Animal Nutrition 1 (2015) 77–84

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original research article

# The influence of graded levels of available phosphorus on growth performance, muscle antioxidant and flesh quality of young grass carp (*Ctenopharyngodon idella*)

Jing Wen [a], Weidan Jiang [a, b, c], Lin Feng [a, b, c], Shengyao Kuang [d], Jun Jiang [a, b, c], Ling Tang [d], Xiaoqiu Zhou [a, b, c, *], Yang Liu [a, b, c, *]

[a] Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China
[b] Fish Nutrition and Safety Production University Key Laboratory of Sichuan Province, Sichuan Agricultural University, Chengdu 611130, China
[c] Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Chengdu 611130, China
[d] Animal Nutrition Institute, Sichuan Academy of Animal Science, Chengdu 610066, China

ARTICLE INFO

*Article history:*
Received 19 December 2014
Accepted 10 February 2015
Available online 22 May 2015

*Keywords:*
Young grass carp (*Ctenopharyngodon idella*)
Phosphorus
Growth
Flesh quality
Antioxidant enzyme

ABSTRACT

Growth, muscle composition, meat quality characteristics and antioxidant capacity in muscle of young grass carp (*Ctenopharyngodon idella*) (initial weight 282.9 ± 3.3 g) fed graded levels of phosphorus (1.0, 2.5, 3.8, 5.6, 7.8 and 9.5 g/kg diet) for 8 wk were investigated. Results indicated that percentage weight gain, feed intake, feed efficiency, serum phosphorus and alkaline phosphatase were improved with optimal phosphorus supplementations ($P < 0.05$). Muscle protein content and water holding capacity were significantly elevated, while moisture, lipid and ash contents were significantly decreased with dietary phosphorus to a certain level ($P < 0.05$). The meat shear force value and hydroxyproline content were not influenced by different levels of phosphorus. Muscle anti-hydroxyl radical, superoxide dismutase, catalase, glutathione S-transferase activities and glutathione content were significantly improved ($P < 0.05$). Conversely, anti-superoxide anion, glutathione reducase and glutathione peroxidase activities were decreased ($P < 0.05$) with dietary phosphorus to a certain level. These results indicated that suitable dietary phosphorus improved growth performance, meat quality and muscle antioxidant capacity. Dietary available phosphorus requirement of young grass carp for percentage weight gain was 4.0 g/kg diet.



## 1. Introduction

Grass carp (*Ctenopharyngodon idella*) is one of the widely cultured species in China and its total output exceeded 4,200,000 tons in 2011 (Yang et al., 2013). Successful intensive farming depends on artificial feeds (Borlongan and Satoh, 2001). The formulation of feeds is based on the nutrition requirements of fish (Borlongan and Satoh, 2001). Phosphorus is one essential major mineral to fish (Pimentel-Rodrigues and Oliva-Teles, 2001). The concentration of this element is low in freshwater (Boyd, 1981), and thus fish cannot absorb enough phosphorus from water (Lall, 1991). Dietary phosphorus is the main source of phosphorus for grass carp (Liang et al., 2012). Dietary phosphorus deficiency could result in low feed efficiency and poor growth of juvenile grass carp (Liang et al., 2012) and juvenile Jian carp (*Cypinus carpio* var. Jian) (Xie et al., 2011). Currently, phosphorus requirement studies have focused on juvenile fish, such as juvenile grass carp (Liang et al., 2012) and juvenile Jian carp (Xie et al., 2011). However, nutrient requirements may be different with fish growth stages. It has been

* Corresponding authors.
*E-mail addresses:* xqzhouqq@tom.com, zhouxq@sicau.edu.cn (X. Zhou), kyckgk@hotmail.com (Y. Liu).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2015.05.004

reported that the phosphorus requirement of rainbow trout (*Oncorhynchus mykiss*) decreased with an increase in fish size (Lellis et al., 2004). The phosphorus requirement for young grass carp has not yet been studied. Thus, it is necessary to address the phosphorus requirement of young grass carp.

Muscle tissue of fish comprises the main edible portion for people (Periago et al., 2005). Fish flesh containing an abundant polyunsaturated fatty acid and balanced amino acid profile is becoming more and more popular with consumers (Martınez-Alvarez et al., 2005; Khan et al., 2011). It is well known that water, protein and lipid content, water holding capacity, pH, collagen concentration, hydroxyproline content, as well as the mechanical properties of the flesh (cohesiveness) together make up the most important flesh quality traits (Periago et al., 2005). Those muscle characteristics have been shown to be influenced by diet, genetics, exercise, and environment (Johnston, 1999, 2001). Among those factors, nutrition is an important one (Izquierdo et al., 2003). It has been reported that low calcium levels in diets can decrease pork drip loss in pig (Shelton et al., 2004). However, no studies have been conducted to investigate the effects of phosphorus on fish flesh quality. Zhou et al. (2004) found that phosphorus could enhance muscle calcium content in orange-spotted groupers (*Epinephebs coioides*). This indicates that phosphorus may influence fish muscle firmness. However, no study has yet addressed this, and so this requires investigation.

Phosphorus is essential for phospholipid bilayer (Zubay, 1983). Phospholipid is a major component of the eukaryotic membranes (Stanley and Parkin, 1991). Phospholipid of fish contains large quantities of polyunsaturated fatty acids (PUFAs) (Liang and Hultin, 2005). However, PUFAs are highly susceptible to the attack of reactive oxygen species (ROS) (Martınez-Alvarez et al., 2005), which produce malondialdehyde (MDA) (Fang et al., 2002). Oxidation of membranal phospholipids leads to a decrease in fluidity of the biomembrane and disruption of muscle cell membrane increasing exudative loss from meat (Buckley et al., 1995). Lipid peroxidation is known to catalyze protein oxidation (Adams et al., 2001). After oxidation, the flesh tenderness may be changed due to myosin oxidized and aggregated (Martinaud et al., 1997). Superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) are the main ROS which can result in oxidative damage (Martınez-Alvarez et al., 2005). To defend against the ROS, fish have developed enzymes and nonenzymatic compounds to prevent oxidative damage. These include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and glutathione (GSH). Phosphorus is a fundamental element of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) (Pasek, 2008). NADPH could protect CAT in bovine liver from inactivation (Kirkman et al., 1999). In organisms, depending on the NADPH, GR can reduce glutathione disulfide (GSSG) into GSH (Davies, 2000). Furthermore, the endogenous GSH synthesis needs energy supplied by ATP (Fang et al., 2002). This indicates that phosphorus may have an effect on the flesh quality through the influence of the enzyme and nonenzymatic antioxidant system in the muscle of fish. However, no study with phosphorus focused on this topic, and so warrants investigation.

The present study aimed to investigate the effect of phosphorus on growth performance, lipid peroxidation, and protein oxidation and antioxidant status in muscle as well as flesh quality of young grass carp. Through investigation of effects of phosphorus on muscle antioxidant, the study aimed to reveal the mechanisms by which antioxidants affected flesh quality. At the same time, the dietary phosphorus requirement of young grass carp was estimated.

## 2. Materials and methods

### 2.1. Experimental design and diets

The formulation of the basal diet is given in Table 1. Fish meal (Pesquera Lota Protein Ltd., Villagram, Chile), casein (Hulunbeier Sanyuan Milk Co., Ltd., Inner Mongolia, China) and gelatin (Rousselot Gelatin Co., Ltd., Guangdong, China) were used as the dietary protein sources. Fish oil (CAI. Pesquera Camanchaca S.A., Santiago, Chile) and soybean oil (Kerry Oils & Grains Industrial Co. Ltd., Chengdu, China) were used as the dietary lipid sources. The basal diet contained crude protein 306.8 g/kg diet and crude fat 44.5 g/kg diet, which were formulated to meet the nutrient requirements of young grass carp. The basal diet was mixed with monosodium phosphate ($NaH_2PO_4 \cdot 2H_2O$, AR) to get experimental diets containing graded levels of phosphorus. The dietary total phosphorus was determined by the method of the AOAC (2000), and final total phosphorus levels of the six experimental diets were 3.4 (unsupplemented control), 5.6, 6.6, 8.3, 11 and 13 g/kg diet, respectively. The experiment diets mixtures were pelleted and stored at −20 °C until used as described by Berdikova-Bohne et al. (2007).

