption is increased by increasing expression of claudin2 (GaffneyStomberg et al., 2011) and claudin7 (Thongon and Krishnamra, 2012). Previous study also showed the integrity of TJ can be damaged by altered level of TJ protein expression (Landy et al., 2016). Dysfunction of TJ can lead to the disruption of intestinal barrier function. Emerging evidence showed the expression levels of TJ protein is modulated by valine, folic acid, and isoleucine in fish (Luo et al., 2014; Shi et al., 2016; Zhao et al., 2014). In piglets, dietary Glu supplementation increased the relative mRNA expression of ZO-1 and occludin in jejunal mucosa (Luo et al., 2014). However, information regarding the effects of Glu on TJ proteins in fish remains scarce.

Thus, this study was conducted to test the following hypothesis: dietary Glu improved growth, digestive and absorptive capability, and intestinal physical barrier function by enhancing digestion and absorption enzyme activities and TJ gene expressions in Jian carp.

2. Materials and methods

Feeding management of fish was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Sichuan Agricultural University, China.

2.1. Experimental design and diets

Feed formulation and chemical composition of diets are presented in Tables 1 and 2. Crystalline amino acids (Donboo Amino Acid, Nantong, Jiangsu, China) were used to simulate the amino acid pattern of diets. Soybean meal, canola meal, corn gluten meal, and fish meal were used as dietary protein sources. All feed ingredients were purchased from Xinnong Feed Company (Shanghai, China). Soybean oil and wheat flour were used as dietary lipid and carbohydrate sources, respectively. The basal diet was formulated to meet the nutrient requirements of carp based on NRC (National Research Council) (2011). The 5 isoenergetic diets were supplemented with Glu (0, 4, 8, 16, 32 g/kg) to provide Glu at the concentrations of 53.4 (control), 57.2, 60.6, 68.4, and 83.4 g/kg diet. Increased Glu levels were compensated by decreasing equal levels of wheat flour. Differences in the amino acid composition of diets were negligible (Table 2), except for diets Glu. Dietary Glu content was analyzed via high performance liquid chromatography as described by Buentello and Gatlin (2002). All dry ingredients were ground through a 60-mesh screen. The diets were prepared by mixing the dry ingredients with the oil and water using a mixer. Then each diet was extruded in a twin-screw extruder (MY-165) with a 2-mm die (EXT50A, Yang gong Machine, China). The processing conditions were as follows: 100 r/min screw speed, 127 °C, and 3.04 to 4.56 MPa. Floating extruded pellets were air-dried and stored at 4 °C in plastic bags until being used.

2.2. Fish management and feeding

The feeding trial was conducted at Experiment Station of Ya'an, Sichuan Agricultural University, China. Carp, obtained from the Tongwei fisheries (Sichuan, China), and were acclimatised to the experimental (outdoor) conditions for 4 wk. Fish were fed 3 times daily with the control diet to satiation during this period. A total of

Table 1
Composition and nutrient contents of the experimental diets (g/kg, as fed basis).

Item	Dietary *L*-glutamate level, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
Ingredients					
Soybean meal	290	290	290	290	290
Canola meal	220	220	220	220	220
Corn gluten meal	60	60	60	60	60
Fish meal	60	60	60	60	60
Wheat flour	277	273	269	261	245
Soy oil	50	50	50	50	50
Vitamin premix[1]	10	10	10	10	10
Trace mineral premix[2]	10	10	10	10	10
Choline chloride	10	10	10	10	10
Monocalcium phosphate	2	2	2	2	2
Lysine	6	6	6	6	6
Methionine	2	2	2	2	2
Threonine	3	3	3	3	3
L-glutamate	0	4	8	16	32
Total	1,000	1,000	1,000	1,000	1,000
Analyzed chemical composition					
Crude protein	306.0	312.4	316.0	316.3	322.3
Crude lipid	60.9	61.9	61.5	60.1	60.0
Crude ash	70.6	71.4	73.1	70.4	69.9

[1] Per kilogram of vitamin premix contained the following: 400,000 IU of vitamin A as retinyl acetate; 240,000 IU of vitamin D_3; 10.000 g of vitamin E; 0.100 g of vitamin K as menadione sodium bisulfate; 0.010 g of cyanocobalamin; 0.100 g of *D*-biotin; 0.500 g of folic acid; 0.102 g of vitamin B_1 as thiamin nitrate; 6.667 g of vitamin C as ascorbyl acetate; 2.800 g of niacin; 51.800 g of meso-inositol; 2.461 g of *D*-pantothenic acid as calcium-*D*-pantothenate; 0.500 g of riboflavine; 0.740 g of vitamin B_6 as pyridoxine hydrochloride. All ingredients were diluted with corn starch to 1 kg.

[2] Per kilogram of mineral premix contained the following: 13.730 g Fe of as $FeSO_4 \cdot 7H_2O$; 0.300 g Cu of as $CuSO_4 \cdot 5H_2O$; 4.869 g Zn of as $ZnSO_4 \cdot 7H_2O$; 1.200 g Mn of as $MnSO_4 \cdot H_2O$; 0.110 g I of as KI; 0.025 g Se of as $NaSeO_3$. All ingredients were diluted with $CaCO_3$ to 1 kg.

600 fish with similar sizes (mean initial weight 126.40 ± 0.21 g) were randomly assigned into 15 outdoor concrete tanks (2 m × 1.5 m × 1 m), resulting in 40 fish in each tank. During the experimental period, fish were reared under natural light conditions. Water temperature was measured at 08:00, 13:00, and 18:00 daily. Average water temperature was 25.5 ± 3.0 °C. Continuous flowing water were maintained at the rate of 0.7 L/min in each tank.

Table 2
Amino acid composition of experimental diets (g/kg, dry matter basis).

Item	Dietary *L*-glutamate level, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
Essential amino acids					
Lysine	20.7	20.7	20.6	20.6	20.6
Methionine	6.8	6.8	6.8	6.7	6.7
Threonine	15.3	15.3	15.3	15.3	15.2
Arginine	18.7	18.7	18.6	18.6	18.5
Leucine	26.4	26.4	26.4	26.3	26.2
Histidine	7.1	7.1	7.1	7.1	7.1
Isoleucine	13.6	13.5	13.5	13.5	13.4
Phenylalanine	14.5	14.5	14.5	14.5	14.5
Valine	14.2	14.2	14.2	14.2	14.2
Non-essential amino acids					
Alanine	14.6	14.5	14.5	14.5	14.4
Aspartate	25.4	25.4	25.4	25.3	25.2
Cystine	5.0	5.0	4.9	4.9	4.9
Glutamine	2.5	2.6	2.6	2.6	2.5
Glutamate	53.4	57.2	60.6	68.4	83.4
Glycine	14.6	14.6	14.5	14.5	14.4
Proline	16.5	16.5	16.4	16.3	16.2
Serine	14.8	14.8	14.8	14.8	14.7
Tyrosine	10.8	10.8	10.8	10.8	10.7

The pH was maintained at 7.0 ± 0.5. Water was continuously aerated using air stones to adjust the dissolved oxygen (>5.0 mg/L). Each of the 5 diets was fed to 3 replicates of fish 3 times daily (07:20, 13:20, and 18:20) to satiation for 63 d. Uneaten feed was collected 1 h after feeding, dried and weighed to calculate feed intake according to Zhao et al. (2015).

2.3. Sample collection and analysis

After a fasting period of 24 h, fish in each tank were weighed and counted at the initiation and termination of the feeding trail. Prior to sampling, fish were anaesthetized in benzocaine bath (50 mg/L). Fifteen fish from the same population before the experiment and 5 fish from each tank at the end of feeding trail were selected for determination of initial and final carcass proximate composition respectively. The proximate compositions of fish carcass and feed were measured according to AOAC (Horwitz, 2000). The hepatopancreas and intestines of another 6 fish from each tank were quickly removed, weighed and frozen in liquid nitrogen, then stored at −70 °C until analysis.

