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Effects of dietary histidine on antioxidant capacity in juvenile Jian carp (*Cyprinus carpio* var. Jian)

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Abstract In the present study, we tested the hypothesis that dietary histidine could improve antioxidant capacity of juvenile Jian carp (*Cyprinus carpio* var. Jian). A total of 1,200 juvenile Jian carp were fed graded levels of histidine at 2.3 (unsupplemented control), 4.4, 6.3, 8.6, 10.8 and 12.7 g/kg diet for 60 days. Results showed that the content of malondialdehyde (MDA) and protein carbonyl (PC) in serum and all tissues apparently decreased with increasing histidine levels up to an optimal level and increased thereafter. Anti-superoxide anion (ASA) capacity, glutathione peroxidase (GPX) activities and glutathione (GSH) content in serum and all tissues, anti-hydroxyl radical (a-HR) capacity, catalase (CAT) and glutathione-*S*-transferase (GST) activities in serum, muscle and intestine, superoxide dismutase (SOD) activities in serum and intestine, as well as glutathione reductase (GR) activity in serum, muscle and hepatopancreas were improved by dietary histidine. Fish fed diet with 8.6 g/kg histidine had lower serum glutamate-pyruvate transaminase (GPT) activity than that fed with control diet, whereas pattern of glutamate–oxaloacetate transaminase (GOT) activity was opposite. The present results suggested that histidine could improve antioxidant capacity and inhibit lipid peroxidation and protein oxidation of juvenile Jian carp.

Keywords *Cyprinus carpio* var. Jian · Histidine · Antioxidant · Oxidative stress

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Introduction

Histidine is one of the essential amino acids for fish, for example, Pacific salmon (*Oncorhynchus shawytscha*) (Halver et al. 1957), Indian major carp (*Cirrhinus mrigala*) (Ahmed and Khan 2005) and Atlantic salmon (*Salmo salar* L.) (Breck et al. 2005) and many other species. Our previous study in juvenile Jian carp (*Cyprinus carpio* var. Jian) indicated that dietary histidine promoted growth, increased protein deposition and improved digestion and absorption ability, which may be partly related to its beneficial effects on intestine and hepatopancreas growth (Zhao et al. 2012). The growth and development of tissue and organs in fish rely on the structural integrity of cells

(Hamlin et al. 2000). Chen et al. (2009) noted that the structural and functional integrity of intestinal epithelial cells in carp were associated with their antioxidant status. Our laboratory studies have demonstrated that the antioxidant status of Jian carp could be affected by glutamine (Lin and Zhou 2006) and methionine hydroxy analogue (Feng et al. 2011). However, only a little was known about the relationship between histidine and antioxidant defense of fish. It has been reported that histidine had an inhibition effect on lipid peroxidation in fish muscle sarcoplasmic reticulum suspension experimental system in vitro (Erickson and Hultin 1988, 1992). It suggested that dietary histidine may have beneficial effects on fish antioxidant status, which needs to be investigated.

Most components of cellular structure are likely to be the potential targets of reactive oxygen species (ROS), and the most susceptible substrates for oxidation are polyunsaturated fatty acids in the biomembrane (Mourente et al. 2007). On mammals, the lipid oxidation levels in kidney and liver of mice decreased with the administration of histidine (Lee et al. 2005). Histidine was also effective in inhibiting the oxidation of low-density lipoprotein in bovine serum (Decker et al. 2001). The effects of histidine on lipid peroxidation inhibition may be related to the interaction with toxic oxygen species (Wade and Tucker 1998). Hydroxyl radical ($^{\cdot}OH$), one kind of toxic oxygen species, can react with a wide variety of biomolecules and lead to oxidative damage of membrane lipids, proteins and nucleic acids (Mourente et al. 2007). Meanwhile, singlet oxygen ($^1O_2$) is a biologically nonradical toxic oxygen species that is highly reactive and potentially deleterious to biological systems (Wade and Tucker 1998). It has been demonstrated that histidine has preventive effects on $^{\cdot}OH$ generation in extracellular fluid of rat striatum (Obata et al. 2001). Besides, histidine has been recognized as a scavenger of the $^{\cdot}OH$ by interfering with the redox reactions (Nagy and Floyd 1984; Wade and Tucker 1998) and $^1O_2$ by direct interactions with the imidazole ring (Foote and Clennan 1995; Wade and Tucker 1998). Moreover, toxic oxygen species or free radicals are considered to cause extensive oxidative damage to cells (Mourente et al. 2007). To prevent oxidative damage, fish have developed antioxidant defense system, which is mainly constituted of antioxidant enzymes and non-enzyme antioxidant (Martínez-Álvarez et al. 2005; Shiau and Hsu 2002). Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and enzymes dependent on glutathione (glutathione peroxidase, GPX, glutathione reductase, GR and glutathione-*S*-Transferase, GST), serve as crucial part in antioxidant system (Martínez-Álvarez et al. 2005). Glutathione (GSH), the most abundant thiol-containing substance of low molecular weight in cells, is an effective non-enzyme antioxidant against free radicals and other oxidants (Mourente et al. 2007). To our knowledge, no research has been conducted to study the effect of histidine on the activities of antioxidant enzymes and GSH content in fish. Studies in mice have demonstrated that histidine supplement elevated CAT and GPX activities in kidney and liver (Lee et al. 2005; Liu et al. 2008). Post-intake of histidine increased GSH content in liver of mice (Liu et al. 2008). Taken together, dietary histidine improved the structure and function of fish tissues and organs may be partly related to the improvement of antioxidant defense, which warrants further investigation.

We conducted a series of studies to explore the effects of dietary histidine on juvenile Jian carp. Part 1 investigated the effects of histidine on growth performance, digestive and absorptive capacity of Jian carp (Zhao et al. 2012). This study was the second part, which shared the same growth trial with part 1, and the aim was to study the effects of dietary histidine on antioxidant capacity of Jian carp in serum, muscle, intestine and hepatopancreas. The present data can partly provide theoretical evidence for the effects of histidine on growth, protein deposition, digestive and absorptive ability of fish.