### 2.2. Experimental procedure

Experimental grass carp were obtained from Bai-long Lake Fisheries (Sichuan, China). Fish were acclimated to the experimental conditions for 2 wk. Exactly 540 grass carp with an average initial weight 282.9 ± 3.3 g were randomly assigned into 18

**Table 1**
Ingredients and nutrient composition of the basal diet.

| Item | Content |
|---|---|
| **Ingredients, g/kg** | |
| Fish meal | 93.75 |
| Casein | 187.97 |
| Gelatin | 91.97 |
| L-threonine | 1.97 |
| DL-methionine | 0.56 |
| Corn starch | 249.45 |
| a-starch | 248.83 |
| Cellulose | 50.00 |
| Fish oil | 20.1 |
| Soya bean oil | 18.9 |
| Choline chloride | 6.00 |
| Ethoxyquin | 0.50 |
| Vitamin premix[1] | 10.00 |
| Mineral mixture[2] | 20.00 |
| Monosodium phosphate mixture[3] | – |
| **Nutrient composition, g/kg** | |
| Crude protein[4] | 306.80 |
| Crude fat[4] | 44.50 |
| Total phosphorus[4] | 3.40 |
| Available phosphorus | 1.00 |

[1] Vitamin premix (g/kg mixture): retinyl acetate (500,000 IU/g), 0.80 g; cholecalciferol (500,000 IU/g), 0.48 g; dl-α-tocopherol acetate (50%), 20.00 g; menadione (23%), 0.22 g; cyanocobalamin (1%), 0.1 g; D-biotin (2%), 5.00 g; folic acid (96%), 0.52 g; thiamin hydrochloride (98%), 0.12 g; ascoryl acetate (93%), 7.16 g; niacin (99%), 2.58 g; meso-inositol (99%), 52.33 g; calcium-D-pantothenate (90%), 2.78 g; riboflavine (80%), 0.99 g; pyridoxine (98%), 0.62 g; All ingredients were diluted with corn starch to 1 kg.

[2] Per kilogram of mineral premix: $MgSO_4 \cdot H_2O$ (15% Mg), 230.67 g; $FeSO_4 \cdot H_2O$ (30% Fe), 25.00 g; $CuSO_4 \cdot 5H_2O$ (25% Cu), 0.60 g; $ZnSO_4 \cdot H_2O$ (34.5% Zn), 4.35 g; $MnSO_4 \cdot H_2O$ (31.8% Mn), 2.04 g; KI (0.38% I), 1.45 g; $NaSeO_3$ (1% Se), 2.5 g; All ingredients were diluted with corn starch to 1 kg.

[3] Monosodium phosphate mixture: premix was added to obtain graded level of phosphorus.

[4] Proximate analysis of basal diet (g/kg): crude protein, crude fat and total phosphorus were determined according to the methods of the AOAC (2000).

experimental cages (1.4 m × 1.4 m × 1.4 m). Each cage was equipped with a disc of 100 cm diameter of 1 mm gauze in the bottom. Each experimental diet was randomly fed to fish of triplicate cages to apparent satiation four times each day for 8 wk. The uneaten feed was collected 30 min after each meal as described by our previous study (Tang et al., 2013). Water temperature and pH were 26 ± 2 °C and 7.0 ± 0.5, respectively. Dissolved oxygen was kept higher than 6.0 mg/L. Before and during the experiment, daily water samples were collected and analyzed. Average waterborne phosphorus concentration was below 0.049 mg/L. Procedures used in this study were conducted according to the guidelines provided by the University of Sichuan Agricultural Animal Care Advisory Committee.

To determine the phosphorus availability from either the basal diet or monosodium phosphate, each of diets 1 or 3 (which were all contained with 5 g/kg $Cr_2O_3$) was fed to triplicates (30 fish in each replicate) four times to satiation. After a 10-d feeding period, faecal collection was conducted 6 h after the first meal at 0700. Faeces were stripped from all fish by applying gentle pressure in the anal area according to the procedure of Austreng (1978). Faecal samples were collected and stored at −20 °C as described by Zhang et al. (2006). The corresponding available phosphorus levels in the diets were calculated based on the apparent phosphorus digestibility in this study as described by Roy and Lall (2003). Those procedures were all carried out according to guidelines followed in our previous laboratory study by Xie et al. (2011).

### 2.3. Sample collection and analysis

At the start and end of the feeding trial, fish in each cage were weighed. Prior to sampling, the fish were starved for 12 h according to Sugiura et al. (2000), and then anaesthetized (ethylene glycol monophenyl ether, 0.3 mL/L) as described by Coutinho et al. (2012). Eighteen fish from each treatment were selected. Blood was extracted from the caudal vein into a 5 mL tube, stored at −4 °C overnight and then centrifuged at 3,000 × *g* for 10 min and serum was removed and stored at −20 °C until analysis. The muscle samples were selected as described by Periago et al. (2005), with the trunk musculature cross section at the level of before and after the dorsal fin of each sample. Subsequently, the left half of one of these samples was trimmed into 3 muscle blocks of approximately equal size. Blocks were snap frozen over liquid nitrogen, and then stored in a −80 °C freezer until using.

Fish muscle pH was measured at 0 and 24 h post mortem with the method described by Periago et al. (2005). Cooking loss was measured using the method of Srikanchai et al. (2010). The maximum shear force value was determined according to Taylor et al. (2002).

Alkaline phosphatase (AKP) and phosphorus in the serum were measured with the methods used by Yang et al. (2006). Samples (50 μL) were mixed with 1.0 mL of disodium phenyl phosphate reaction solution. Absorbance was monitored at 520 nm. Proximate composition of diets and muscle were analyzed according to the procedure of AOAC (2000). Crude protein was measured by the Kjeldhal method, using Kjeltec (Tecator) digestion and distillation units. Crude lipid content was determined by extraction with petroleum ether in a Soxhletextractor. Moisture content was measured by drying using an oven at 105 °C. Ash content was determined by the combustion method using a muffle furnace at 550 °C. Muscle samples were homogenized on ice in 10 volumes (w/v) of ice-cold physiological saline and centrifuged at 6,000 × *g* at 4 °C for 20 min, and then the supernatant was conserved at −70 °C for enzyme activity analysis. Muscle protein content was determined according the method of Bradford (1976). As described, the protein concentration and protein retention (PR) were measured using the Coo-massie Brilliant Blue dye binding technique compared with the standard bovine serum albumin. Muscle MDA and protein carbonyl (PC) residues content were measured by the methods of Rueda-Jasso et al. (2004) and Dalle-Donne et al. (2002), respectively. When the MDA equivalents were determined, samples were mixed with trichloroacetic acid and centrifuged. Then, the thiobarbituric acid (TBA) was added to the supernatant. The mixture was heated in water at 95 °C for 40 min. MDA forms a red adduct with TBA, which has an absorbance at 532 nm. With a minor modification using the 2,4-dinitrophenylhydrazine (DNPH) reagent, the carbonyl content was calculated from the peak absorbance at 340 nm, using an absorption coefficient of 22,000/M/cm. Anti-superoxide anion (ASA) ($O_2^{\bullet -}$ scavenging ability) and anti-hydroxy radical (AHR) (OH• scavenging ability) adopted the method usually used by our laboratory listed in the article of Feng et al. (2012). In brief, superoxide radicals ($O_2^{\bullet -}$) were generated resulted from the oxidizing reaction in the body. Using the gross reagent, an electron acceptor was used to develop a coloration reaction (absorbance at 550 nm). Vitamin C was used as the standard agent. Afterward, AHR was assayed based on the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\bullet}OH^- + {\bullet}OH$). A coloration reaction (absorbance at 550 nm) was also developed using the gross reagent. SOD, CAT, GSH, GST and GR concentrations were examined by the methods of Wang et al. (2009); Aebi (1984); Vardi et al. (2008); Lushchak et al. (2001) and Lora et al. (2004). The SOD activity was measured as follows. The assay mixture contained 50 mmol/L phosphate buffer (pH 7.8), 1.08 mmol/L diethylenetriaminepentaacetic acid (DETAPAC), 0.06 mmol/L nitro blue tetrazolium (NBT), 0.16 mmol/L xanthine solution and 30 μL of sample extraction buffer. After the addition of 0.19 U/mL of xanthine oxidase, the absorbance change at 550 nm was monitored. Activity of CAT was measured as follows. The reaction mixture contained 100 mmol/L $KPO_4$ buffer (pH 7.0), 10 mmol/L $H_2O_2$ and 50 μL of sample extraction buffer in a total volume of 1 mL. Decrease in $H_2O_2$ was monitored by measuring absorbance at 240 nm. GST activity was measured through the reaction mixture containing 5 mmol/L GSH, 1 mmol/L 1-chloro-2, 4-dinitrobenzene (CDNB), 50 mmol/L KPi buffer (pH 7.0) plus 0.5 mmol/L EDTA and 40 μL of sample extraction buffer. The adduct formed between GSH and CDNB was monitored at 340 nm. Chromic oxide was determined using an atomic absorption spectrometer (Thermo Elemental Solar M6, Waltham, MA, USA) after perchloric acid digestion Chromic oxide was determined after perchloric acid digestion according to Ye et al. (2012).