The intestine and hepatopancreas samples were each homogenized in 10 volumes (wt/vol) of ice-cold physiological saline solution and centrifuged at 6,000 × *g* for 20 min at 4 °C. The supernatant was collected for enzyme activity analysis. Trypsin, amylase, and lipase activities were determined according to Zhao et al. (2015). Trypsin activity was determined using ρ-toluenesulphonyl-l-arginine methyl esther as a substrate in 0.05-mol/L Tris–HCl buffer, pH = 9.0. Amylase was assayed using 1% soluble starch as a substrate in 0.02-mol/L phosphate buffer, pH = 8.0. Lipase activity was measured at 405 nm by the rate of methylresorufin formation at 37 °C using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The activities of alkaline phosphatase (AKP) and NKA are assayed according to Hong et al. (2015). To measure AKP activity, 50 μL of samples was mixed with 1.0 mL of reaction solution containing disodium phenyl phosphate. Absorbance was monitored at 520 nm. The NKA, creatine kinase (CK) and gamma-glutamyl transpeptidase (γ-GT) activities were determined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The intestine and hepatopancreas protein content was determined using the Coomassie Brilliant Blue dye binding technique with bovine serum albumin as the standard following the method of Bradford (1976). The intestinal malondialdehyde (MDA) content was analysed using the thiobarbituric acid reaction. The MDA forms a red adduct with thiobarbituric acid, with an absorbance at 532 nm. The protein carbonyl (PCO) content was determined according to the method described by Jiang et al. (2015a,b,c) with a minor modification using 2,4-dinitrophenylhydrazine (DNPH) reagent. The GSH content and activities of SOD, CAT, and GR in the intestine were determined by the commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The intestinal superoxide anion ($\cdot O_2^-$) (SAS) and hydroxyl radical ($\cdot OH^-$) (HRS) scavenging activities were measured as our previous study described by Jiang et al. (2015b). The SAS was determined by Superoxide Anion Free Radical Detection Kit. Superoxide radicals were generated by the action of xanthine and xanthine oxidase, with the electron acceptor added, a colouration reaction is developed using the griess reagent. The colouration degree is directly proportional to the quantity of superoxide anion in the reaction. The HRS was determined by a Hydroxyl Free Radical Detection Kit. Hydroxyl radicals are generated in the Fenton reaction, with the electron acceptor added, a colouration reaction is developed using the griess reagent. The colouration degree is directly proportional to the quantity of hydroxyl radicals in the reaction.

2.4. Real-time quantitative PCR

The procedures of RNA isolation, reverse transcription and real-time quantitative PCR were similar to the previous study (Jiang et al., 2016). The total RNA was extracted from the intestine using RNAiso Plus kit (TaKaRa, Dalian, China) according to the manufacturer's instructions followed by DNAse I treatment. The RNA purity and integrity were assessed spectrophotometric (A260:A280 ratio) analysis and agarose gel (1%) electrophoresis, respectively. Subsequently, the 2 μL of total RNA was used to synthesize cDNA using the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). Specific primers for ZO-1 were designed according to sequences of Jian carp cloned in our laboratory (Table 3), and the primers for Cu/Zn superoxide dismutase (CuZnSOD), CAT, GPx1a, GPx1b, GST, GR, nuclear factor erythroid 2-related factor 2 (Nrf2), Keap1, occludin1, claudin2 (Jiang et al., 2016), claudin3, claudin7, and protein kinase C (PKC) were designed using the published sequences of Jian carp (Table 3). All of the real-time quantitative PCR analyses were performed in a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Elongation factor 1A (EF1a) was used as a reference gene to normalize cDNA loading, according to the results of our preliminary experiment concerning the evaluation of internal control genes. The amount of the target gene was calculated based on the threshold cycle number (CT), and the CT for each sample was determined using the CFX Manager software. All of the primer amplification efficiencies were approximately 100%. The gene expression results were analyzed using the $2^{-\Delta\Delta CT}$ method according to Jiang et al. (2015a,b,c).

Table 3
The primers and annealing temperature used for real-time quantitative PCR.

Item	Sequence (5′-3′)	Annealing temperature, °C	GenBank ID
CuZnSOD	F: TGGCGAAGAAGGCTGTTTGT R: TTCACTGGAGACCCGTCACT	60.4	JF342355
CAT	F: CTGGAAGTGGAATCCGTTTG R: CGACCTCAGCGAAATAGTTG	54	JF411604
GPx1a	F: GTGACGACTCTGTGTCCTTG R: AACCTTCTGCTGTATCTCTTGA	60.4	JF411605
GPx1b	F: TATGTCCGTCCTGGCAATGG R: ATCGCTCGGGAATGGAAGTT	60.4	JF411606
GST	F: TCTCAAGGCACCCGTCTG R: TCTCCAAGTATCCATCCCACA	56.6	DQ411314.1
GR	F: GAGAAGTACGACACCATCCA R: CACACCTATTGAACTGAGATTGAG	56	JF411607
Nrf2	F: TTCCCGCTGGTTTACCTTAC R: CGTTTCTTCTGCTTGTCTTT	60	JX462955
Keap1	F: GCTCTTCGGAAACCCCT R: GCCCCAAGCCCACTACA	60	JX470752
ZO-1	F: GCGAAATGACACGGGCTAT R: CTCTGTTGTGGTTGAGTGTAGGC	65	KY290394
Occludin1	F: ATCGGTTCAGTACAATCAGG R: GACAATGAAGCCCATAACAA	55.5	KF975606
Claudin2	F: CTGGAGTTGATGGGTTTCTTTTG R: AGACCTTTCATGCTTTCTACCG	63.5	Syakuri et al. (2013)
Claudin3	F: GCACCAACTGTATCGAGGATG R: GGTTGTAGAAGTCCCGAATGG	56.6	JQ767157.1
Claudin7	F: CTTCTATAACCCCTTCACACCAG R: ACATGCCTCCACCCATTATG	56	JQ767155.1
PKC	F: AAATCCACCAAGCGACCT R: CGAACCCTCCsCACAGACG	60	JX470751.1
EF1a	F: TCACCATTGACATTGCTCTC R: TGTTCTTGATGAAGTCTCTGT	56	AF485331

CuZnSOD = Cu/Zn superoxide dismutase; F = forward; R = reverse; CAT = catalase; GPx1a = glutathione peroxidase 1a; GPx1b = glutathione peroxidase 1b; GST = glutathione S-transferase; GR = glutathione reductase; Nrf2 = nuclear factor erythroid 2-related factor 2; ZO-1 = zonula occludens protein-1; PKC = protein kinase C; EF1a = elongation factor 1a.

2.5. Statistical analysis

Results were presented as means ± stand error (SE). The general linear models (GLM) procedure of SAS software (SAS Institute Inc., 2006) was used to determined treatment effects, and considered significant when $P < 0.05$ as described (Kabaroff et al., 2006). Orthogonal polynomial contrasts were used to test quadratic effects of dietary Glu level as described by Mahmoud et al. (2017). The quadratic regression analysis model was used to determine the optimal dietary Glu supplementation levels based on different indices, and R^2 between 0.7 and 1 indicated a good fit of the regression equation to the data, according to Xu et al. (2018). Pearson correlation coefficient analysis was conducted using the Bivariate Correlation program.

3. Results

Dietary Glu did not have a significant effect on the survival rate of Jian carp (Table 4). No pathological signs were observed during the trail. Effect of graded levels of dietary Glu on growth parameters are presented in Table 4. The final body weight (FBW) and percent weight gain (PWG) were improved with increasing dietary Glu levels up to 83.4 g/kg diet ($P < 0.05$). The feed intake (FI) of fish was the highest for fish fed 83.4 g of Glu/kg diet ($P < 0.05$). The feed conversion ratio (FCR) was the highest for fish fed control diet, and significantly decreased with increasing dietary Glu levels up to 68.4 g/kg diet ($P < 0.05$). Body composition and net nutrient deposition of fish fed diets with graded levels of Glu are presented in Table 5. The body moisture of 57.2, 60.6, and 68.4 g of Glu/kg groups was significantly lower than that of the other groups ($P < 0.05$). Fish body crude protein and crude lipid contents, and lipid production value (LPV) were improved with increasing levels of dietary Glu up to 68.4 g/kg diet and depressed thereafter ($P < 0.05$). The ash content was significantly higher for fish fed Glu supplemented diets compared with control diet ($P < 0.05$). The protein production value (PPV) and ash production value (APV) were the highest for fish fed the diet with 68.4 and 83.4 g of Glu/kg diet, respectively ($P < 0.05$). Based on the quadratic regression analysis of PWG, the optimal dietary Glu levels were estimated to be 81.97 g/kg diet (Fig. 1).