Materials and methods

Experimental diets

Formulation of the basal diet was the same as our previous study (Zhao et al. 2012) and was presented in Table 1. Fish meal (Pesquera Lota Protein Ltd., Villagram, Chile) and gelatin (Rousselot Gelatin Co., Ltd., Guangdong, China) were used as dietary protein sources. Crystalline amino acids (Jiangsu Nantong Eastern Amino Acid Co. Ltd., Nantong, China) were used to simulate the amino acid profile of diets with 34 g/kg whole chicken egg protein, except for histidine. The histidine concentrations in fish meal and

Table 1 Composition and nutrients content of the basal diet

Ingredients	g/kg	Nutrients content[a]	g/kg
Fish meal	153.0	Crude protein	328.0
Gelatin	70.0	Crude lipid	61.1
Amino acid mix[b]	187.0	n-3	10.0
Amino acid premix[c]	50.0	n-6	10.0
Fish oil	16.3	Available phosphorus	6.0
Soybean oil	18.9		
α-starch	230.0		
Corn starch	208.3		
Vitamin premix[d]	10.0		
Trace mineral premix[e]	10.0		
Ca $(H_2PO_4)_2$	24.7		
Choline chloride (500 g/kg)	1.3		
Cellulose	20.0		
Ethoxyquin (300 g/kg)	0.5		

[a] Crude protein and crude fat were measured value. Available phosphorus, n-3 and n-6 contents were calculated according to NRC (1993) and Bell (1984)

[b] Amino acid mix (g/kg): arginine, 11.01 g; isoleucine, 13.60 g; leucine, 22.29 g; lysine, 17.69 g; methionine, 8.42 g; cystine, 0.50 g; phenylalanine, 14.90 g; tyrosine, 12.30 g; threonine, 12.80 g; tryptophan, 4.11 g; valine, 16.75 g; glycine, 52.63 g

[c] L-histidine hydrochloride monohydrate was added to obtain graded level of histidine. Each mixture was made isonitrogenous with addition of reduced amounts of glycine and compensated with appropriate amounts of corn starch. Per kilogram of amino acid premix composition from diet 1 to 6 was as follows (g/kg): L-histidine hydrochloride monohydrate 0.00, 41.44, 82.88, 124.31, 165.75, 207.19 g, glycine 298.63, 238.90, 179.18, 119.45, 59.73, 0.00 g and corn starch 701.37, 719.66, 737.94, 756.24, 774.52, 792.81 g, respectively

[d] Per kilogram of vitamin premix (g/kg): retinyl acetate (500,000 IU/g), 0.80 g; cholecalciferol (500,000 IU/g), 0.48 g; DL-α-tocopherol acetate (500 g/kg), 20.00 g; menadione (500 g/kg), 0.20 g; cyanocobalamin (100 g/kg), 0.01 g; D-biotin (200 g/kg), 0.50 g; folic acid (960 g/kg), 0.52 g; thiamin nitrate (980 g/kg), 0.10 g; ascorbyl acetate (920 g/kg), 7.24 g; niacin (980 g/kg), 2.85 g; meso-inositol (980 g/kg), 52.86 g; calcium-D-pantothenate (980 g/kg) 2.51 g; riboflavine (800 g/kg), 0.63 g; pyridoxine hydrochloride (980 g/kg), 0.76 g. All ingredients were diluted with corn starch to 1 kg

[e] Per kilogram of mineral premix (g/kg): $FeSO_4 \cdot 7H_2O$ (197 g/kg Fe), 69.70 g; $CuSO_4 \cdot 5H_2O$ (250 g/kg Cu), 1.20 g; $ZnSO_4 \cdot 7H_2O$ (225 g/kg Zn), 21.64 g; $MnSO_4 \cdot H_2O$ (318 g/kg Mn), 4.09 g; KI (38 g/kg I), 2.90 g; $NaSeO_3$ (10 g/kg Se), 2.50 g. All ingredients were diluted with $CaCO_3$ to 1 kg

gelatin were measured before the formulation by the method of Llames and Fontaine (1994). Experimental diets were supplemented with L-histidine hydrochloride monohydrate to provide histidine levels at 2.5 (unsupplemented control), 4.5, 6.5, 8.5, 10.5, 12.5 g/kg diet. All diets were made isonitrogenous with the addition of appropriate amounts of glycine. Zinc, ferrum, pyridoxine, pantothenic acid, inositol, thiamin and riboflavin were formulated to meet the nutrient requirements of Jian carp according to our laboratory's studies (He et al. 2009; Wen et al. 2009; Jiang et al. 2009a, b; Li et al. 2010; Huang et al. 2011; Tan et al. 2011; Ling et al. 2010). The levels of other nutrients met the requirements for common carp according to the NRC (1993). Procedures for diet preparation and storage were the same as our previous study (Zhao et al. 2012). The histidine concentration in the experimental diets were measured to be 2.3 (unsupplemented control), 4.4, 6.3, 8.6, 10.8 and 12.7 g/kg diet as described by Llames and Fontaine (1994).

Feeding management

Juvenile Jian carp were obtained from Tong Wei Hatchery (Sichuan, China). Feeding management was the same as described in our previous study (Zhao et al. 2012). Fish were adapted to the experimental environment for 4 weeks. A total of 1,200 fish with an average initial weight of 8.76 ± 0.02 g were randomly assigned to 24 experimental aquaria (90 $L \times 30$ $W \times 40$ H cm), each of which was connected to a closed recirculating water system with continuous aeration. Feeding management was conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of Animal Nutritional Institute, Sichuan Agricultural University. Water change rates in each aquarium were maintained at 1.2 L/min, and the water was drained through biofilters in order to decrease microorganism, reduce ammonia concentration and remove solid substances in the water. Dissolved oxygen was higher than 5 mg/L; temperature and pH of the water were maintained at 26 ± 1 and 7.0 ± 0.3 °C, respectively. The experimental units were maintained under natural light and dark cycle. For the feeding trial, each of six experimental diets was fed to quadruplicate of fish six times daily from 1 to 30 days and four times daily from 31 to 60 days. Fish were fed to satiation, and uneaten feed was removed by siphoning after each meal.

Sample collection and analysis

At the end of the feeding trial, fish were anaesthetized in a benzocaine bath (50 mg/L) 12 h after the last

feeding according to the method described by Bohne et al. (2007). Blood of 15 fish collected from each aquarium was drawn from the caudal vein into heparinized syringes, stored at 4 °C overnight, and centrifuged at 3,000×*g* at 4 °C for 10 min, then stored at −70 °C until analyzed. The hepatopancreas, intestine and muscle of the same 15 fish were removed, weighed and frozen in liquid nitrogen, then stored at −70 °C until analyzed. Tissue samples of six fish from each aquarium were homogenized in 10 volumes (w/v) of ice-cold physiological saline and centrifuged at 6,000×*g* at 4 °C for 20 min respectively, and then, supernatants were collected for antioxidant parameters analysis. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University.