### 2.4. Calculation and statistical analysis

Apparent phosphorus digestibility in diet 1 and diet 3 was calculated according to NRC (2011) by the Equation D (g/kg) = $[1 - (Cr_D \times P_F)/(Cr_F \times P_D)] \times 100$, where $Cr_D$ was the concentration of $Cr_2O_3$ in the diet, $P_F$ was the concentration of phosphorus in the faeces, $Cr_F$ was the concentration of $Cr_2O_3$ in the faeces and $P_D$ was the concentration of phosphorus in the diet.

According to Roy and Lall (2003), apparent phosphorus availability in the monosodium phosphate ($D_i$) was determined according to the Equation $D_i$ (g/kg) = $100 \times (P_2 \times D_2 - P_1 \times D_1)/I$, where $P_2$ was the concentration of phosphorus in the diet containing monosodium phosphate, $D_2$ was the apparent phosphorus digestibility in the diet containing monosodium phosphate, $P_1$ was the concentration of phosphorus in the basal diet, $D_1$ was the apparent phosphorus digestibility in the basal diet, and I was the concentration of inorganic phosphorus in the diet containing monosodium phosphate. Apparent digestibility of phosphorus in Diet 1 and Diet 3 were 29.82% and 58.65%, respectively. Apparent digestibility of phosphorus in monosodium phosphate was

calculated to be 89%. According to these values, available phosphorus contents in the experimental diets were estimated to be 1.0 (Diet 1, unsupplemented control), 2.5 (Diet 2), 3.8 (Diet 3), 5.6 (Diet 4), 7.8 (Diet 5) and 9.5 g/kg (Diet 6), respectively.

All of the data were subjected to one-way analysis of variance (ANOVA), followed by the Duncan method to determine significant differences among the treatment groups at the level of $P < 0.05$ through SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The data were presented as means ± SD. Broken line models and the quadratic regression analysis models were used to estimate the requirement of dietary phosphorus.

## 3. Results

### 3.1. Growth performance, serum alkaline phosphates and phosphorus

Table 2 shows the growth performance and serum indicators of young grass carp fed with graded levels of available phosphorus. The percent weight gain (PWG) were significantly improved with dietary available phosphorus levels up to 3.8 g/kg ($P < 0.05$), where the response reached a plateau. Specific growth rate (SGR) and feed efficiency (FE) followed a similar pattern to PWG when the dietary available phosphorus levels contained up ≥ 5.6 g/kg ($P < 0.05$). Feed intake (FI) showed quadratic responses to dietary available phosphorus concentrations ($Y_{FI} = -6.84x^2 + 95.91x + 229.32$, $R^2 = 0.89$, $P < 0.05$). The available phosphorus requirement of young grass carp established by the broken-line regression method based on PWG was 4.0 g/kg diet (Fig. 1).

Data of serum phosphorus and AKP are presented in Table 2. With the dietary available phosphorus levels up to 5.6 g/kg, serum phosphorus was significantly improved ($P < 0.05$) and thereafter remained constant ($P > 0.05$). Serum AKP activities were significantly quadratic in response to the increase of dietary phosphorus levels ($P < 0.05$) ($Y_{serum\ P} = 2.24x + 10.04$, $R^2 = 0.99$, $P < 0.05$; $Y_{AKP} = -0.29\ x^2 + 3.36x + 5.65$, $R^2 = 0.97$, $P < 0.05$) (Fig. 2 and Fig. 3).

### 3.2. Muscle composition and meat quality

The water, lipid, protein, ash, calcium and phosphorus contents of the muscle from the six treatments fed with different levels of available phosphorus are shown in Table 3. Muscle moisture content was the highest for fish fed with available phosphorus at 9.5 g/kg and the lowest for fish fed the diet containing available phosphorus at 3.8 g/kg ($P < 0.05$). The treatment groups with dietary available phosphorus levels of 3.8 and 5.6 g/kg had higher crude protein contents than other groups ($P < 0.05$). Muscle lipid content in the treatment groups containing available phosphorus 5.6–9.5 g/kg was lower than that in the treatment groups containing available phosphorus 1.0–3.8 g/kg ($P < 0.05$). Ash content was the lowest in the diet supplemented with available phosphorus at 5.6 g/kg ($P < 0.05$). There were no differences between the

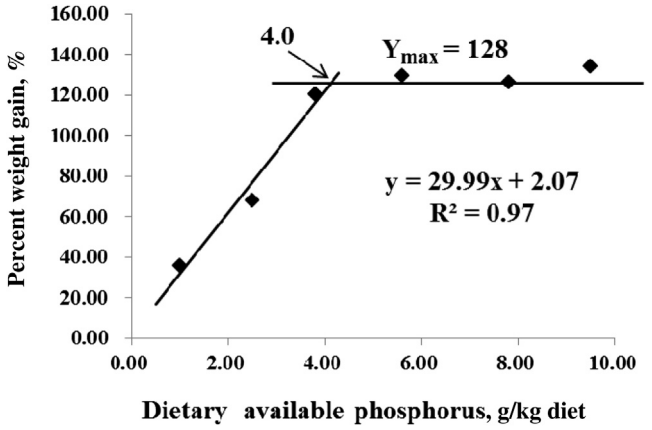


**Fig. 1.** Effect of dietary available phosphorus on percent weight gain in young grass carp fed experimental diets for 8 wk. The dietary available phosphorus requirement of young grass carp was 4.0 g/kg.

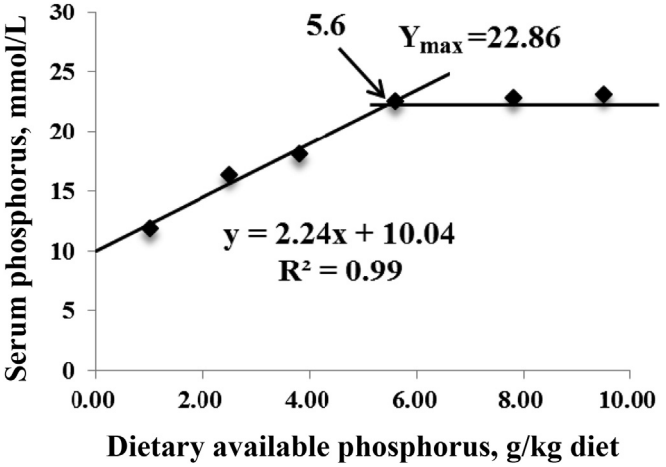


**Fig. 2.** Effect of dietary available phosphorus on serum phosphorus in young grass carp fed experimental diets for 8 wk. The dietary available phosphorus requirement of young grass carp was 5.6 g/kg.