The effects of dietary Glu on intestine somatic index (ISI), hepatosomatic index (HSI), relative gut length (RGL), and hepatopancreas protein (HPC) and intestinal protein contents (IPC) are shown in Table 6. The HPC significantly increased and obtained maximum values when Glu level was at 68.4 g/kg diet ($P < 0.05$). The ISI and IPC also showed similar trends. Fish fed diets containing 57.2, 60.6, and 68.4 g of Glu/kg diet had significantly higher RGL than those fed other diets ($P < 0.05$).

The effects of dietary Glu on trypsin, chymotrypsin, lipase and amylase are presented in Table 7. Trypsin and chymotrypsin activities in the hepatopancreas were the highest for fish fed 68.4 g of Glu/kg diet ($P < 0.05$). Lipase activity in the hepatopancreas was the lowest for fish fed the control diet, and the highest for fish fed diet supplemented with 83.4 g/kg Glu ($P < 0.05$). Amylase activity in the hepatopancreas was significantly improved with increasing dietary Glu levels up to 60.6 g/kg diet and decreased thereafter ($P < 0.05$). The activities of trypsin and lipase in the intestine increased with increasing dietary Glu levels in up to 68.4 and 60.6 g/kg diets, respectively, and decreased thereafter ($P < 0.05$). Fish fed diets containing 68.4 and 83.4 g of Glu/kg diets had higher level of chymotrypsin activity in the intestine than those fed other levels diets ($P < 0.05$). Amylase activity in the intestine was the highest for fish fed 60.6 g of Glu/kg diet ($P < 0.05$).

The brush border enzyme activities in proximal intestine (PI), mid intestine (MI) and distal intestine (DI) for Jian carp fed graded

Table 4
The IBW, FBW, survival, FI, PWG, and FCR of carp fed diets with graded levels of *L*-glutamate for 9 weeks[1].

Item	Dietary *L*-glutamate levels, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
IBW, g/fish	126.33 ± 0.17	126.33 ± 0.17	125.50 ± 0.00	126.33 ± 0.17	125.50 ± 0.00
FBW, g/fish	306.04 ± 8.97^{a}	309.17 ± 3.31^{ab}	311.12 ± 4.89^{abc}	326.49 ± 2.42^{bc}	328.52 ± 5.40^{c}
Survival rate, %	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
FI, g/fish	263.53 ± 3.01^{b}	248.51 ± 4.12^{a}	251.36 ± 4.21^{a}	257.15 ± 2.40^{ab}	278.36 ± 2.43^{c}
PWG[2]	142.23 ± 6.83^{a}	144.74 ± 2.90^{a}	145.95 ± 3.86^{ab}	158.44 ± 1.97^{b}	159.70 ± 4.27^{b}
FCR[3]	1.48 ± 0.09^{b}	1.36 ± 0.01^{ab}	1.36 ± 0.01^{ab}	1.28 ± 0.01^{a}	1.38 ± 0.03^{ab}
Regression					
$Y_{FBW} = -0.03021X^2 + 4.971X + 124.60$				$R^2 = 0.7566$	$P = 0.07$
$Y_{PWG} = -0.02416X^2 + 3.961X - 2.081$				$R^2 = 0.7503$	$P = 0.079$
$Y_{FCR} = 0.000639X^2 - 0.09038X + 4.467$				$R^2 = 0.7088$	$P = 0.077$

IBW = initial body weight; FBW = final body weight; FI = feed intake; PWG = percent weight gain; FCR = feed conversion ratio.
[a-c] Mean values within the same row with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SE.
[2] PWG = Weight gain (g)/Initial weight (g) × 100.
[3] FCR = Feed intake (g)/Wet weight gain (g).

Table 5
Body composition (% as wet tissue), PPV, LPV and APV of carp fed diets with graded levels of *L*-glutamate for 9 weeks[1].

Item	Dietary *L*-glutamate levels, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
Moisture	73.83 ± 1.27^{b}	66.81 ± 1.29^{a}	66.54 ± 0.27^{a}	64.30 ± 0.32^{a}	71.84 ± 0.83^{b}
Crude protein	15.05 ± 0.11^{a}	17.24 ± 0.24^{c}	17.31 ± 0.07^{cd}	18.07 ± 0.35^{d}	16.29 ± 0.31^{b}
Crude lipid	7.95 ± 0.53^{a}	8.94 ± 0.24^{ab}	9.01 ± 0.28^{ab}	10.1 ± 0.08^{b}	8.14 ± 0.46^{a}
Ash	2.83 ± 0.04^{a}	3.08 ± 0.10^{b}	3.18 ± 0.04^{b}	3.21 ± 0.03^{b}	3.06 ± 0.08^{b}
PPV[2]	34.28 ± 1.27^{a}	37.26 ± 1.04^{ab}	36.96 ± 0.42^{ab}	40.16 ± 1.61^{b}	37.24 ± 0.88^{ab}
LPV[3]	124.30 ± 12.7^{a}	130.81 ± 2.91^{a}	131.60 ± 5.15^{a}	156.65 ± 1.82^{b}	128.34 ± 8.10^{a}
APV[4]	25.91 ± 1.02^{a}	25.88 ± 1.08^{a}	26.96 ± 0.72^{a}	28.78 ± 0.22^{ab}	30.29 ± 1.04^{b}
Regression					
$Y_{Moisture} = 0.03847X^2 - 5.286X + 245.30$				$R^2 = 0.8734$	$P = 0.089$
$Y_{Crude\ protein} = -0.01087X^2 + 1.515X - 34.46$				$R^2 = 0.8590$	$P = 0.110$
$Y_{Crude\ lipid} = -0.008351X^2 + 1.151X - 29.72$				$R^2 = 0.8208$	$P = 0.06$
$Y_{Ash} = -0.001326X^2 + 0.1871X - 3.333$				$R^2 = 0.7771$	$P = 0.104$
$Y_{PPV} = -0.01749X^2 + 2.489X - 48.66$				$R^2 = 0.7259$	$P = 0.10$
$Y_{LPV} = -0.1106X^2 + 15.45X - 389.60$				$R^2 = 0.6080$	$P = 0.228$
$Y_{APV} = -0.002347X^2 + 0.4815X + 6.515$				$R^2 = 0.8236$	$P < 0.05$

PPV = protein production value; LPV = lipid production value; APV = ash production value.
[a-d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SE.
[2] PPV = Fish protein gain (g)/Total protein intake (g) × 100.
[3] LPV = Fish lipid gain (g)/Total lipid intake (g) × 100.
[4] APV = Fish ash gain (g)/Total ash intake (g) × 100.

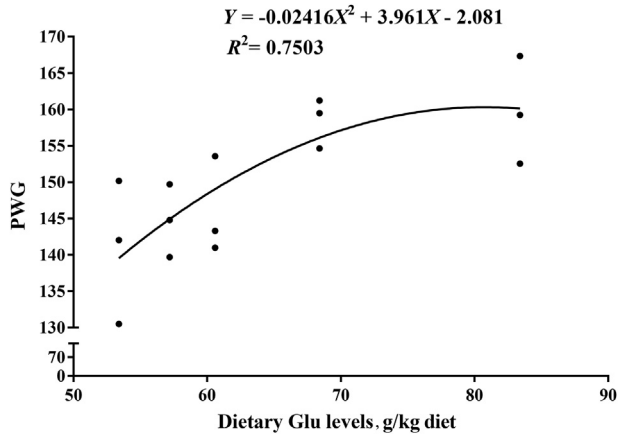

Fig. 1. The quadratic regression analysis of percent weight gain (PWG) of Jian carp fed diets containing graded levels of *L*-glutamate (Glu). Values are means of 3 replicates, with 40 fish in each replicate.

levels of Glu are presented in Table 8. The AKP activity in all intestinal segments was the highest for fish fed 83.4 g of Glu/kg diet ($P < 0.05$). The NKA activity in PI and DI was the highest for fish fed 68.4 g of Glu/kg diet ($P < 0.05$), and the lowest for fish fed control diet. The activity of NKA in MI was increased with increasing Glu levels up to 68.4 g/kg diet and decreased thereafter ($P < 0.05$). The γ-GT activity in MI was not different for fish fed diets containing ≤68.4 g of Glu/kg diet ($P > 0.05$), and was significantly increased in diet containing 83.4 g of Glu/kg diet ($P < 0.05$). The γ-GT activity in DI was the highest for fish fed 83.4 g of Glu/kg diet and the lowest for fish fed control diet ($P < 0.05$). The CK activity in PI, MI, and DI was significantly improved with increasing dietary Glu levels up to 68.4 g/kg diet ($P < 0.05$), where the response reached a plateau ($P > 0.05$).