The protein concentration of tissue samples were determined by the method of Bradford (1976). Malondialdehyde (MDA) content was assayed as described by Livingstone et al. (1990) using the thiobarbituric acid reaction. Protein carbonyl (PC) content was determined according to the method described by Armenteros et al. (2009). The protein carbonyl content was calculated from the peak absorbance at 370 nm, using an absorption coefficient of 21,000/M cm. The anti-superoxide anion (ASA) capacity (O_2^--scavenging ability) and anti-hydroxyl radical (a-HR) capacity ($^{\cdot}OH$-scavenging ability) were determined by the method described by Zhang et al. (2005) and Jiang et al. (2010), respectively. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities were assayed as described by Zhang et al. (2008). Catalase (CAT) activity was determined by the decomposition of hydrogen peroxide (Aebi 1984). Glutathione-*S*-transferase (GST) activity was measured by monitoring the formation of adduct between GSH and 1-chloro-2, 4-dinitrobenzene (CDNB) (Lushchak et al. 2001). Glutathione reductase (GR) activity was measured according to the method described by Lora et al. (2004). GSH content was determined as described by Vardi et al. (2008). GOT and GPT activities in serum were determined by the method of Bergmeyer and Bernt (1974a, b), respectively.

Calculations and statistical analysis

Results were presented as mean ± SD. All data were subjected to a one-way analysis of variance (ANOVA) followed by the Duncan's multiple-range test to determine significant differences among treatment means at the level of $P < 0.05$ through SPSS 13.0 (SPSS Inc., Chicago, USA). The parameters with significant differences were subjected to a second-degree polynomial regression analysis.

Results

Serum antioxidant parameters

Effects of graded levels of dietary histidine on antioxidant parameters in serum of juvenile Jian carp are given in Table 2 and 3. MDA and PC content significantly decreased with increasing histidine levels up to 10.8 and 8.6 g/kg, respectively, and increased thereafter ($P < 0.05$). ASA capacity was the lowest in fish fed the diet with histidine concentration at 2.3 g/kg (unsupplemented control). a-HR capacity was improved with the increase in dietary histidine levels and was significantly higher in fish fed diets with 4.4, 6.3 and 8.6 g/kg histidine compared with other groups ($P < 0.05$). SOD activities in fish fed diets with 10.8 and 12.7 g/kg histidine were significantly higher than that in fish fed other diets ($P < 0.05$). The CAT, GPX, GST, GR activities and GSH content were significantly enhanced with the increase in dietary histidine levels up to 10.8, 6.3, 8.6, 8.6 and 8.6 g/kg, respectively, and decreased with further increase in dietary histidine concentration ($P < 0.05$). The relationship between serum a-HR capacity, CAT activity, GSH content and dietary histidine levels were described by the following quadratic equation: $Y_{\text{a-HR}} = -5.2929x^2 + 75.727x + 64.55$, $R^2 = 0.904$, $P < 0.05$; $Y_{\text{CAT}} = -0.0585x^2 + 0.9881x + 1.3566$, $R^2 = 0.976$, $P < 0.01$; $Y_{\text{GSH}} = -0.1685x^2 + 2.635x + 1.7548$, $R^2 = 0.970$, $P < 0.01$.

Muscle antioxidant parameters

MDA, PC, and GSH content, ASA and a-HR capacities, SOD, CAT, GPX, GST, GR activities and in muscle are presented in Table 4 and 5. MDA content of the fish fed diet containing 2.3 g/kg (unsupplemented control) histidine was found to be significantly higher than those fed other dietary levels ($P < 0.05$), while no significant differences among other groups were evident ($P > 0.05$). PC content significantly

Table 2 Antioxidant status in serum of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	MDA (nmol/mL)	PC (nmol/mL)	ASA (U/L)	a-HR (U/mL)
2.3	11.47 ± 0.49^{c}	81.16 ± 5.99^{e}	252.2 ± 18.8^{a}	197.6 ± 12.3^{a}
4.4	9.14 ± 1.05^{b}	61.25 ± 3.75^{d}	296.4 ± 17.4^{b}	322.3 ± 10.9^{c}
6.3	8.71 ± 0.80^{b}	54.99 ± 3.98^{c}	291.4 ± 17.7^{b}	318.5 ± 22.2^{c}
8.6	8.12 ± 0.83^{ab}	39.08 ± 4.17^{a}	289.9 ± 18.1^{b}	336.0 ± 22.1^{c}
10.8	7.47 ± 0.44^{a}	45.16 ± 3.59^{b}	303.4 ± 10.2^{b}	237.9 ± 9.10^{b}
12.7	8.89 ± 1.05^{b}	62.16 ± 4.35^{d}	299.0 ± 20.1^{b}	187.3 ± 11.0^{a}

Values are mean ± SD of four groups (6 fish per group)

MDA malondialdehyde, *PC* protein carbonyl, *ASA* anti-superoxide anion, *a-HR* anti-hydroxy radical

[a–e] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

Table 3 Antioxidant enzyme activity and glutathione content in serum of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	SOD (U/mL)	CAT (U/mL)	GPX (U/0.1 mL)	GST (U/mL)	GR (U/L)	GSH (mg/mL)
2.3	123.9 ± 6.2^{a}	3.43 ± 0.40^{a}	113.6 ± 11.0^{b}	83.1 ± 4.0^{a}	192.9 ± 18.0^{a}	7.13 ± 0.51^{a}
4.4	129.8 ± 8.4^{a}	4.34 ± 0.25^{b}	118.0 ± 8.9^{bc}	132.0 ± 10.6^{b}	209.0 ± 18.0^{ab}	9.57 ± 0.27^{c}
6.3	133.0 ± 5.4^{a}	5.33 ± 0.20^{c}	129.6 ± 12.4^{c}	168.7 ± 11.7^{c}	209.0 ± 18.0^{ab}	11.87 ± 0.62^{d}
8.6	132.6 ± 18.0^{a}	5.60 ± 0.25^{c}	114.2 ± 9.7^{b}	376.2 ± 16.8^{e}	297.4 ± 22.0^{d}	12.32 ± 0.62^{d}
10.8	149.3 ± 10.5^{b}	5.24 ± 0.25^{c}	97.7 ± 5.8^{a}	257.9 ± 24.4^{d}	265.3 ± 22.0^{c}	10.20 ± 0.63^{c}
12.7	150.1 ± 9.0^{b}	4.43 ± 0.38^{b}	88.5 ± 9.7^{a}	154.3 ± 13.0^{c}	233.1 ± 18.0^{b}	8.13 ± 0.36^{b}