**Table 2**
Initial body weight, final body weight, percent weight gain, feed intake, feed efficiency, specific growth rate, serum phosphorus (n = 6) and serum alkaline phosphatase (n = 6) activity of young grass carp (*Ctenopharyngodon idella*) fed diets with graded levels of available phosphorus for 8 wk.

| APL, g/kg | IBW, g/fish | FBW, g/fish | PWG[1], % | FI, g/fish | FE[2], % | SGR[3], %/d | Serum phosphorus, mmol/L | Serum AKP, king's unit/mL |
|---|---|---|---|---|---|---|---|---|
| 1.0 | 285.3 ± 3.7 | 388.7 ± 6.4$^{a}$ | 36.23 ± 2.87$^{a}$ | 326.8 ± 2.4$^{a}$ | 0.32 ± 0.02$^{a}$ | 0.6 ± 0.04$^{a}$ | 11.95 ± 0.90$^{a}$ | 8.57 ± 0.75$^{a}$ |
| 2.5 | 280.4 ± 2.5 | 471.3 ± 20.8$^{b}$ | 68.08 ± 7.92$^{b}$ | 383.2 ± 4.1$^{b}$ | 0.50 ± 0.05$^{b}$ | 0.9 ± 0.08$^{b}$ | 16.36 ± 1.56$^{b}$ | 12.07 ± 1.12$^{b}$ |
| 3.8 | 281.1 ± 2.3 | 620.8 ± 6.8$^{c}$ | 120.85 ± 4.10$^{c}$ | 547.4 ± 3.0$^{d}$ | 0.62 ± 0.02$^{c}$ | 1.4 ± 0.03$^{c}$ | 18.18 ± 1.62$^{bc}$ | 15.00 ± 1.26$^{c}$ |
| 5.6 | 281.3 ± 1.3 | 647 ± 30.3$^{cd}$ | 130.0 ± 11.83$^{c}$ | 542.4 ± 3.8$^{d}$ | 0.67 ± 0.09$^{cd}$ | 1.5 ± 0.09$^{cd}$ | 22.60 ± 1.35$^{bcd}$ | 14.84 ± 1.61$^{c}$ |
| 7.8 | 286.1 ± 4.3 | 648.3 ± 15.8$^{cd}$ | 126.64 ± 6.84$^{c}$ | 542.7 ± 3.0$^{d}$ | 0.671 ± 0.05$^{cd}$ | 1.5 ± 0.05$^{cd}$ | 22.86 ± 1.96$^{cd}$ | 13.86 ± 1.12$^{c}$ |
| 9.5 | 282.9 ± 2.3 | 663.4 ± 21.2$^{d}$ | 134.50 ± 6.23$^{c}$ | 533.2 ± 2.7$^{c}$ | 0.71 ± 0.05$^{d}$ | 1.5 ± 0.03$^{d}$ | 23.12 ± 1.62$^{d}$ | 11.42 ± 0.85$^{b}$ |

APL = levels of available phosphorus; IBW = initial body weight; FBW = final body weight; PWG = percent weight gain; FI = feed intake; FE = feed efficiency; SGR = specific growth rate; AKP = alkaline phosphatase.
All data were expressed as means ± SD.
[a,b,c,d]Mean values with the different superscripts in the same column were significantly different ($P < 0.05$).
[1] PWG = 100 × weight gain (g/fish)/initial weight (g/fish).
[2] FE = 100 × weight gain (g)/dry diet intake (g).
[3] SGR = 100 × [ln (mean final body weight) - ln (mean initial body weight)]/days.

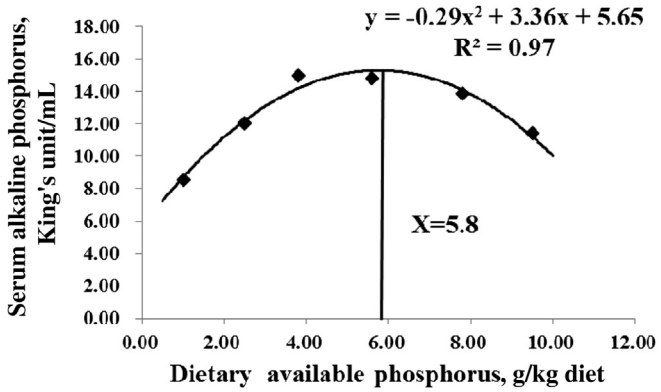


**Fig. 3.** Quadratic regression analysis of dietary available phosphorus on serum alkaline phosphorus in young grass carp fed experimental diets for 8 wk. The dietary available phosphorus requirement of young grass carp was 5.8 g/kg diet.

groups with available phosphorus levels of 2.5 and 3.8 g/kg ($P > 0.05$). Calcium and phosphorus contents in muscle were the lowest in the diet supplemented with available phosphorus at 5.6 g/kg diet ($P < 0.05$).

As shown in Table 4, postmortem muscle pH of the grass carp fed a diet containing available phosphorus at 2.5 g/kg had the lowest pH at 0 and 24 h ($P < 0.05$), while other groups showed no difference ($P > 0.05$). With the dietary available phosphorus levels up to 3.8 and 5.6 g/kg, meat cooking losses were significantly decreased and thereafter increased ($P < 0.05$). There were no differences in meat shear value and hydroxyproline content among six treatment groups fed various levels of available phosphorus ($P > 0.05$).

### 3.3. Muscle oxidative status and antioxidant ability

The effects of dietary available phosphorus levels on muscle oxidative status and antioxidant ability are shown in Table 5 and Table 6. MDA content in muscle cells was significantly decreased with dietary available phosphorus levels up to 3.8 g/kg and thereafter elevated ($P < 0.05$). Similar patterns were found in PC content with the lowest value occurring when available phosphorus was supplied at 5.6 g/kg. AHR was found in the unsupplemented phosphorus treatment group to be lower than that in the group containing available phosphorus 3.8 g/kg ($P < 0.05$). The ASA content was higher in fish fed diets containing unsupplemented phosphorus compared to those in the other groups ($P < 0.05$). CAT, SOD and GSH were significantly improved with dietary available phosphorus levels up to 3.8 g/kg and thereafter decreased ($P < 0.05$). Conversely, the content of GR was significantly decreased with dietary available phosphorus levels up to 5.6 g/kg and thereafter plateaued ($P < 0.05$). GST content in the treatment group fed the unsupplemented available phosphorus diet was lower than those in other groups. Glutathione peroxides (GPx) in the treatment groups containing available phosphorus 1.0–3.8 g/kg were higher than those in other treatment groups ($P < 0.05$).

## 4. Discussion

The study herein showed that growth performance of young grass carp was significantly influenced by the dietary available phosphorus levels. The treatment group fed a phosphorus deficient diet obtained poor PWG, and was improved by optimum phosphorus supplementation. The enhancement of fish growth was always related to the improvement of FI and FE in fish as reported in juvenile grouper (*Epinephelus coioides*) (Ye et al., 2006). In the present study, FI and FE were elevated with the provision of dietary available phosphorus to optimal levels. Correlation analysis showed that the PWG positively correlated to FI ($r = +0.986$, $P < 0.01$) and FE ($r = +0.984$, $P < 0.01$), suggesting that the enhanced growth performance caused by phosphorus was most likely attributed to the elevated FI and FE. Phosphorus in blood was used as indicator of phosphorus nutrition status in fish (Skonberg et al., 1997). In addition, AKP activity in serum is the most common clinical index of bone formation in humans (Cashman and Flynn, 1999). In the present study, serum phosphorus and AKP activity were significantly increased with optimal available phosphorus supplementation, indicating that dietary optimum phosphorus supplementation improved its nutrition status.