The effect of dietary Glu on antioxidant parameters in the hepatopancreas and intestine are displayed in Table 9. The contents of MDA in the hepatopancreas and intestine, and PCO in the intestine were significantly decreased with the increased dietary Glu levels up to 68.4 g/kg diet, and then increased with further increase of dietary Glu levels ($P < 0.05$). The PCO content in the hepatopancreas was reduced in fish fed diets supplemented with Glu

Table 6
The HSI, HPC, ISI, RGL and IPC of carp fed diets with graded levels of *L*-glutamate for 9 weeks (%)[1].

Item	Dietary *L*-glutamate levels, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
Hepatopancreas					
HIS[2]	2.79 ± 0.15	2.89 ± 0.11	3.10 ± 0.16	2.73 ± 0.14	2.74 ± 0.23
HPC[3], %	7.27 ± 0.88^{a}	9.74 ± 0.57^{ab}	9.60 ± 1.14^{ab}	10.99 ± 0.50^{b}	10.28 ± 0.88^{ab}
Intestine					
ISI[4]	2.42 ± 0.11^{a}	2.67 ± 0.04^{b}	2.55 ± 0.03^{ab}	2.63 ± 0.07^{b}	2.61 ± 0.07^{b}
RGL[5]	167.42 ± 3.00^{a}	196.63 ± 7.72^{b}	193.73 ± 4.15^{b}	188.19 ± 5.73^{b}	184.24 ± 6.58^{ab}
IPC[6], %	4.25 ± 0.10^{a}	4.30 ± 0.31^{a}	4.36 ± 0.29^{ab}	6.10 ± 0.25^{c}	5.27 ± 0.25^{bc}

HSI = hepatosomatic index; HPC = hepatopancreas protein content; ISI = intestine somatic index; RGL = relative gut length; IPC = intestinal protein content.
[a-c] Mean values within the same row with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SE.
[2] HSI = 100 × Wet hepatopancreas weight (g)/Wet body weight (g).
[3] HPC = 100 × Hepatopancreatic protein (g)/Wet hepatopancreas weight (g).
[4] ISI = 100 × Wet intestine weight (g)/Wet body weight (g).
[5] RGL = 100 × Intestine length (cm)/Total body length (cm).
[6] IPC = 100 × Intestinal protein (g)/Wet intestine weight (g).

Table 7
The activities (U/g tissue) of trypsin, chymotrypsin, lipase, and amylase in the hepatopancreas and whole intestine of carp fed diets with graded levels of *L*-glutamate for 9 weeks[1].

Item	Dietary *L*-glutamate levels, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
Hepatopancreas					
Trypsin	395.04 ± 7.61^{a}	581.17 ± 3.44^{b}	561.78 ± 9.67^{b}	695.05 ± 5.12^{d}	620.50 ± 11.3^{c}
Chymotrypsin	520.35 ± 47.20^{a}	740.59 ± 10.33^{b}	649.78 ± 21.19^{b}	898.72 ± 30.95^{c}	752.20 ± 35.50^{b}
Lipase	1.32 ± 0.16^{a}	2.51 ± 0.17^{bc}	1.87 ± 0.24^{ab}	2.50 ± 0.23^{bc}	3.19 ± 0.55^{c}
Amylase	2,001.53 ± 58.72^{a}	2,494.38 ± 38.63^{bc}	2,516.04 ± 42.86^{c}	2,142.84 ± 119.45^{ab}	2,242.13 ± 99.48^{abc}
Intestine					
Trypsin	25.24 ± 3.77^{a}	27.39 ± 2.41^{ab}	29.50 ± 2.42^{abc}	38.66 ± 0.93^{c}	36.03 ± 1.81^{bc}
Chymotrypsin	102.82 ± 1.03^{a}	96.34 ± 1.00^{a}	102.23 ± 5.85^{a}	124.41 ± 5.75^{b}	120.83 ± 4.09^{b}
Lipase	1.06 ± 0.02^{a}	1.45 ± 0.16^{ab}	2.90 ± 0.60^{c}	2.43 ± 0.33^{bc}	2.07 ± 0.20^{abc}
Amylase	419.39 ± 3.04^{b}	399.67 ± 20.81^{b}	518.15 ± 20.12^{c}	439.57 ± 11.94^{b}	324.98 ± 19.76^{a}
Regression					
$Y_{\text{Hpeatopancreas trypsin}} = -0.7840X^2 + 113.9X - 3{,}428$				$R^2 = 0.8904$	$P = 0.106$
$Y_{\text{Hpeatopancreas chymotrypsin}} = -0.9685X^2 - 139.9X - 4{,}173$				$R^2 = 0.7433$	$P = 0.225$
$Y_{\text{Hpeatopancreas lipase}} = -0.0008520X^2 + 0.1681X - 4.931$				$R^2 = 0.6358$	$P = 0.266$
$Y_{\text{Intestine trypsin}} = -0.02936X^2 + 4.435X - 129.30$				$R^2 = 0.7925$	$P = 0.087$
$Y_{\text{Intestine chymotrypsin}} = -0.03535X^2 + 5.704X - 107.70$				$R^2 = 0.6516$	$P = 0.288$
$Y_{\text{Intestine lipase}} = -0.005404X^2 + 0.7691X - 24.54$				$R^2 = 0.5863$	$P = 0.318$
$Y_{\text{Intestine amylase}} = -0.4199X^2 + 54.39X - 1{,}294$				$R^2 = 0.6691$	$P = 0.299$

[a-d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SE.

($P < 0.05$). The activities of CAT in the hepatopancreas, GST in the hepatopancreas, and HRS in the intestine were significantly increased with the increased dietary Glu levels up to 68.4 g/kg diet ($P < 0.05$), and plateaued thereafter ($P > 0.05$). The activities of GPx in the hepatopancreas and intestine, GST in the intestine and SAS in the intestine were significantly improved with increasing dietary Glu levels up to 68.4 and 60.6 g/kg diet, and decreased thereafter ($P < 0.05$). The GR activity and GSH content in the intestine were the highest for fish fed the diet with 83.4 and 60.6 g of Glu/kg diet, respectively ($P < 0.05$), and the lowest for fish fed control diet ($P < 0.05$). The HRS activity in the hepatopancreas was significantly increased in response to diet with 57.2 g of Glu/kg diet ($P < 0.05$). According to a quadratic regression analysis model run on MDA content in the hepatopancreas

$$Y_{\text{Hepatopancreas}} = 0.016\,X^2 - 2.274X + 85.791,\ R^2 = 0.914,\ P < 0.05$$

and the intestine

$$Y_{\text{Intestine}} = 0.014\,X^2 - 1.998X + 78.75,\ R^2 = 0.943,\ P < 0.05,$$

the optimal dietary Glu level was estimated to be 71.06 (22.5% of crude protein) and 71.36 g/kg diet (22.6% of crude protein).

The gene expressions of antioxidant enzymes, Nrf2 and Keap1 in the intestine are displayed in Figs. 2 and 3. The CAT and GR mRNA levels increased with increasing dietary Glu levels up to 60.6 g/kg diet ($P < 0.05$) and plateaued thereafter ($P > 0.05$). The GPx1a and GST mRNA levels gradually increased with increasing Glu levels up to 68.4 g/kg diet, and then decreased ($P < 0.05$). No significant differences were found in CuZnSOD and GPx1b mRNA expressions among groups ($P > 0.05$). The Nrf2 mRNA level was the highest for fish fed 60.6 g of Glu/kg diet ($P < 0.05$). The Keap1 mRNA level showed an inverse trend with respect to Nrf2 mRNA expression. As shown in Figs. 4 and 5, ZO-1, occludin2, claudin3, and PKC mRNA levels increased with increasing dietary Glu levels up to 68.4 g/kg diet ($P < 0.05$) and plateaued thereafter ($P > 0.05$). Occludin1 mRNA level increased with increasing dietary Glu levels up to 83.4 g/kg diet ($P < 0.05$). Claudin7 mRNA level gradually increased with increasing Glu levels up to 68.4 g/kg diet, and then significantly decreased ($P < 0.05$).