Values are mean ± SD of four groups (6 fish per group)

SOD superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *GST* glutathione-*S*-transferase, *GR* glutathione reductase, *GSH* glutathione

[a–e] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

decreased with dietary histidine levels up to 8.6 g/kg and significantly increased thereafter ($P < 0.05$). ASA capacity was significantly improved with increasing dietary histidine levels up to 8.6 g/kg diet ($P < 0.05$) and plateaued thereafter ($P > 0.05$). a-HR capacity followed a similar pattern to that observed in ASA capacity. There was no significant difference in SOD activities among the groups ($P > 0.05$). CAT, GPX and GR activities significantly increased with increasing dietary histidine levels up to 6.3 g/kg, after that significantly decreased ($P < 0.05$). GST activity was minimal in fish fed diet with 2.3 g/kg histidine and was maximum in fish fed diet with 8.6 g/kg histidine. Fish fed diets of histidine levels at 2.3 and 12.7 g/kg diet had lower GSH content than fish fed other diets. Regression analysis showed that muscle MDA, PC content, ASA capacity, GST activity and GSH content were quadratic response to graded levels of dietary histidine ($Y_{\mathrm{MDA}} = 0.0146x^2 - 0.2699x + 3.7818$, $R^2 = 0.967$, $P < 0.01$; $Y_{\mathrm{PC}} = 0.0129x^2 - 0.1646x + 1.8524$, $R^2 = 0.861$, $P = 0.051$; $Y_{\mathrm{ASA}} = -0.011x^2 + 0.8036x + 30.935$, $R^2 = 0.903$, $P < 0.05$; $Y_{\mathrm{GST}} = -0.4353x^2 + 7.7412x + 161.9$, $R^2 = 0.875$, $P < 0.05$; $Y_{\mathrm{GSH}} = -0.1352x^2 + 1.9996x + 10.57$, $R^2 = 0.852$, $P = 0.060$).

Intestine antioxidant parameters

As shown in Table 6, MDA and PC content, ASA and a-HR capacities in intestine were significantly affected by dietary histidine. MDA content was reduced with the increase in dietary histidine levels up to 8.6 g/kg diet and then significantly increased ($P < 0.05$). PC content was the lowest for fish fed diet containing 4.4, 6.3 and 8.6 g/kg histidine and was the highest for fish fed diet with 12.7 g/kg histidine. Both ASA and a-HR capacities were significantly improved with increasing dietary histidine levels up to 8.6 g/kg diet and declined thereafter ($P < 0.05$). Effects of graded levels of dietary histidine on SOD, CAT, GPX, GST, GR

Table 4 Antioxidant status in muscle of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	MDA (nmol/mg protein)	PC (nmol/mg protein)	ASA (mU/mg protein)	a-HR (U/mg protein)
2.3	3.28 ± 0.18^{b}	1.49 ± 0.15^{b}	32.97 ± 1.92^{a}	570.6 ± 30.9^{a}
4.4	2.79 ± 0.15^{a}	1.43 ± 0.15^{ab}	34.45 ± 2.60^{a}	563.3 ± 37.4^{a}
6.3	2.68 ± 0.23^{a}	1.42 ± 0.09^{ab}	34.18 ± 2.88^{a}	652.8 ± 48.9^{b}
8.6	2.58 ± 0.20^{a}	1.29 ± 0.09^{a}	37.89 ± 3.82^{b}	649.4 ± 48.9^{b}
10.8	2.57 ± 0.24^{a}	1.53 ± 0.14^{b}	38.93 ± 2.50^{b}	659.9 ± 73.9^{b}
12.7	2.69 ± 0.24^{a}	1.89 ± 0.16^{c}	38.84 ± 1.52^{b}	653.3 ± 43.1^{b}

Values are mean ± SD of four groups (6 fish per group)

MDA malondialdehyde, *PC* protein carbonyl, *ASA* anti-superoxide anion, *a-HR* anti-hydroxy radical

$^{a–c}$ Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

Table 5 Antioxidant enzyme activity and glutathione content in muscle of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GPX (U/mg protein)	GST (U/mg protein)	GR (mU/mg protein)	GSH (mg/g protein)
2.3	9.90 ± 0.59^{a}	0.76 ± 0.09^{a}	53.31 ± 3.35^{a}	175.3 ± 15.0^{a}	55.53 ± 3.89^{b}	14.21 ± 1.18^{a}
4.4	10.07 ± 0.69^{a}	1.14 ± 0.06^{b}	60.21 ± 5.30^{b}	191.6 ± 18.0^{ab}	67.92 ± 4.78^{c}	17.48 ± 1.26^{b}
6.3	10.02 ± 0.34^{a}	1.70 ± 0.16^{c}	83.57 ± 4.67^{c}	192.3 ± 15.3^{ab}	122.93 ± 7.53^{f}	17.41 ± 1.43^{b}
8.6	10.34 ± 0.73^{a}	1.16 ± 0.12^{b}	57.80 ± 3.73^{ab}	196.4 ± 12.5^{b}	91.90 ± 3.82^{e}	17.09 ± 1.39^{b}
10.8	10.04 ± 0.89^{a}	0.85 ± 0.09^{a}	56.02 ± 4.61^{ab}	191.8 ± 3.5^{ab}	81.29 ± 5.24^{d}	17.33 ± 0.72^{b}
12.7	10.20 ± 0.81^{a}	0.87 ± 0.08^{a}	60.31 ± 3.04^{b}	191.9 ± 14.3^{ab}	48.48 ± 4.09^{a}	13.82 ± 1.27^{a}

Values are mean ± SD of four groups (6 fish per group)

SOD superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *GST* glutathione-*S*-transferase, *GR* glutathione reductase, *GSH* glutathione