**Table 3**
Muscle moisture, crude protein, lipid, ash, calcium and phosphorus of young grass carp fed diets containing graded levels of available phosphorus for 8 wk.

| APL, g/kg | Moisture, % | CP, % | Lipid, % | Ash, % | Ca, % | P, % |
|---|---|---|---|---|---|---|
| 1.0 | 77.26 ± 0.16$^{b}$ | 17.17 ± 0.07$^{a}$ | 4.07 ± 0.12$^{b}$ | 1.61 ± 0.02$^{e}$ | 0.49 ± 0.004$^{d}$ | 0.298 ± 0.004$^{e}$ |
| 2.5 | 77.08 ± 0.10$^{b}$ | 17.26 ± 0.36$^{a}$ | 4.08 ± 0.14$^{b}$ | 1.54 ± 0.01$^{d}$ | 0.41 ± 0.01$^{c}$ | 0.293 ± 0.002$^{de}$ |
| 3.8 | 76.58 ± 0.13$^{a}$ | 17.89 ± 0.27$^{b}$ | 3.90 ± 0.17$^{b}$ | 1.53 ± 0.02$^{d}$ | 0.38 ± 0.02$^{c}$ | 0.287 ± 0.009$^{cd}$ |
| 5.6 | 77.77 ± 0.11$^{cd}$ | 18.06 ± 0.11$^{b}$ | 2.93 ± 0.22$^{a}$ | 1.29 ± 0.03$^{a}$ | 0.17 ± 0.01$^{a}$ | 0.245 ± 0.002$^{a}$ |
| 7.8 | 77.68 ± 0.04$^{c}$ | 17.49 ± 0.22$^{a}$ | 3.19 ± 0.20$^{a}$ | 1.39 ± 0.01$^{b}$ | 0.32 ± 0.01$^{b}$ | 0.268 ± 0.002$^{b}$ |
| 9.5 | 77.92 ± 0.02$^{d}$ | 17.39 ± 0.14$^{a}$ | 3.21 ± 0.09$^{a}$ | 1.45 ± 0.03$^{c}$ | 0.39 ± 0.05$^{c}$ | 0.276 ± 0.002$^{bc}$ |

APL = levels of available phosphorus; CP = crude protein; Ca = calcium; P = phosphorus.
All data were expressed as means ± SD (n = 6).
$^{a,b,c,d,e}$Mean values with the different superscripts in the same column were significantly different ($P < 0.05$).

**Table 4**
Mean values and standard deviation of pH, hydroxyproline, shear force and cooking loss in flesh of young grass carp fed graded levels of phosphorus for 8 wk.

| APL, g/kg | pH | | Cooking loss, % | Hydroxyproline, nmol/mg protein | Shear force, N |
|---|---|---|---|---|---|
| | 0 h | 24 h | | | |
| 1.0 | 6.15 ± 0.11$^{b}$ | 6.36 ± 0.13$^{b}$ | 0.17 ± 0.02$^{a}$ | 2.19 ± 0.12$^{a}$ | 18.06 ± 0.15$^{bc}$ |
| 2.5 | 6.01 ± 0.15$^{a}$ | 6.17 ± 0.07$^{a}$ | 0.10 ± 0.01$^{a}$ | 2.22 ± 0.09$^{a}$ | 17.68 ± 1.03$^{bc}$ |
| 3.8 | 6.12 ± 0.08$^{ab}$ | 6.22 ± 0.09$^{ab}$ | 0.10 ± 0.01$^{a}$ | 2.23 ± 0.12$^{a}$ | 15.41 ± 0.93$^{a}$ |
| 5.6 | 6.21 ± 0.11$^{b}$ | 6.28 ± 0.05$^{ab}$ | 0.14 ± 0.01$^{a}$ | 2.25 ± 0.14$^{a}$ | 15.98 ± 1.02$^{a}$ |
| 7.8 | 6.22 ± 0.08$^{b}$ | 6.32 ± 0.23$^{b}$ | 0.18 ± 0.02$^{a}$ | 2.26 ± 0.13$^{a}$ | 17.22 ± 1.09$^{b}$ |
| 9.5 | 6.13 ± 0.1$^{ab}$ | 6.27 ± 0.10$^{ab}$ | 0.18 ± 0.02$^{a}$ | 2.22 ± 0.11$^{a}$ | 18.45 ± 0.76$^{c}$ |

APL = levels of available phosphorus.
All data were expressed as means ± SD (n = 6).
$^{a,b,c}$Mean values with the different superscripts in the same column were significantly different ($P < 0.05$).

**Table 5**
Malondialdehyde, protein carbonyl content, anti-superoxide anion, anti-hydroxy radical, superoxide dismutase and catalase in muscle of young grass carp fed graded levels of available phosphorus for 8 wk.

| APL, g/kg | MDA, nmol/mg protein | PC, nmol/mg protein | ASA, U/g protein | AHR, U/mg protein | SOD, U/mg protein | CAT, U/mg protein |
|---|---|---|---|---|---|---|
| 1.0 | 1.23 ± 0.05$^{b}$ | 16.48 ± 1.65$^{e}$ | 203.24 ± 18.49$^{c}$ | 1176.68 ± 93.95$^{a}$ | 14.61 ± 0.59$^{b}$ | 2.03 ± 0.10$^{a}$ |
| 2.5 | 1.19 ± 0.11$^{b}$ | 8.96 ± 0.47$^{c}$ | 170.02 ± 13.28$^{b}$ | 1256.97 ± 42.53$^{ab}$ | 15.71 ± 0.55$^{c}$ | 3.76 ± 0.27$^{c}$ |
| 3.8 | 1.04 ± 0.10$^{a}$ | 6.69 ± 0.47$^{b}$ | 144.80 ± 12.97$^{a}$ | 1344.09 ± 28.35$^{b}$ | 17.47 ± 0.65$^{d}$ | 4.37 ± 0.23$^{d}$ |
| 5.6 | 1.22 ± 0.07$^{b}$ | 5.28 ± 0.55$^{a}$ | 151.44 ± 10.72$^{a}$ | 1260.61 ± 73.34$^{ab}$ | 16.12 ± 0.44$^{c}$ | 2.50 ± 0.18$^{b}$ |
| 7.8 | 1.25 ± 0.09$^{b}$ | 11.78 ± 1.01$^{d}$ | 154.57 ± 15.82$^{a}$ | 1270.30 ± 98.64$^{ab}$ | 14.94 ± 0.86$^{b}$ | 2.12 ± 0.18$^{a}$ |
| 9.5 | 1.41 ± 0.14$^{c}$ | 12.43 ± 1.40$^{d}$ | 154.64 ± 14.77$^{a}$ | 1253.20 ± 89.05$^{ab}$ | 13.74 ± 0.54$^{a}$ | 2.21 ± 0.17$^{a}$ |

APL = levels of available phosphorus; MDA = malondialdehyde; PC = protein carbonyl; ASA = anti-superoxide anion; AHR = anti-hydroxy radical; SOD = superoxide dismutase; CAT = catalase.
All data were expressed as means ± SD (n = 6).
[a,b,c,d,e]Mean values with the different superscripts in the same column were significantly different ($P < 0.05$).