Correlation analysis (Table 10) demonstrated that Glu was positively correlated with intestinal trypsin ($r = 0.810$, $P = 0.096$), AKP ($r_{PI} = 0.971$, $P < 0.01$; $r_{MI} = 0.880$, $P < 0.05$; $r_{DI} = 0.874$, $P = 0.053$), γ-GT

Table 8
The activities of AKP, NKA, γ-GT and CK in PI, MI, DI of carp fed diets with graded levels of *L*-glutamate for 9 weeks[1].

Item	Dietary *L*-glutamate levels, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
AKP, U/g tissue					
PI	4.33 ± 0.26^{a}	3.89 ± 1.02^{a}	4.41 ± 0.44^{a}	5.68 ± 0.51^{ab}	8.22 ± 0.97^{b}
MI	2.72 ± 0.07^{a}	3.91 ± 0.73^{ab}	3.71 ± 0.48^{ab}	5.96 ± 0.92^{b}	5.97 ± 0.28^{b}
DI	1.47 ± 0.15^{a}	2.01 ± 0.41^{ab}	2.15 ± 0.04^{ab}	2.23 ± 0.10^{ab}	2.54 ± 0.45^{b}
NKA, μmol of phosphorus released/g tissue per h					
PI	160.21 ± 0.88^{a}	193.45 ± 12.6^{bc}	170.23 ± 2.20^{ab}	215.93 ± 4.13^{bc}	157.36 ± 6.21^{a}
MI	132.27 ± 2.13^{a}	160.47 ± 2.76^{b}	182.67 ± 2.83^{c}	185.44 ± 5.25^{c}	127.47 ± 0.80^{a}
DI	128.74 ± 5.93^{a}	174.46 ± 1.57^{c}	147.67 ± 0.75^{ab}	195.78 ± 2.89^{d}	156.49 ± 4.84^{bc}
γ-GT, U/g tissue					
PI	0.93 ± 0.00	0.97 ± 0.14	0.93 ± 0.10	0.96 ± 0.12	1.07 ± 0.26
MI	0.93 ± 0.16^{a}	1.13 ± 0.30^{a}	1.23 ± 0.10^{ab}	1.30 ± 0.11^{ab}	1.78 ± 0.24^{b}
DI	1.72 ± 0.03^{a}	2.02 ± 0.09^{ab}	1.78 ± 0.05^{a}	2.39 ± 0.15^{b}	2.52 ± 0.35^{b}
CK, U/g tissue					
PI	22.08 ± 1.74^{a}	23.37 ± 1.99^{a}	23.99 ± 1.03^{a}	35.20 ± 2.02^{b}	39.58 ± 2.04^{b}
MI	21.72 ± 1.23^{a}	22.73 ± 0.48^{a}	24.51 ± 0.78^{ab}	27.70 ± 1.25^{b}	27.79 ± 1.31^{b}
DI	14.97 ± 0.55^{a}	17.86 ± 0.79^{b}	17.96 ± 0.40^{b}	18.94 ± 0.86^{b}	17.96 ± 0.73^{b}
Regression					
$Y_{PI\ AKP} = 0.003225X^2 - 0.3003X + 10.88$				$R^2 = 0.8603$	$P < 0.05$
$Y_{MI\ AKP} = -0.005623X^2 + 0.8821X - 28.43$				$R^2 = 0.8124$	$P = 0.079$
$Y_{DI\ AKP} = -0.001373X^2 + 0.2180X - 6.118$				$R^2 = 0.6144$	$P = 0.117$
$Y_{PI\ NKA} = -0.2034X^2 + 27.79X - 744.10$				$R^2 = 0.6308$	$P = 0.332$
$Y_{MI\ NKA} = -0.2640X^2 + 35.84X - 1026$				$R^2 = 0.9654$	$P < 0.05$
$Y_{DI\ NKA} = -0.1914X^2 + 27.01X - 764$				$R^2 = 0.6240$	$P = 0.365$
$Y_{DI\ \gamma\text{-}GT} = -0.0006641X^2 + 0.1183X - 2.71$				$R^2 = 0.6853$	$P = 0.175$
$Y_{PI\ CK} = -0.01106X^2 + 2.164X - 63.58$				$R^2 = 0.8905$	$P = 0.072$
$Y_{MI\ CK} = -0.01193X^2 + 1.853X - 43.67$				$R^2 = 0.8691$	$P < 0.05$
$Y_{DI\ CK} = -0.01156X^2 + 1.659X - 40.11$				$R^2 = 0.7352$	$P = 0.137$

AKP = alkaline phosphatase; NKA = Na^+, K^+-ATPase; γ-GT = γ-glutamyl transpeptidase; CK = creatine kinase; PI = proximal intestine; MI = mid intestine; DI = distal intestine.
[a-d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SE.

($r_{PI} = 0.895$, $P < 0.05$; $r_{MI} = 0.982$, $P < 0.01$; $r_{DI} = 0.889$, $P < 0.05$), and CK ($r_{PI} = 0.954$, $P < 0.05$; $r_{MI} = 0.892$, $P < 0.05$) activities. Secondly, weight gain appeared positive correlations with AKP ($r_{PI} = 0.913$, $P < 0.05$; $r_{MI} = 0.960$, $P < 0.05$; $r_{DI} = 0.817$, $P = 0.091$), γ-GT ($r_{PI} = 0.756$, $P = 0.139$; $r_{MI} = 0.878$, $P = 0.050$; $r_{DI} = 0.939$, $P < 0.05$), and CK ($r_{PI} = 0.996$, $P < 0.01$; $r_{MI} = 0.968$, $P < 0.01$). Thirdly, intestinal MDA was negatively correlated with intestinal trypsin ($r = -0.897$, $P < 0.05$), AKP ($r_{MI} = -0.881$, $P < 0.05$; $r_{PI} = -0.840$, $P = 0.075$), and CK ($r_{MI} = -0.870$, $P = 0.054$) activities.

4. Discussion

4.1. Effect of dietary glu on growth performance

In the present study, the growth performance of Jian carp was significantly influenced by dietary Glu levels. The FBW and PWG were improved in diet supplemented with 68.4 and 83.4 of Glu g/kg, which was in agreement with reports for grass carp (Zhao et al., 2015), Atlantic salmon (Oehme et al., 2010), and rainbow trout (Chika et al., 2016). The enhancement of fish growth may be attributed to the fact that feed efficiency was improved with appropriate dietary Glu. There is growing evidence that some of the traditionally classified nonessential amino acids (NEAA, e.g. glycine) are important regulators of key metabolic pathways, which play enormous roles in multiple signaling pathways, thereby regulating gene expression, intracellular protein turnover, nutrient metabolism, and oxidative defense (Xiao et al., 2014; Xie et al., 2016). In order to avoid the influence of other NEAA, all diets in the present study were not maintained isonitrogenous by supplementation of other NEAA as the study on glycine by Xie et al. (2014). Although a higher protein level was found in diet supplemented with Glu at 83.4 g/kg. However, a better growth performance is observed in diet supplemented with 81.97 g of Glu/kg. Therefore, a high level of Glu may result in adverse effects on growth performance. Fish weight gain is primarily attributed to the accretion of protein and fat (Bureau et al., 2000). The present study showed that Glu significantly enhanced carp body protein and lipid contents, and dietary protein and lipids unitization, which were in accordance with the results for grass carp (Zhao et al., 2015), gilthead seabream (Caballero-Solares et al., 2015), and rainbow trout (Chika et al., 2016). However dietary Glu supplementation has no effect on growth and feed utilization in Atlantic salmon *S. salar* (Larsson et al., 2014). One of reasons for this difference is that the ratio of dietary Glu to fat level is too low, which are both the main energy source of fish. Recent study indicates Glu contributes the major tissues of approximately 80% of ATP production in the liver, proximal intestine, and skeletal muscle of zebrafish (*Danio rerio*) and hybrid striped bass (*Morone saxatilis* ♀ + *Morone chrysops* ♂) (Jia et al., 2017). In grass carp (Zhao et al., 2015), gilthead seabream (Caballero-Solares et al., 2015), and rainbow trout (Chika et al., 2016), the ratios of dietary Glu to protein and fat level are 18.8% and 143.2%, 17.8% and 52.4%, and 23.3% and 74.5%, respectively. In Atlantic salmon, the ratio of dietary Glu to protein is 17.9%, but the ratio of dietary Glu to fat level only is 18% (Larsson et al., 2014). Therefore, it is needed to further study on the appropriate proportions of dietary fat and Glu. The present result also firstly showed the optimal dietary Glu level was 81.97 g/kg diet (116.5% of dietary crude lipid and 26.4% of dietary crude protein) for Jian carp growth.