$^{a–f}$ Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

activities and GSH content in intestine are given in Table 7. SOD activities were the lowest in fish fed diet with 2.3 g/kg histidine and were the highest in fish fed with 8.6 and 10.8 g/kg dietary histidine. CAT and GPX activities were significantly enhanced with the increase in dietary histidine levels up to 8.6 and 6.3 g/kg diet, respectively, and decreased thereafter ($P < 0.05$). Fish fed diets with 6.3 and 8.6 g/kg histidine had significantly higher GST activities than those fed other diets ($P < 0.05$). No significant differences in GR activities among the groups were evident ($P > 0.05$). The GSH content was significantly lower in fish fed the diet with histidine concentration at 2.3 g/kg (unsupplemented control) than that in other groups ($P < 0.05$). The second-degree polynomial regression equations about the relationship between intestinal PC content, SOD, CAT, GST activity and dietary histidine levels were presented as following: $Y_{PC} = 0.0137x^2 - 0.1668x + 2.645$, $R^2 = 0.908$, $P < 0.05$; $Y_{SOD} = -0.1685x^2 + 2.8623x + 32.701$, $R^2 = 0.868$, $P < 0.05$; $Y_{CAT} = -0.751x^2 + 12.908x + 13.026$, $R^2 = 0.854$, $P = 0.056$; $Y_{GST} = -3.2315x^2 + 46.649x + 39.586$, $R^2 = 0.920$, $P < 0.05$.

Hepatopancreas antioxidant parameters

MDA and PC content, ASA and a-HR capacities in hepatopancreas of juvenile Jian carp fed diets containing graded levels of histidine are presented in Table 8. MDA content was the highest in fish fed diets with 2.3 and 4.4 g/kg histidine and the lowest in fish fed diet with 8.6 g/kg histidine. PC content significantly decreased with the increase in dietary histidine levels and was significantly lower in fish fed diets with 6.3 and 8.6 g/kg histidine compared with that in other groups ($P < 0.05$). ASA capacity was the highest in fish fed diet with 8.6 g/kg histidine, followed by those

Table 6 Antioxidant status in intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	MDA (nmol/mg protein)	PC (nmol/mg protein)	ASA (mU/mg protein)	a-HR (U/mg protein)
2.3	4.42 ± 0.48^{c}	2.30 ± 0.21^{ab}	281.0 ± 24.3^{a}	472.9 ± 45.5^{a}
4.4	4.29 ± 0.22^{c}	2.24 ± 0.24^{a}	379.6 ± 31.0^{bc}	480.5 ± 54.0^{a}
6.3	3.45 ± 0.23^{b}	2.16 ± 0.19^{a}	551.7 ± 57.4^{e}	539.6 ± 44.2^{b}
8.6	2.51 ± 0.25^{a}	2.11 ± 0.18^{a}	457.3 ± 44.1^{d}	702.9 ± 43.4^{d}
10.8	3.14 ± 0.27^{b}	2.52 ± 0.18^{bc}	393.9 ± 24.7^{c}	624.5 ± 41.3^{c}
12.7	4.54 ± 0.36^{c}	2.72 ± 0.21^{c}	347.0 ± 26.4^{b}	630.8 ± 46.3^{c}

Values are mean ± SD of four groups (6 fish per group)

MDA malondialdehyde, *PC* protein carbonyl, *ASA* anti-superoxide anion, *a-HR* anti-hydroxy radical

[a–e] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

Table 7 Antioxidant enzyme activity and glutathione content in intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GPX (U/mg protein)	GST (U/mg protein)	GR (mU/mg protein)	GSH (mg/g protein)
2.3	38.57 ± 2.86^{a}	40.09 ± 1.78^{a}	44.47 ± 5.74^{a}	139.4 ± 8.2^{b}	127.5 ± 9.0^{a}	12.63 ± 1.04^{a}
4.4	42.34 ± 2.90^{bc}	54.43 ± 3.43^{b}	68.01 ± 2.95^{cd}	166.8 ± 11.8^{c}	123.1 ± 7.0^{a}	20.45 ± 1.06^{bc}
6.3	42.95 ± 1.81^{bc}	59.54 ± 3.89^{bc}	72.21 ± 5.20^{d}	202.9 ± 15.9^{d}	121.7 ± 8.9^{a}	21.99 ± 1.07^{c}
8.6	44.81 ± 2.45^{c}	76.21 ± 7.36^{d}	62.16 ± 5.59^{bc}	205.7 ± 8.3^{d}	118.9 ± 9.4^{a}	20.48 ± 1.01^{bc}
10.8	45.43 ± 3.30^{c}	61.31 ± 3.27^{c}	62.13 ± 6.77^{bc}	180.6 ± 16.9^{c}	124.3 ± 9.1^{a}	20.48 ± 1.54^{bc}
12.7	41.05 ± 3.40^{ab}	56.11 ± 5.07^{bc}	59.83 ± 5.68^{b}	101.0 ± 9.1^{a}	123.6 ± 8.3^{a}	19.44 ± 1.80^{b}

Values are mean ± SD of four groups (6 fish per group)

SOD superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *GST* glutathione-*S*-transferase, *GR* glutathione reductase, *GSH* glutathione

[a–d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

with 10.8 and 12.7 g/kg histidine, and was the lowest in fish fed diet of histidine level at 2.3 g/kg. No significant differences in a-HR capacities among the groups were found ($P > 0.05$). SOD, CAT, GPX, GST, GR activities and GSH content in hepatopancreas of juvenile Jian carp fed graded levels of dietary histidine are presented in Table 9. SOD activities were lowest in fish fed diets with 4.4, 6.3 and 8.6 g/kg histidine and were highest in fish fed diets containing 10.8 and 12.7 g/kg histidine. CAT activity was the highest in fish fed unsupplemented control diet and then significantly decreased ($P < 0.05$), while no significant differences were found among the other groups ($P > 0.05$). GST activities were gradually reduced with dietary histidine up to 6.3 g/kg diet and after that significantly increased ($P < 0.05$). GPX, GR activities and GSH content were significantly improved with the increase in dietary histidine levels up to 8.6 g/kg diet and decreased with levels further increasing ($P < 0.05$). Hepatopancreas PC content, ASA capacity, GPX and GR activity to dietary levels of histidine relationship were described by quadratic regression analysis: $Y_{PC} = 0.0255x^2 - 0.3713x + 2.3742$, $R^2 = 0.901$, $P < 0.05$; $Y_{ASA} = -1.156x^2 + 27.308x + 278.21$, $R^2 = 0.907$, $P < 0.05$; $Y_{GPX} = -2.7072x^2 + 41.234x + 17.337$, $R^2 = 0.911$, $P < 0.05$, $Y_{GR} = = -1.4145x^2 + 18.868x + 52.031$, $R^2 = 0.923$, $P < 0.05$.