**Table 6**
Glutathione-S-transferase, glutathione peroxidase, glutathione reductase activities and glutathione content in muscle of young grass carp fed graded levels of available phosphorus for 8 wk.

| APL, g/kg | GST, U/mg protein | GPx, U/mg protein | GR, U/g protein | GSH, mg/g protein |
|---|---|---|---|---|
| 1.0 | 78.69 ± 7.71$^{a}$ | 63.53 ± 4.06$^{b}$ | 12.72 ± 0.80$^{d}$ | 1.48 ± 0.13$^{a}$ |
| 2.5 | 96.71 ± 9.63$^{b}$ | 65.81 ± 10.24$^{b}$ | 11.44 ± 0.53$^{c}$ | 1.37 ± 0.12$^{a}$ |
| 3.8 | 100.98 ± 6.52$^{b}$ | 65.08 ± 5.09$^{b}$ | 9.63 ± 0.44$^{b}$ | 2.59 ± 0.19$^{c}$ |
| 5.6 | 106.24 ± 8.35$^{b}$ | 53.34 ± 3.95$^{a}$ | 7.94 ± 0.53$^{a}$ | 2.17 ± 0.23$^{b}$ |
| 7.8 | 107.35 ± 8.73$^{b}$ | 48.07 ± 2.82$^{a}$ | 8.52 ± 0.34$^{a}$ | 2.09 ± 0.20$^{b}$ |
| 9.5 | 101.25 ± 7.98$^{b}$ | 47.95 ± 4.12$^{a}$ | 7.86 ± 0.60$^{a}$ | 2.05 ± 0.21$^{b}$ |

APL = levels of available phosphorus; GST = glutathione-S-transferase; GPx = glutathione peroxidase; GR = glutathione reductase; GSH = glutathione.
All data were expressed as means ± SD (n = 6).
[a,b,c,d]Mean values with the different superscripts in the same column were significantly different ($P < 0.05$).

Muscle mass represents more than 60% of body mass in most teleosts (Alami-Durante et al., 2010). The present study showed that muscle protein was increased with dietary phosphorus levels up to an optimal concentration. The phosphorus-increased muscle protein content may be related to the decreases of deamination of amino acid and ammonia excretion. The main end product of dietary protein metabolism is ammonia in teleosts (Wood, 1958). Yang et al. (2006) found that phosphorus could decrease deamination of amino acid and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). However, lipid content in the muscle of fish was increased in the phosphorus deficient treatment group. Phosphorus deficiency resulting in higher accumulation of muscle lipid may be due to the inhibition of β-oxidation of fatty acids. Roy and Lall (2003) indicated that phosphorus deficiency inhibited ATP-dependent esterification of free fatty acid with CoA to depress fatty acid. In addition, muscle ash, calcium and phosphorus contents in the muscle of young grass carp were significantly decreased with dietary phosphorus to optimal levels. However, it was reported that whole body ash, calcium and phosphorus were increased in some fish such as juvenile grass carp (Liang et al., 2012), juvenile Jian carp (Xie et al., 2011) and juvenile milkfish (*Chanos chanos* Forsskal) (Borlongan and Satoh, 2001). This may be due to the fact that 80% of the phosphorus and 99% of the calcium were deposited in the bones of fish (FAO, 1980).

Fish muscle tissue is the main edible portion for people (Periago et al., 2005). In our study, we investigated the influence of different levels of phosphorus on meat quality, such as the meat pH, hydroxyproline content, shear force value and water holding capacity. Meat pH played an important role in the evolution of postmortem flesh of Atlantic cod (*Gadus morhua* L.) (Kristoffersen et al., 2006). The present study showed that flesh pH of young grass carp fed diets with available phosphorus at 2.5 g/kg was lower than those of other groups. Pork pH can be affected by lactate acid and buffering capacity (Kylä-Puhju et al., 2004). When the ATP is exhausted, porcine muscle pH begins to decline due to the production of lactic acid (Kylä-Puhju et al., 2004). Phosphorus compounds also have a buffering capacity in the regulation of meat pH (Kylä-Puhju et al., 2004). However, the reason for the lower pH in a certain group needs further study. Collagen constitutes 3–10% of the proteins in fish muscle (Delbarre-Ladrat et al., 2006) and its concentration has previously been estimated by hydroxyproline (Johnston et al., 2006). Our study found that variation in hydroxyproline content resulted in no differences among treatment groups. Protocollagen proline hydroxylase is an important enzyme in collagen synthesis (Saito et al., 2001). Takeda et al. (1976) indicated that ATP could improve the activity of protocollagen proline hydroxylase. In our study the phosphorus deficient treatment group may have also supplied enough ATP for collagen synthesis, contributing to the equal collagen content in treatment groups. In our study, meat shear force value had no difference among six treatment groups. Fish flesh firmness is affected by collagen content (Hatae et al., 1986). We speculated that the lack of variation of hydroxyproline content among treatment groups could have been the reason for no changes in shear force values. Water holding capacity is an important factor for fish flesh quality (Ofstad et al., 1996). Meat cooking loss can negatively related to the muscle water holding capacity (Srikanchai et al., 2010). In our study, the cooking loss of young grass carp was the lowest in fish fed with optimal available phosphorus levels, suggesting that optimal phosphorus increased the water holding capacity. Phosphorus-increased water holding capacity of fish may be related to the changes to the gap in the muscle. Olsson et al. (2003) observed that gaps in the extracellular matrix and widened intermyofibrillar space in Atlantic halibut muscle contributed to the low water holding capacity. The calpain hydrolyzed sarcomeric associated proteins such as titin (Geesink et al., 2000) could decrease the attachments of fiber to fiber (Taylor et al., 2002). Low calcium content may reduce the activity of calpain (Salem et al., 2004). Thus suitable dietary phosphorus could reduce the activity of calpains as muscle calcium content was found to be the lowest in optimal phosphorus levels in the present study. Liu et al. (2011) found that cooking loss in pork was also increased due to protein oxidation. Therefore, we investigated the influence of dietary phosphorus on muscle oxidative status and antioxidant ability of fish.

MDA and PC are the most easily assayed biomarkers for oxidative damage to lipid (Valko et al., 2007) and protein (Dalle-Donne et al., 2003). In our study, we observed that MDA and PC contents in the muscle of young grass carp were decreased with optimal dietary available phosphorus supplementations, suggesting that phosphorus could reduce fish muscle lipid and protein oxidation. Lipid peroxidation is generally caused by ROS (Valko et al., 2007). The $O_2^{\bullet -}$ and hydroxy radical (OH•) are the main ROS (Martınez-Alvarez et al., 2005). Thus we determined the capacity of scavenging $O_2^{\bullet -}$ and OH• with elevated dietary phosphorus levels. Our study showed that AHR was improved, while ASA content was reduced in treatment groups supplemented with certain levels of phosphorus. Koh and Kim (2001) found that rabbit muscle metallothioneins could scavenge $O_2^{\bullet -}$. The expression of metallothioneins was positively correlated with zinc levels in human nasopharyngeal cancer cells (Jayasurya et al., 2000). Zhou et al. (2004) reported that phosphorus deficiency increased zinc content in the muscle of juvenile orange-spotted grouper. This information indicated that phosphorus deficiency may work by increasing muscle zinc content to improve metallothioneins content to enhance ASA activity. The content of metallothioneins was not measured in our study and needed further investigated.

To scavenge ROS, fish have developed non-enzymatic and enzymatic antioxidant systems (Martınez-Alvarez et al., 2005). In this study, we observed that CAT and SOD activities were improved with optimal phosphorus supplementations. Phosphorus participates in the composition of NADPH. Yang et al. (2011) suggested that NADPH might protect CAT in freshwater mussel (*Cristaria plicata*). Therefore, we speculated that phosphorus improved the CAT activity through elevating the NADPH content. GSH as a major antioxidant can reduce ROS (Elia et al., 2006). The present study showed GSH activity was increased with dietary available phosphorus to the optimal level. GSH homeostasis is maintained through intracellular synthesis or regeneration from its oxidized form glutathione disulfide (GSSG) (Fang et al., 2002). In organisms, the synthesis of GSH needs energy supplied by ATP (Fang et al., 2002). Sugiura et al. (2000) reported that the muscle ATP activity was elevated in rainbow trout fed optimal phosphorus. Therefore, fish feed containing suitable phosphorus may promote the synthesis of GSH. Depending on NADPH, GR can catalyze the reduction in GSSG back to GSH (Elia et al., 2006). Our study showed that the activity of GR was decreased with dietary available phosphorus levels up to an optimal level. This might be because GR was consumed during reduction of GSSG resulting in the low activity in fish fed optimal levels of phosphorus. GST is able to detoxify from xenobiotics via conjugation of their electrophylic group with the nucleo-philic group of GSH to facilitate their excretion from cells (Elia et al., 2006). In our study, the phosphorus deficient treatment group had the lowest GST activity which was positively correlated with the GSH concentration ($r = +0.945$, $P < 0.05$). GPx utilizes the reducing power of GSH to detoxify $H_2O_2$ and other organic peroxides (Davies, 2000). The present results showed that GPx declined with available phosphorus levels up to optimum.