4.2. Effect of dietary glu on digestive and absorptive capacity

Fish growth is related to the digestive and absorptive capacity (Gisbert et al., 2009), which can be reflected by digestive organ growth and development, as well as activities of intestinal enzymes related to digestion and absorption (Yan and Qiu-Zhou, 2006). In

Table 9
The activities of MDA, PCO, SOD, CAT, GPx, GST, GR, GSH, SAS, HRS in the hepatopancreas and whole intestine of carp fed diets with graded levels of *L*-glutamate for 9 weeks[1].

Item	Dietary *L*-glutamate levels, g/kg diet				
	53.4	57.2	60.6	68.4	83.4
Hepatopancreas					
MDA, nmol/mg protein	11.52 ± 0.09^{c}	9.36 ± 0.26^{b}	9.03 ± 0.11^{b}	6.81 ± 0.25^{a}	9.88 ± 0.93^{b}
PCO, nmol/mg protein	4.76 ± 0.30^{b}	2.78 ± 0.04^{a}	2.65 ± 0.39^{a}	2.68 ± 0.24^{a}	3.10 ± 0.42^{a}
SOD, U/mg protein	563.00 ± 1.79	560.12 ± 15.4	581.75 ± 5.96	582.13 ± 8.77	556.88 ± 7.93
CAT, U/mg protein	51.23 ± 3.47^{a}	59.23 ± 4.04^{ab}	65.92 ± 3.76^{bc}	76.41 ± 1.53^{d}	73.84 ± 0.86^{cd}
GPx, U/mg protein	4,280.54 ± 7.01^{ab}	4,360.52 ± 50.05^{b}	4,650.92 ± 26.93^{c}	4,708.27 ± 42.00^{c}	4,204.55 ± 11.85^{a}
GST, U/mg protein	66.72 ± 2.36^{a}	70.77 ± 4.62^{ab}	80.23 ± 1.01^{bc}	82.42 ± 2.49^{c}	79.40 ± 0.53^{bc}
GR, U/g protein	56.91 ± 0.16	61.70 ± 2.73	63.23 ± 0.05	63.98 ± 3.00	58.90 ± 2.57
GSH, μmol/g protein	31.96 ± 0.02	31.09 ± 1.44	33.73 ± 2.22	34.78 ± 1.06	32.38 ± 1.59
SAS, U/g protein	3.38 ± 0.01	3.49 ± 0.35	3.93 ± 0.16	3.62 ± 0.16	3.56 ± 0.26
HRS, U/mg protein	613.87 ± 10.31^{a}	709.42 ± 44.05^{b}	699.34 ± 4.85^{ab}	680.39 ± 16.68^{ab}	635.89 ± 12.25^{ab}
Intestine					
MDA, nmol/mg protein	11.64 ± 1.04^{c}	9.13 ± 0.00^{b}	8.67 ± 0.17^{b}	6.63 ± 0.22^{a}	8.09 ± 0.52^{ab}
PCO, nmol/mg protein	3.20 ± 0.14^{b}	2.57 ± 0.13^{a}	2.59 ± 0.11^{a}	2.48 ± 0.02^{a}	3.07 ± 0.06^{b}
SOD, U/mg protein	282.06 ± 5.46	295.86 ± 2.90	300.74 ± 3.84	300.77 ± 8.87	286.37 ± 11.94
CAT, U/mg protein	2.01 ± 0.03^{a}	2.85 ± 0.19^{b}	4.14 ± 0.07^{c}	4.41 ± 0.06^{c}	4.30 ± 0.15^{c}
GPx, U/mg protein	3,324.10 ± 99.56^{a}	3,409.41 ± 31.67^{ab}	3,563.85 ± 26.08^{b}	3,622.51 ± 60.46^{b}	3,248.96 ± 97.05^{a}
GST, U/mg protein	61.86 ± 0.31^{a}	67.29 ± 1.19^{b}	67.72 ± 0.30^{b}	69.87 ± 1.93^{b}	63.00 ± 0.10^{a}
GR, U/g protein	26.02 ± 0.73^{a}	44.75 ± 1.70^{b}	39.59 ± 1.72^{b}	45.69 ± 1.32^{b}	45.78 ± 2.83^{b}
GSH, μmol/g protein	17.21 ± 0.09^{a}	26.44 ± 1.70^{ab}	35.94 ± 2.74^{b}	34.60 ± 4.62^{b}	29.34 ± 0.37^{b}
SAS, U/g protein	14.06 ± 0.08^{a}	17.70 ± 0.86^{b}	17.92 ± 0.23^{b}	15.38 ± 0.39^{ab}	14.73 ± 0.73^{a}
HRS, U/mg protein	944.44 ± 4.67^{a}	997.33 ± 10.03^{ab}	1,025.80 ± 8.83^{b}	1,099.33 ± 8.40^{c}	1,085.20 ± 17.35^{c}
Regression					
$Y_{\text{Hpeatopancreas MDA}} = 0.016X^2 - 2.274X + 85.791$				$R^2 = 0.914$	$P < 0.05$
$Y_{\text{Hpeatopancreas PCO}} = 0.00667X^2 - 0.9472X + 35.79$				$R^2 = 0.6262$	$P = 0.311$
$Y_{\text{Hpeatopancreas CAT}} = -0.06043X^2 + 9.038X - 259.6$				$R^2 = 0.9325$	$P < 0.05$
$Y_{\text{Hpeatopancreas GPX}} = -2.163X^2 + 294.7X - 5{,}324$				$R^2 = 0.9162$	$P = .068$
$Y_{\text{Hpeatopancreas GST}} = -0.04764X^2 + 6.942X - 168.3$				$R^2 = 0.8219$	$P = 0.076$
$Y_{\text{Intestine MDA}} = 0.014X^2 - 1.988X + 78.75$				$R^2 = 0.943$	$P < 0.05$
$Y_{\text{Intestine PCO}} = 0.03029X^2 - 0.4138X + 16.53$				$R^2 = 0.7863$	$P = 0.15$
$Y_{\text{Intestine CAT}} = -0.00664X^2 + 0.9811X - 31.38$				$R^2 = 0.9245$	$P = 0.066$
$Y_{\text{Intestine GPx}} = -1.521X^2 + 206.2X - 3{,}367$				$R^2 = 0.8365$	$P < 0.05$
$Y_{\text{Intestine GST}} = -0.03305X^2 + 4.532X - 85.18$				$R^2 = 0.8740$	$P = 0.058$
$Y_{\text{Intestine GR}} = -0.04461X^2 + 6.594X - 194.40$				$R^2 = 0.6523$	$P = 0.323$
$Y_{\text{Intestine GSH}} = -0.06204X^2 + 8.814X - 274.80$				$R^2 = 0.7774$	$P = 0.149$
$Y_{\text{Intestine HRS}} = -0.3534X^2 + 53.15X - 888.30$				$R^2 = 0.9698$	$P < 0.01$

MDA = malondialdehyde; PCO = protein carbonyl; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; GST = glutathione S-transferase; GR = glutathione reductase; GSH = glutathione; SAS = superoxide anion scavenging ability; HRS = hydroxyl radical scavenging ability.
[a-d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$).
[1] Values are means ± SE.