Serum GOT and GPT activities

Effects of graded levels of dietary histidine on GOT and GPT activities in serum of juvenile Jian carp are shown in Table 10. GOT activities were lowest in fish fed diets with 2.3 and 4.4 g/kg histidine, and significantly increased with dietary histidine levels up to 10.8 g/kg diet and then decreased ($P < 0.05$). GPT activity was highest in fish fed the unsupplemented control diet and was lowest in those fed diet of histidine level at 8.6 g/kg. Regression analysis

Table 8 Antioxidant status in hepatopancreas of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	MDA (nmol/mg protein)	PC (nmol/mg protein)	ASA (mU/mg protein)	a-HR (U/mg protein)
2.3	5.54 ± 0.51^{d}	1.64 ± 0.09^{d}	346.2 ± 28.9^{a}	618.5 ± 35.9^{a}
4.4	5.87 ± 0.59^{d}	1.30 ± 0.12^{b}	357.6 ± 28.6^{ab}	629.8 ± 42.8^{a}
6.3	4.90 ± 0.36^{c}	1.01 ± 0.08^{a}	398.0 ± 25.1^{bc}	624.9 ± 51.0^{a}
8.6	3.24 ± 0.22^{a}	0.96 ± 0.08^{a}	445.8 ± 38.7^{d}	657.8 ± 28.1^{a}
10.8	3.59 ± 0.16^{ab}	1.51 ± 0.12^{c}	439.2 ± 25.5^{cd}	649.9 ± 46.2^{a}
12.7	3.98 ± 0.31^{b}	1.71 ± 0.15^{d}	433.0 ± 54.8^{cd}	658.1 ± 63.6^{a}

Values are mean ± SD of four groups (6 fish per group)

MDA malondialdehyde, *PC* protein carbonyl, *ASA* anti-superoxide anion, *a-HR* anti-hydroxy radical

[a–d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

Table 9 Antioxidant enzyme activity and glutathione content in hepatopancreas of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

His (g/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GPX (U/mg protein)	GST (U/mg protein)	GR (mU/mg protein)	GSH (mg/g protein)
2.3	56.94 ± 3.73^{ab}	33.79 ± 3.44^{b}	97.9 ± 5.9^{a}	143.7 ± 12.9^{d}	89.1 ± 6.5^{b}	3.21 ± 0.28^{a}
4.4	54.29 ± 4.05^{a}	27.79 ± 2.76^{a}	140.6 ± 7.9^{c}	119.0 ± 8.2^{ab}	106.2 ± 5.9^{c}	3.35 ± 0.33^{a}
6.3	51.68 ± 3.76^{a}	27.57 ± 2.18^{a}	178.3 ± 16.4^{d}	109.1 ± 6.5^{a}	111.1 ± 5.6^{c}	5.59 ± 0.31^{c}
8.6	55.49 ± 3.81^{a}	27.37 ± 1.53^{a}	177.5 ± 15.3^{d}	129.0 ± 10.2^{bc}	118.7 ± 6.2^{d}	6.30 ± 0.39^{d}
10.8	61.34 ± 6.03^{b}	27.55 ± 1.01^{a}	130.1 ± 13.7^{c}	132.1 ± 11.0^{cd}	83.7 ± 6.5^{b}	4.75 ± 0.40^{b}
12.7	61.65 ± 6.19^{b}	27.23 ± 1.62^{a}	112.4 ± 8.4^{b}	131.6 ± 10.6^{cd}	65.5 ± 5.2^{a}	3.19 ± 0.26^{a}

Values are mean ± SD of four groups (6 fish per group)

SOD superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *GST* glutathione-*S*-transferase, *GR* glutathione reductase, *GSH* glutathione

[a–d] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

showed that serum GOT activity was quadratic response to graded levels of dietary histidine ($Y_{GOT} = 0.007x^2 + 1.5892x + 14.617$, $R^2 = 0.881$, $P < 0.05$).

Histidine requirement

As shown in Fig. 1a, on subjecting the serum MDA content and dietary histidine levels to second-degree polynomial regression analysis, optimum histidine level was found at 9.2 g/kg diet or 2.80 g/100 g protein. The relationship was described by the following equation: $Y_{MDA} = 0.0743x^2 - 1.3717x + 14.122$, $R^2 = 0.933$, $P < 0.05$. The PC content in serum to dietary levels of histidine relationship was described by quadratic regression analysis ($Y_{PC} = 0.9769x^2 - 16.89x + 116.49$, $R^2 = 0.932$, $P < 0.05$) (Fig. 1b). Based on the above equation, the optimum histidine level was estimated to be 8.6 g/kg diet or 2.62 g/100 g protein.

Discussion

Fish are a rich source of the n-3 polyunsaturated fatty acids (PUFA), which are vital constituents for cell membrane structure and function, but which are also highly susceptible to attack by oxygen and other organic radicals (Mourente et al. 2007). The highly unsaturated fatty acid composition of fish muscle makes it extremely susceptible to oxidation stress (Olsen and Henderson 1997). Intestine and pancreas are the main digestive organs for stomachless fish such as carp, and their antioxidant status play an important role for fish growth (Jiang et al. 2010). Study in vitro showed that histidine exerted an inhibition effect

Table 10 Activity of glutamate–oxaloacetate transaminase (GOT, U/L) and glutamate-pyruvate transaminase (GPT, U/L) in serum of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets with graded levels of histidine

	Dietary histidine levels (g/kg)					
	2.3	4.4	6.3	8.6	10.8	12.7
GOT	19.62 ± 0.87[a]	20.15 ± 0.81[a]	24.08 ± 1.12[b]	28.00 ± 2.08[c]	36.92 ± 1.62[e]	33.53 ± 1.46[d]
GPT	7.11 ± 0.69[b]	6.80 ± 0.47[ab]	6.58 ± 0.53[ab]	6.04 ± 0.40[a]	6.58 ± 0.88[ab]	6.63 ± 0.39[ab]

Values are mean ± SD of four groups (6 fish per group)

[a–e] Mean values within the same row with different superscripts are significantly different ($P < 0.05$)

on lipid peroxidation in fish muscle sarcoplasmic reticulum suspension experimental system (Erickson and Hultin 1988, 1992). Therefore, the present study investigated the effects of dietary histidine on oxidative stress and antioxidant responses in serum, muscle, intestine and hepatopancreas of Jian carp.