Based on the regression analysis for PWG, the available phosphorus requirement for young grass carp was estimated to be 4.0 g/kg, which was lower than that for juvenile grass carp (8.49 g/kg) (Liang et al., 2012). Similar results were also observed in rainbow trout (Lellis et al., 2004). Shearer (1984) indicated that calcium and phosphorus contents decreased in post-juvenile rainbow trout. Perhaps, this is the reason why dietary phosphorus requirement decreased as fish size increased. Plasma phosphorus and AKP were also indicators of dietary phosphorus status in fish nutrition studies (Zhang et al., 2006). The dietary phosphorus optimal supplementation levels estimated by regression analysis based on serum phosphorus and AKP were 5.6 and 5.8 g/kg diet, respectively.

## 5. Conclusion

Briefly, this study demonstrated that available phosphorus supplementation significantly improved the growth performance and flesh water holding capacity in grass carp, preventing lipid, protein oxidation of muscle by enhancing free radical-scavenging abilities and enzymatic antioxidant capacities such as SOD, CAT and GST activities and GSH content. Dietary phosphorus was important for growth performance and the requirement of available phosphorus based on the PWG for the young grass carp was 4.0 g/kg. Further study is needed to examine the specific molecule mechanisms whereby phosphorus mediates antioxidant defenses in fish.

## Acknowledgments

This research was supported by the National Department Public Benefit Research Foundation (Agriculture) of China (201003020), Science and Technology Support Programme of Sichuan Province of China (2011NZ0071) and Major Scientific and Technological Achievement Transformation Project of Sichuan Province of China (2012NC0007). The authors would like to thank the personnel of these teams for their kind assistance.

## References

Adams S, Green P, Claxton R, Simcox S, Williams MV, Walsh K, et al. Reactive carbonyl formation by oxidative and non-oxidative pathways. Front Biosci 2001;6:A17–24.
Aebi H. Catalase in vitro. Method Enzymol 1984;105:121–6.
Alami-Durante H, Médale F, Cluzeaud M, Kaushik S. Skeletal muscle growth dynamics and expression of related genes in white and red muscles of rainbow trout fed diets with graded levels of a mixture of plant protein sources as substitutes for fishmeal. Aquaculture 2010;303:50–8.
AOAC (Association of Official Analytical Chemists). Official Methods of Analysis of the AOAC International. 17th ed. Gaithersburg, MD: AOAC International; 2000.
Austreng E. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture 1978;13:265–72.
Berdikova Bohne VJ, Hamre K, Arukwe A. Hepatic metabolism, phase I and II biotransformation enzymes in Atlantic salmon (Salmo Salar, L) during a 12 week feeding period with graded levels of the synthetic antioxidant, ethoxyquin. Food Cheml Toxicol 2007;45:733–46.
Borlongan I, Satoh S. Dietary phosphorus requirement of juvenile milkfish, *Chanos chanos* (Forsskal). Aquac Res 2001;32:26–32.
Boyd CE. Water quality in warmwater fish ponds. Opelika. AL: Craftmaster Printers Inc; 1981.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
Buckley D, Morrissey P, Gray J. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. J Anim Sci 1995;73:3122–30.
Cashman KD, Flynn A. Optimal nutrition: calcium, magnesium and phosphorus. P Nutr Soc 1999;58:477–87.
Coutinho F, Peres H, Guerreiro I, Pousão-Ferreira P, Oliva-Teles A. Dietary protein requirement of sharpsnout sea bream (*Diplodus puntazzo*) juveniles. Aquaculture 2012;37:391–7.
Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 2003;329:23–38.
Davies KJ. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. Iubmb Life 2000;50:279–89.
Delbarre-Ladrat C, Chéret R, Taylor R, Verrez-Bagnis V. Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. Critl Rev Food Sci 2006;46:409–21.
Elia AC, Anastasi V, Dörr AJM. Hepatic antioxidant enzymes and total glutathione of *Cyprinus carpio* exposed to three disinfectants, chlorine dioxide, sodium hypochlorite and peracetic acid, for superficial water potabilization. Chemosphere 2006;64:1633–41.
Fang YZ, Yang S, Wu GY. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872–9.
FAO. Aquaculture development and coordination programme. Fish feed technology. In: Lectures presented at the FAO/UNDP Training Course in fish feed technology, Seattle, Washington, 9 October – 15 December 1978; 1980. ADCP/REP/80/11-Fish Feed Technology.
Feng L, Zhao B, Chen G, Jiang WD, Liu Y, Jiang J, et al. Effects of dietary histidine on antioxidant capacity in juvenile Jian carp (*Cyprinus carpio* var. Jian). Fish Physiol Biochem 2012;39:559–71.