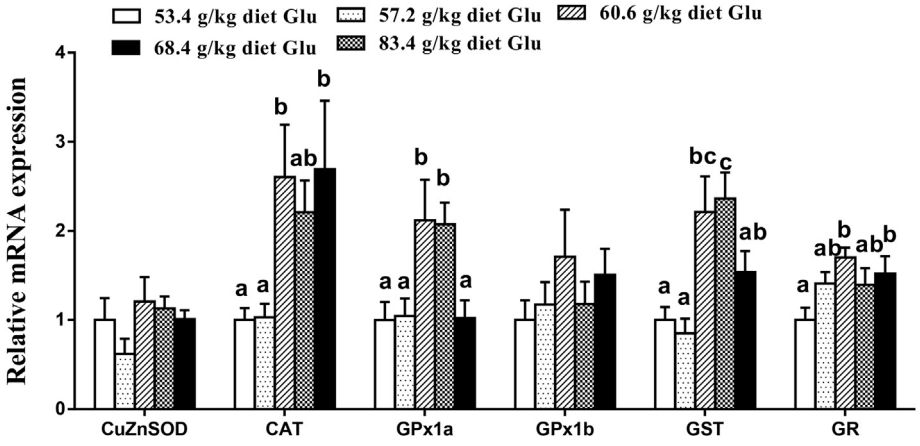

Fig. 2. Effects of dietary *L*-glutamate (Glu) on Cu/Zn superoxide dismutase (CuZnSOD), catalase (CAT), glutathione peroxidase 1a (GPx1a), glutathione peroxidase 1b (GPx1b), glutathione S-transferase (GST), and glutathione reductase (GR) gene expressions in the intestine of Jian carp. Values are means ± SE of 3 replicates with 6 fish in each replicate. Data columns with different letters denote significant difference ($P < 0.05$).

the present study, there were significant improvements in HPC, ISI, HSI, and RGL. These results indicated that Glu promoted the growth and development of the fish intestine and hepatopancreas. Intestinal growth and development are related with the intestinal cell proliferation and differentiation. In parenteral mice, Glu could prevent intestinal mucosal atrophy via promotion of intestine

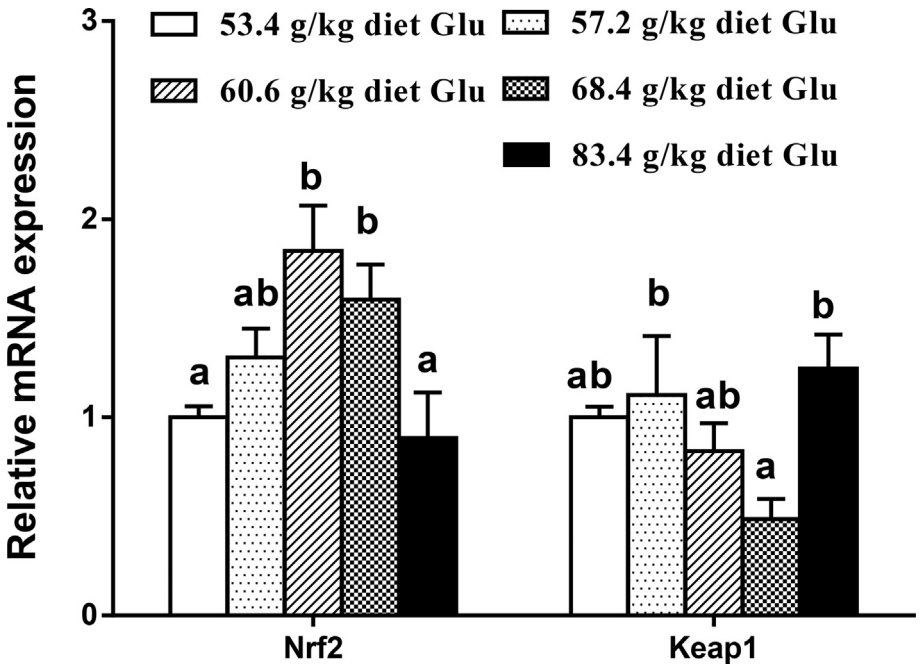

Fig. 3. Effects of dietary *L*-glutamate (Glu) on nuclear factor erythroid 2-related factor 2 (Nrf2) and Keap1 gene expressions in the intestine of Jian carp. Values are means ± SE of 3 replicates with 6 fish in each replicate. Data column with different letters denote significant difference ($P < 0.05$).

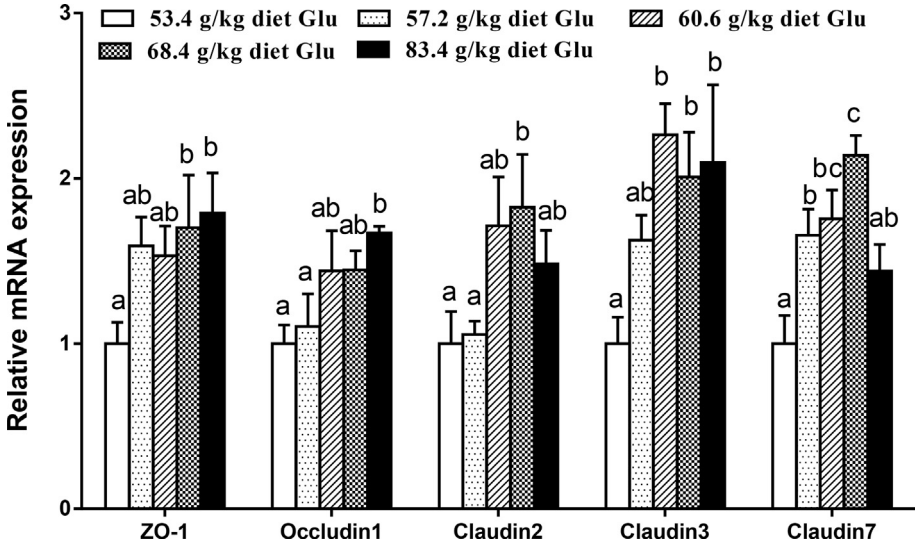

Fig. 4. Effects of dietary *L*-glutamate (Glu) on zonula occludens protein-1 (ZO-1), occludin1, claudin2, claudin3, and claudin7 gene expressions in the intestine of Jian carp. Values are means ± SE of 3 replicates with 6 fish in each replicate. Data columns with different letters denote significant difference ($P < 0.05$).

epithelial cell proliferation (Xiao et al., 2014). The previous studies showed Glu could improve the proliferation, differentiation, and function of fish enterocytes (Jiang et al., 2015a,b,c), and promote intestinal nucleotide synthesis and cell-proliferation in rainbow trout (Chika et al., 2016). Meanwhile, the activities of trypsin, chymotrypsin, amylase, and lipase in whole intestine, as well as trypsin, chymotrypsin, and lipase in the hepatopancreas were improved by dietary appropriate Glu levels. All these data above suggested that Glu enhanced the digestive capacity of Jian carp, which agreed with our previous study of grass carp (Zhao et al., 2015). Intestinal absorptive ability is also the basis of utilizing nutrient adequately (Wen et al., 2009). The enzymes located in the intestinal brush border section are responsible for the final stages of degradation and assimilation of the food. The AKP is considered to be involved in absorption of nutrients such as lipid, glucose, calcium, and inorganic phosphatase (Tengjaroenkul et al., 2000). The NKA (Gal-Garber et al., 2003; Geering, 1990) and γ-GT (Griffith and Meister, 1980; Ogawa et al., 1998) play a crucial role in absorption of most of amino acids and glucose. The CK has a key role in the energy metabolism of cells (Wallimann and Hemmer, 1994). In the present study, NKA and CK activities in all intestine segments, AKP activity in MI, γ-GT activity in DI significantly increased with increasing dietary Glu levels. Correlation analysis showed activities of intestinal brush-border enzymes was positively related

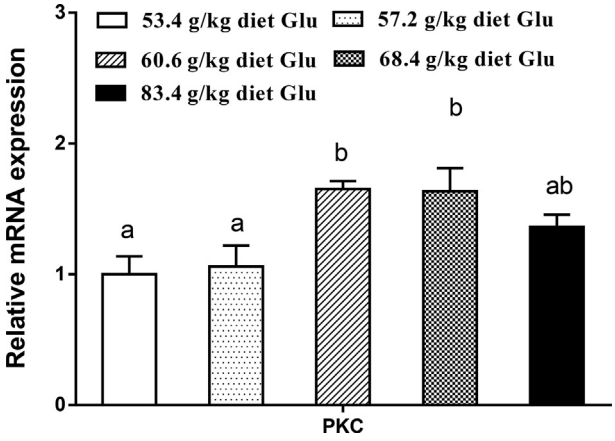

Fig. 5. Effects of dietary *L*-glutamate (Glu) on protein kinase C (PKC) gene expressions in the intestine of Jian carp. Values are means ± SE of 3 replicates with 6 fish in each replicate. Data columns with different letters denote significant difference ($P < 0.05$).

to dietary Glu level. These results suggested that dietary Glu could improve the absorption ability in fish. Similarly, dietary Glu improved the activities of brush-border enzymes in grass carp (Zhao et al., 2015).