Lipid peroxidation can be defined as the oxidative deterioration of PUFA and is an important consequence of oxidative stress, as indicated by the levels of malondialdehyde (MDA), which is a key metabolite production derived from lipid oxidation (Mourente et al. 2007). The current study showed that MDA content was reduced with histidine supplement in serum and tissues, which indicated that lipid peroxidation was suppressed by histidine. Similar results were documented with a fish muscle sarcoplasmic reticulum suspension experimental system in vitro (Erickson and Hultin 1988, 1992). Free radicals also catalyze the oxidation of amino acid residues in proteins, forming protein carbonyls (PC). PC content is the most widely used marker of oxidative modification of proteins (Uchida and Kawakishi 1993a). In our study, optimal level of dietary histidine decreased the PC content in serum and tissues, suggesting that protein oxidation were also inhibited by histidine. Decker et al. (2001) noted that histidine was effective in inhibiting formation of carbonyls on bovine serum albumin. It has been established that oxidative modification of proteins involves the conversion of amino acids to their oxidized forms and histidine residue is one of the major sites of damage during radical attack upon proteins, whereas supplement of histidine may donate itself to free radicals resulting in the stabilization of the protein (Uchida 2003). The findings of the present study suggest that histidine alleviated the oxidative damage of Jian carp in different tissues and organs and ensured the normal function of various tissues and organs as well as the whole body. Based on serum MDA and PC content data, the dietary histidine requirements of juvenile Jian carp were estimated to be 9.2 g/kg diet (2.80 g/100 g protein) and 8.6 g/kg diet (2.62 g/100 g protein), respectively, which were a little higher than that based on specific growth rate (7.8 g/kg diet or 2.38 g/100 g protein) (Zhao et al. 2012).

Increased ROS generation by monovalent reduction in cellular aerobic metabolism is responsible for increased oxidative injury to lipids and proteins (Livingstone 2003). Wade and Tucker (1998) implied that the effects of histidine to inhibit lipid peroxidation may be related to its interaction with toxic oxygen species. In this work, we determined the scavenging ability of histidine against superoxide radicals ($O_2^{\cdot-}$) and hydroxyl radical ($^{\cdot}OH$), two agents strongly involved in oxidative damage (Kohen and Nyska 2002). $O_2^{\cdot-}$ yielded from electron leakage in the mitochondrial respiratory transport chain imply a high toxicity to compound (Klotz and Sies 2009). In the present study, $O_2^{\cdot-}$-scavenging ability (indicated by ASA capacity) was enhanced with histidine supplement in serum and tissues. Spectroscopic evidence has established the generation of singlet oxygen ($^1O_2$) in the water-induced dismutation of $O_2^{\cdot-}$ and in the electron transfer reaction or Haber–Weiss reaction of $O_2^{\cdot-}$ (Khan and Kasha 1994). Histidine has been recognized as an efficient quencher of $^1O_2$ and may accelerate the consumption of $O_2^{\cdot-}$ through scavenging the product $^1O_2$ (Foote and Clennan 1995). Thus, histidine may act as $O_2^{\cdot-}$-scavenger indirectly through its interaction with $^1O_2$. Furthermore, cellular $O_2^{\cdot-}$ is mainly reduced to form hydrogen peroxide (H_2O_2) and further reduced to generated $^{\cdot}OH$ (Klotz and Sies 2009). The present study showed that $^{\cdot}OH$-scavenging ability (indicated by a-HR capacity) in serum, muscle and intestine were improved by dietary histidine. The effects of histidine to prevent $^{\cdot}OH$ generation or to