Geesink G, Morton J, Kent M, Bickerstaffe R. Partial purification and characterization of Chinook salmon (*Oncorhynchus tshawytscha*) calpains and an evaluation of their role in postmortem proteolysis. J Food Sci 2000;65:1318–24.
Hatae K, Tobimatsu A, Takeyama M, Matsumoto J. Contribution of the connective tissues on the texture difference of various fish species. Bull Jpn Soc Sci Fish 1986;52:2001–7.
Izquierdo M, Obach A, Arantzamendi L, Montero D, Robaina L, Rosenlund G. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquacult Nutr 2003;9:397–407.
Jayasurya A, Bay B, Yap W, Tan N, Tan B. Proliferative potential in nasopharyngeal carcinoma: correlations with metallothionein expression and tissue zinc levels. Carcinogenesis 2000;21:1809–12.
Johnston IA. Muscle development and growth: potential implications for flesh quality in fish. Aquaculture 1999;177:99–115.
Johnston IA. Genetic and environmental determinants of muscle growth patterns. Fish Physiol Biochem 2001;18:141–86.
Johnston IA, Li X, Vieira VL, Nickell D, Dingwall A, Alderson R, et al. Muscle and flesh quality traits in wild and farmed Atlantic salmon. Aquaculture 2006;256:323–36.
Khan N, Qureshi NA, Nasir M, Rasool F, Iqbal KJ. Effect of artificial diet and culture systems on sensory quality of fried fish flesh of Indian major carps. Pak J Zool 2011;43:1177–82.
Kirkman HN, Rolfo M, Ferraris AM, Gaetani GF. Mechanisms of protection of catalase by NADPH Kinetics and stoichiometry. J Bio Chem 1999;274:13908–14.
Koh M, Kim HJ. The effects of metallothionein on the activity of enzymes involved in removal of reactive oxygen species. B Kor Chem Soc 2001;22:362–6.
Kristoffersen S, Tobiassen T, Steinsund V, Olsen RL. Slaughter stress, postmortem muscle pH and rigor development in farmed Atlantic cod (*Gadus morhua* L.). Intl J Food Sci Tech 2006;41:861–4.
Kylä-Puhju M, Ruusunen M, Kivikari R, Puolanne E. The buffering capacity of porcine muscles. Meat Sci 2004;67:587–93.
Lall S. Digestibility, metabolism and excretion of dietary phosphorus in fish. Nutr Strateg Aquac Waste 1991:21–36.
Lellis W, Barrows F, Hardy R. Effects of phase-feeding dietary phosphorus on survival, growth, and processing characteristics of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 2004;242:607–16.
Liang JJ, Liu YJ, Tian LX, Yang HJ, Liang GY. Dietary available phosphorus requirement of juvenile grass carp (Ctenopharyngodon idella). Aquacul Nutr 2012;18:181–8.
Liang Y, Hultin HO. Separation of muscle membrane from alkali-solubilized fish muscle proteins. J Agr Food Chem 2005;53:10012–7.
Liu ZL, Xiong YL, Chen J. Morphological examinations of oxidatively stressed pork muscle and myofibrils upon salt marination and cooking to elucidate the water-binding potential. J Agr Food Chem 2011;59:13026–34.
Lora J, Alonso FJ, Segura JA, Lobo C, Marquez J, Mates JM. Antisense glutaminase inhibition decreases glutathione antioxidant capacity and increases apoptosis in ehrlich ascitic tumour cells. Eur J Biochem 2004;271:4298–306.
Lushchak VI, Lushchak LP, Mota AA, Hermes-Lima M. Oxidative stress and antioxidant defenses in goldfish Carassius auratus during anoxia and reoxygenation. Am J Phys – Reg I 2001;280:R100–7.
Martinaud A, Mercier Y, Marinova P, Tassy C, Gatellier P, Renerre M. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. J Agr Food Chem 1997;45:2481–7.
Martinez-Alvarez RM, Morales AE, Sanz A. Antioxidant defenses in fish: biotic and abiotic factors. Rev Fish Biol Fish 2005;15:75–88.
NRC (National Research Council). Nutrient requirements of fish and shrimp. Washington, DC, USA: National Academies Press; 2011.
Ofstad R, Egelandsdal B, Kidman S, Myklebust R, Olsen RL, Hermansson AM. Liquid loss as effected by post mortem ultrastructural changes in fish muscle: cod (*Gadus morhua* L.) and Salmon (*Salmo salar*). J Sci Food Agr 1996;71:301–12.
Olsson GB, Olsen RL, Ofstad R. Post-mortem structural characteristics and water-holding capacity in Atlantic halibut muscle. LWT – Food Sci Technol 2003;36:125–33.
Pasek MA. Rethinking early earth phosphorus geochemistry. PNAS 2008;105:853–8.
Periago MJ, Ayala MD, López-Albors O, Abdel I, Martínez C, García-Alcázar A, et al. Muscle cellularity and flesh quality of wild and farmed sea bass, *Dicentrarchus labrax* L. Aquaculture 2005;249:175–88.
Pimentel-Rodrigues A, Oliva-Teles A. Phosphorus requirements of gilthead sea bream (*Sparus aurata* L.) juveniles. Aquac Res 2001;32:157–61.
Roy PK, Lall SP. Dietary phosphorus requirement of juvenile haddock (*Melanogrammus aeglefinus* L.). Aquaculture 2003;221:451–68.
Rueda-Jasso R, Conceicao L, Dias J, De Coen W, Gomes E, Rees J, et al. Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese sole (*Solea senegalensis*) juveniles. Aquaculture 2004;231:417–33.
Saito M, Takenouchi Y, Kunisaki N, Kimura S. Complete primary structure of rainbow trout type I collagen consisting of α1 (I) α2 (I) α3 (I) heterotrimers. Eur J Biochem 2001;268:2817–27.
Salem M, Kenney P, Killefer J, Nath J. Isolation and characterization of calpains from rainbow trout muscle and their role in texture development. J Muscle Foods 2004;15:245–55.
Shearer KD. Changes in elemental composition of hatchery-reared rainbow trout, Salmo gairdneri, associated with growth and reproduction. Can J Fish Aquat Sci 1984;41:1592–600.
Shelton J, Southern L, LeMieux F, Bidner T, Page T. Effects of microbial phytase, low calcium and phosphorus, and removing the dietary trace mineral premix on carcass traits, pork quality, plasma metabolites, and tissue mineral content in growing-finishing pigs. J Anim Sci 2004;82:2630–9.
Skonberg DI, Yogev L, Hardy RW, Dong FM. Metabolic response to dietary phosphorus intake in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 1997;157:11–24.
Srikanchai T, Murani E, Wimmers K, Ponsuksili S. Four loci differentially expressed in muscle tissue depending on water-holding capacity are associated with meat quality in commercial pig herds. Mol Biol Rep 2010;37:595–601.
Stanley DW, Parkin KL. Biological membrane deterioration and associated quality losses in food tissues. Crit Rev Food Sci 1991;30:487–553.
Sugiura S, Dong F, Hardy R. Primary responses of rainbow trout to dietary phosphorus concentrations. Aquacult Nutr 2000;6:235–45.
Takeda K, Kawai S, Tetsuka T, Konno K. Stimulation of protocollagen proline hydroxylase activity by nucleoside triphosphates. Biochem Bioph Res Co 1976;69:957–61.
Tang QQ, Feng L, Jiang WD, Liu Y, Jiang J, Li SH, et al. Effects of dietary copper on growth, digestive, and brush border enzyme activities and antioxidant defense of hepatopancreas and intestine for young grass carp (*Ctenopharyngodon idella*). Biol Trace Elem Res 2013;155:370–80.
Taylor R, Fjaera S, Skjervold P. Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment. J Food Sci 2002;67:2067–71.
Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell B 2007;39:44–84.
Vardi N, Parlakpinar H, Ozturk F, Ates B, Gul M, Cetin A, et al. Potent protective effect of apricot and β-carotene on methotrexate-induced intestinal oxidative damage in rats. Food Chem Toxicol 2008;46:3015–22.
Wang AN, Yi XW, Yu HF, Dong B, Qiao SY. Free radical scavenging activity of *Lactobacillus fermentum* in vitro and its antioxidative effect on growing–finishing pigs. J Appl Microbiol 2009;107:1140–8.
Wood J. Nitrogen excretion in some marine teleosts. Can J Physiol Pharm 1958;36:1237–42.
Xie NB, Feng L, Liu Y, Jiang J, Jiang WD, Hu K, et al. Growth, body composition, intestinal enzyme activities and microflora of juvenile Jian carp (*Cyprinus carpio var.* Jian) fed graded levels of dietary phosphorus. Aquacult Nutr 2011;17:645–56.
Yang HJ, Tian LX, Huang JW, Liang GY, Liu YJ. Dietary taurine can improve the hypoxia-tolerance but not the growth performance in juvenile grass carp Ctenopharyngodon idellus. Fish Physiol Biochem 2013;39:1071–8.
Yang XL, Li G, Wen CG, Hu BQ, Deng LR, Pei PZ, et al. A catalase from the freshwater mussel *Cristaria plicata* with cloning, identification and protein characterization. Fish Shellfish Immun 2011;31:389–99.
Yang SD, Lin TS, Liu FG, Liou CH. Influence of dietary phosphorus levels on growth, metabolic response and body composition of juvenile silver perch (*Bidyanus bidyanus*). Aquaculture 2006;253:592–601.
Ye CX, Liu YJ, Tian LX, Mai KS, Du ZY, Yang HJ, et al. Effect of dietary calcium and phosphorus on growth, feed efficiency, mineral content and body composition of juvenile grouper, *Epinephelus coioides*. Aquaculture 2006;255:263–71.
Ye JD, Liu XH, Kong JH, Wang K, Sun YZ, Zhang CX, et al. The evaluation of practical diets on a basis of digestible crude protein, lysine and methionine for *Litopenaeus vannamei*. Aquacult Nutr 2012;18:651–61.
Zhang CX, Mai KS, Ai QH, Zhang WB, Duan QY, Tan BP, et al. Dietary phosphorus requirement of juvenile Japanese seabass, *Lateolabrax japonicus*. Aquaculture 2006;255:201–9.
Zhou QC, Liu YJ, Mai KS, Tian LX. Effect of dietary phosphorus levels on growth, body composition, muscle and bone Mineral concentrations for orange-spotted grouper *Epinephelus coioides* reared in floating cages. J World Aquacult Soc 2004;35:427–35.
Zubay G. Biochemistry Addison-Wesley. Read Mass 1983:138–206.