4.3. Effect of dietary glu on antioxidant capacity in the intestine

The structural integrity of fish intestines is the guarantee of its normal digestion and absorption. The structural and functional integrity of the intestine and enterocytes was closely related to the antioxidant enzyme activities (Jiang et al., 2015a,b,c; Jiang et al., 2013; Shoveller et al., 2005). The MDA is a by-product of lipid peroxidation induced by excessive reactive oxygen species (ROS) and a good marker of lipid peroxidation (Mourente et al., 2007). The PCO is one of the most extensively studied forms of proteins oxidative modification (Wang and Powell, 2010). The contents of MDA and PCO can reflect the antioxidant status of living organisms (Ghosh et al., 2008). In the present study, the contents of MDA and PCO were decreased with increasing dietary Glu levels up to certain values in both the intestine and hepatopancreas, suggesting depressions of the lipid peroxidation and protein oxidation. The Glu is a key transamination partner and is required for the synthesis of GSH in the intestine (Johnson et al., 2003). Previous study reported a strong negative correlation between GSH content and MDA level in the intestinal mucosa of weaned piglets (Wang and Li, 2012). The Glu ameliorated copper induced MDA generation in fish intestines (Jiang et al., 2016). Quadratic regression analysis against MDA content in the hepatopancreas and whole intestine showed the optimal dietary Glu level was 71.06 and 71.36 g/kg diet, respectively. Correlation analysis showed PWG was positively related to activities of trypsin and chymotrypsin in the intestine. Activities of trypsin and chymotrypsin were positively related to MDA contents in the hepatopancreas and intestine. However, there was no significant correlation between Glu and activities of trypsin and chymotrypsin. These results indicated Glu might improve the digestive ability by reducing oxidative damage in the hepatopancreas and increasing the ability of the pancreas to synthesize and secrete protease. The specific mechanism needs a further study.

Table 10
Correlation coefficient of some parameters.

Independent parameters	Dependent parameters	Correlation coefficients	*P*-value
L-glutamate	Intestine trypsin	0.810	0.096
	PI AKP	0.971	<0.01
	MI AKP	0.880	<0.05
	DI AKP	0.874	0.053
	PI γ-GT	0.895	<0.05
	MI γ-GT	0.982	<0.01
	DI γ-GT	0.889	<0.05
	PI CK	0.954	<0.05
	MI CK	0.892	<0.05
Intestine MDA	Intestine trypsin	−0.897	<0.05
	MI AKP	−0.881	<0.05
	PI AKP	−0.840	0.075
	MI CK	−0.870	0.054
Hepatopancreas MDA	Hepatopancreas trypsin	−0.886	<0.05
	Hepatopancreas chymotrypsin	−0.895	<0.05
Intestine trypsin	WG	0.945	<0.05
Intestine chymotrypsin	WG	0.942	<0.05
PI AKP	WG	0.913	<0.05
MI AKP	WG	0.960	<0.05
DI AKP	WG	0.817	0.091
PI γ-GT	WG	0.756	0.139
MI γ-GT	WG	0.878	0.050
DI γ-GT	WG	0.939	<0.05
PI CK	WG	0.996	<0.01
MI CK	WG	0.968	<0.01

PI = proximal intestine; AKP = alkaline phosphatase; MI = mid intestine; DI = distal intestine; γ-GT = γ-glutamyl transpeptidase; CK = creatine kinase; MDA = malondialdehyde; WG = weight gain.

In terrestrial animals, SOD, CAT, GPx, GST, GR, and GSH play a vital role in preventing cellular damage caused by ROS (David et al., 2008; Martínez-álvarez et al., 2005; Rong et al., 2012; Winston and Di Giulio, 1991; Wu et al., 2004). Our present results showed that Glu supplementation increased CAT, GPx, GST, GR activities and GSH content in the intestine. The SAS and HRS activities are 2 indexes used to evaluate the total capacity of scavenging superoxide and hydroxyl radical, respectively (Wu et al., 2004). The present results showed that Glu significantly increased the SAS and HRS activities in the intestine and hepatopancreas. These results indicated that the elevated anti-oxidative capacity of carp may, at least in part, be due to Glu induced the increment of the non-enzymatic content and enzymatic activities. Antioxidant enzymes are proteins, and their activities can be affected by the mRNA levels (Tiedge et al., 1997). In the present study, dietary Glu supplementation up-regulated CAT, GST, GR, and GPx1a mRNA expressions. These results matched a similar pattern to the respective enzyme activity changes. In eukaryote, as a nucleus transcription factor, the intranuclear Nrf2 can promote the transcription of antioxidant genes (Kwak et al., 2004) The Keap1 is identified as an Nrf2-binding protein that prevents Nrf2 translocation to the nucleus and promotes the ubiquitination-proteasomal degradation of Nrf2 (Ma, 2013). The present study showed Glu significantly up-regulated Nrf2 mRNA levels in the intestine of Jian carp. In contrast, Glu significantly decreased Keap1 mRNA expression. This result agreed with the previous study in grass carp that Glu supplementation alleviated oxidative damage induced by copper (Jiang et al., 2016). Thus, the positive effects of Glu on antioxidant enzymes mRNA expression may be partly ascribed to promote Nrf2 nuclear translocation by down-regulating Keap1 mRNA expression. However, the specific action of Glu affecting Nrf2 nuclear translocation needs a further study.

4.4. Effect of dietary glu on intestinal structure integrity

The previous study reported absorption capacity may be associated with TJ permeability of the intestine (Ballard et al., 1995). Our present results also showed that Glu supplementation significantly increased intestinal ZO-1, occludin1, claudin2, claudin3, and claudin7 mRNA expressions. This result was in good agreement with a

report on piglet. The Glu supplementation improved intestinal integrity and up-regulated jejunum ZO-1 and occludin mRNA expression in weaning piglets (Lin et al., 2014). In addition, evidence from animals and cell studies also shows that glutamine is important for intestinal barrier function and regulation of TJ protein (Li and Neu, 2009). As well, it has been shown that prohibiting the conversion from glutamine to Glu inhibits enhancement effect of glutamine on TJ protein expression (Nose et al., 2010; Vermeulen et al., 2011). In fish enterocyte, the expression of glutaminase mRNA level is increased quickly and effectively by glutamine (Jiang et al., 2015a,b,c).. Thus, dietary Glu maybe improve intestinal barrier functions and nutrient absorptive capacities by up-regulating TJ gene expression. Furthermore, TJ permeability is mediated by PKC (Andreeva et al., 2006; Stenson et al., 1993; Stuart and Nigam, 1995). Previous study showed that dietary arginine and medium pre-supplementation with linolenic acid and docosahexaenoic acid affect TJ permeability in Jian carp and intestinal monolayer cells (Usami et al., 2003; Wang et al., 2016). In the current study, optimal dietary Glu caused an up-regulation of the PKC mRNA expression level in Jian carp. Nevertheless, how Glu interacts with PKC is unknown by now and requires further study.

5. Conclusions

The present study demonstrated that dietary Glu increased fish growth by enhancing digestive and absorptive enzyme activities and intestinal physical barrier functions by regulating antioxidant-related signaling molecule and TJ protein gene expressions in fish intestines. The beneficial actions of Glu in intestinal physical barrier functions are closely associated with its ability in increasing Nrf2 and PKC mRNA expression. The optimal dietary Glu levels for Jian carp (126 to 337 g) were estimated to be 81.97 g/kg diet based on growth performance (PWG), 71.06 and 71.36 g/kg diet based on antioxidant-related indices (MDA content in the hepatopancreas and whole intestine, respectively).

Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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