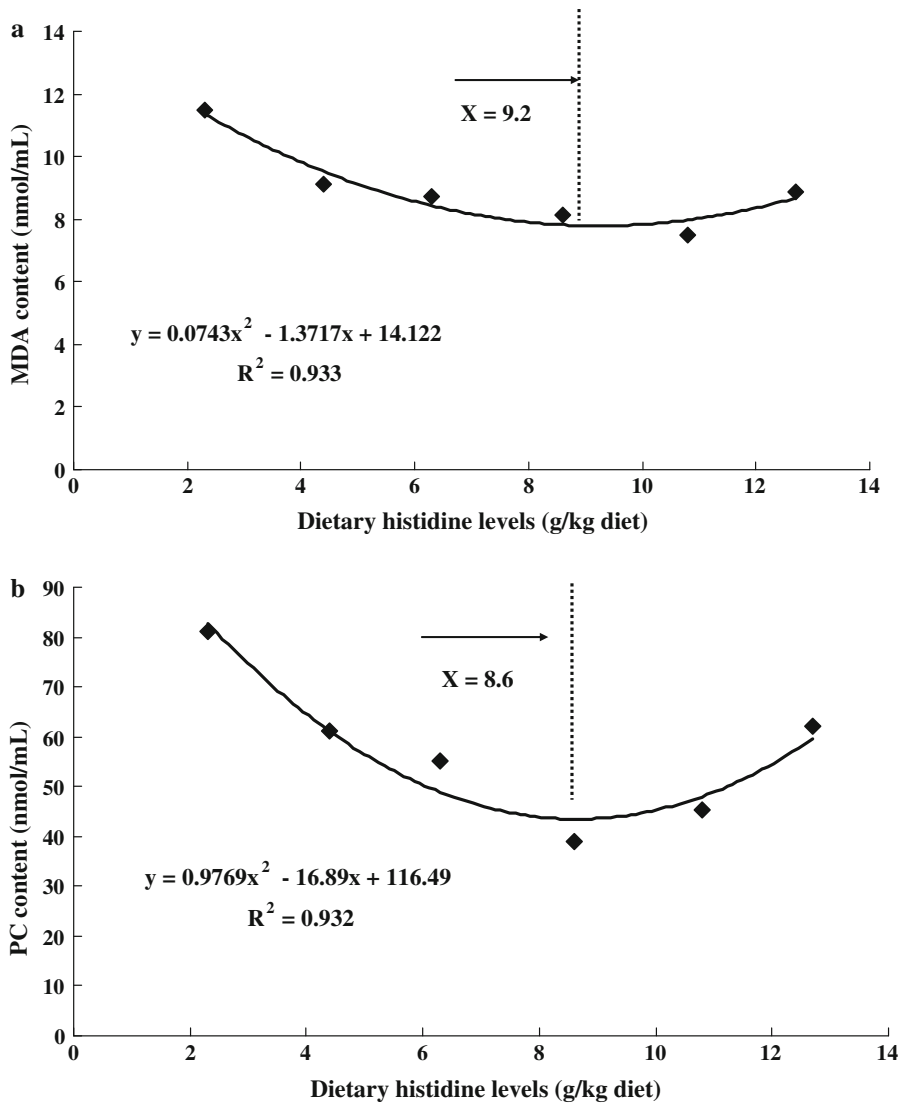

Fig. 1 Quadratic regression analysis of malondialdehyde (MDA) content (**a**) and protein carbonyl (PC) content (**b**) in serum for juvenile Jian carp (*Cyprinus carpio* var. Jian) fed diets containing graded levels of histidine for 60 days. Each point represents the mean of four replicates, with six fish per group. Optimal levels of dietary histidine for serum MDA and PC content were 9.2 and 8.6 g/kg diet, respectively

eliminate ·OH had been established in vitro (Nagy and Floyd 1984; Obata et al. 2001). ·OH is generated from H_2O_2 via the Fenton reaction, which was mediated by divalent metal ion iron or copper (Klotz and Sies 2009). Histidine is an efficient chelator for copper and iron (Chevion 1988). Erickson and Hultin (1988, 1992) suggested that the effects of histidine to inhibit ·OH generation from its ability to coordinate with iron or copper, thus interfering with Fenton reaction that produce the ·OH. Histidine also could form a tight complex with H_2O_2 and thus lower the rate of ·OH formation (Chevion, 1988). Lipid peroxidation occurred when PUFA is attack directly by ·OH (Livingstone 2003). In this study, correlation analysis showed that MDA content was negative correlated to a-HR capacity in muscle ($r = -0.760$, $P = 0.08$) and in intestine ($r = -0.671$, $P = 0.14$). Therefore, the inhibition of lipid peroxidation by histidine may partly due to the promotion of ·OH-scavenging ability. In a word, histidine alleviated the oxidative damage in two main ways: decreasing the generation and/or increasing the elimination of ROS.

In the antioxidative defense of the host system, SOD catalyzes the conversion of $O_2^{\cdot-}$ to H_2O_2 and the dioxygen molecule (Khan and Kasha 1994). The present data showed that SOD activities were improved by dietary histidine in serum and intestine, whereas no difference in muscle. Tansini et al. (2004) reported SOD activity was not affected by histidine in brain of rats. Histidine residues serve as essential

components in the active center of bovine erythrocyte Cu, Zn-SOD (Uchida and Kawakishi 1993b). When treated with its own reaction product H_2O_2, histidine residues at the active site were oxidized, which resulted in the inactivation of the enzyme (Uchida and Kawakishi 1993b). But whether dietary histidine supplementation can protect the histidine residues at the active site of Cu, Zn-SOD awaits investigation. The product of SOD dismutation, H_2O_2, can be removed by the CAT and GPX (Livingstone 2003). Our results demonstrated that CAT and GPX activities in serum, muscle and intestine as well as GPX activity in hepatopancreas were improved with dietary histidine up to an optimum level. Lee et al. (2005) and Liu et al. (2008) noted that histidine supplement elevated CAT and GPX activities in kidney and liver of mice. GST have a cytoprotective role involving elimination of reactive chemical species originating from the oxidative metabolism (Baez et al. 1997). In the present study, GST activity in serum, muscle and intestine were improved by histidine. Interestingly, our results showed that SOD, CAT and GST activities in hepatopancreas decreased with the increase in dietary histidine levels. a-HR capacity in hepatopancreas showed no difference among groups. A possible explanation could be that these antioxidant enzymes activities in hepatopancreas were inactivated by ROS. Growth rate was positive related to energy metabolism and amino acid metabolism, particularly in liver (Chessex et al. 1981). Our previous study has demonstrated that both specific growth rate and protein productive value exhibited positive quadratic relationship with dietary histidine levels (Zhao et al. 2012) $O_2^{\cdot-}$ is mainly generated from respiratory transport chain and $^1O_2$ and $^{\cdot}OH$ were derived from $O_2^{\cdot-}$ (Khan and Kasha 1994). SOD and CAT could be inactivated by $O_2^{\cdot-}$, $^1O_2$ and $^{\cdot}OH$ (Pigeolet et al. 1990; Kim et al. 2001). Thus, fish with higher growth rate may associate with more ROS in liver, which thereby inhibited the activities of antioxidant enzymes. Serum glutamate–oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) activities are usually used as indicators of the function of vertebrate liver (Lin et al. 2010). The histology changes of liver are important indicators of the nutritional and physiological status of fish (Gatta et al. 2011). Chien et al. (2003) suggested GOT and GPT may be indirectly related to oxidant metabolites and also serve as indicators of oxidative status of liver. In the current study, Jian carp fed diet with 8.6 g/kg histidine had lower GPT activity than those fed control diet, while GOT activity in serum increased with the increase in dietary histidine levels. Liu et al. (2008) reported serum GOT and GPT activities were decreased with histidine supplementary in mice, whereas Easter and Baker (1977) reported that they were unaffected by histidine in gravid swine. The reason for these interesting results was not clear. Glutathione (GSH) is a tripeptide containing a thiol group and is an important protective non-enzyme antioxidant against free radicals and other oxidants (Rahman and MacNee 2000). The present study indicated that GSH content in serum and tissues was improved by histidine. Similar result was observed in liver of mice (Liu et al. 2008). GR catalyze the reduction of the oxidized of glutathione (GSSG) to GSH, at the expense of the NADPH (Reed 1990). The present work demonstrated that histidine was effective in promoting the activity of GR in serum, muscle and hepatopancreas. The elevation of GSH content may probably attribute to the promotion of GR activity. Our results suggested that histidine can promote antioxidant enzyme activity and non-enzyme antioxidant content, which contribute to the improvement of antioxidant capacity in Jian carp.

In summary, the present work showed that dietary histidine could elevate antioxidant enzymes activities and GSH content, enhance oxygen species-scavenging ability and thus inhibit lipid peroxidation and protein oxidation of Jian carp. The result of this study could partly provide theoretical evidence for the improvement of growth, protein deposition, digestion and absorption ability by histidine in our previous research. However, further studies should be carried out to reveal the underlying mechanisms of dietary histidine on antioxidant capacity of fish.

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