Received: 5 March 2019 | Revised: 3 July 2019 | Accepted: 8 July 2019

DOI: 10.1111/anu.12967

ORIGINAL ARTICLE

Aquaculture Nutrition WILEY

Effect of dietary vitamin C on growth performance, body composition and biochemical parameters of juvenile Chu's croaker (*Nibea coibor*)

Weiguang Zou[1] | **Zhideng Lin**[1] | **Yisheng Huang**[2] | **Samwel M. Limbu**[3,4] | **Hua Rong**[1] | **Chuanqi Yu**[1] | **Fan Lin**[1] | **Xiaobo Wen**[1]

[1]Guangdong Provincial Key Laboratory of Marine Biology, Shantou University, Shantou, China

[2]School of Food Engineering and Biotechnology, Hanshan Normal University, Chaozhou, China

[3]Laboratory of Aquaculture Nutrition and Environmental Health, School of Life Sciences, East China Normal University, Shanghai, China

[4]Department of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, Dar es Salaam, Tanzania

Correspondence
Xiaobo Wen, Guangdong Provincial Key Laboratory of Marine Biology, Shantou University, 243 University Road, Shantou, Guangdong 515063, China.
Email: wenxbo@stu.edu.cn

Funding information
Technology Foundation and Major Scientific Research Projects of Guangdong University Foundation, Grant/Award Number: 2016KQNCX058; National Nature Science Foundation of China from Guangdong Oceanic and Fishery Science, Grant/Award Number: A201005D06-1

Abstract

Vitamin C is an essential micronutrient for normal physiological and immune functions of fish. However, its requirements and effects in Chu's croaker (*Nibea coibor*) are currently unknown. A 56-day feeding trial was conducted to evaluate the optimal dietary vitamin C requirements based on its effects on growth performance, body composition and biochemical parameters in juvenile Chu's croaker (14.17 ± 0.1 g). Six isoproteic (450 g/kg crude protein) and isolipidic (100 g/kg crude lipid) diets were formulated to contain 2.24 (basal diet), 39.03, 85.01, 171.16, 356.49 and 715.46 mg/kg of vitamin C. The results showed that fish fed on 171.16 mg/kg vitamin C diet had the highest growth performance and feed utilization. Fish fed on the basal diet had higher malondialdehyde (MDA) content and lower activities of antioxidant enzymes in the serum and liver as compared with those fed on vitamin C diets. Polynomial analysis indicated that the optimal dietary vitamin C requirements of juvenile Chu's croaker were 102.28, 98.21, 150.26, 165.38, 71.46, 176.19, 84.84 and 103.78 mg/kg based on weight gain, specific growth rate, liver storage, muscle storage, liver MDA content, liver alanine aminotransferase activity, liver alkaline phosphatase activity and liver superoxide dismutase activity, respectively. We recommend an inclusion level in the range of 71.46–150.26 mg/kg vitamin C in the diets of juvenile Chu's croaker for optimum growth performance, liver function, antioxidant capacity and innate immunity functions.

KEYWORDS
antioxidant capacity, Chu's croaker, growth performance, innate immunity, Nibea coibor, vitamin C

1 | INTRODUCTION

Vitamin C (ascorbic acid, ASA) is a water-soluble micronutrient essential for normal physiological functions and stimulation of the immune response of fish (Dawood & Koshio, 2016; Lim & Lovell, 1978). Specifically, vitamin C acts as a cofactor and plays important roles in many physiological processes involved in the biosynthesis of collagen (Darias, Mazurais, Koumoundouros, Cahu, & Zambonino-Infante, 2011), carnitine and norepinephrine, which are necessary for normal fish growth (Ai et al., 2004; Chen et al., 2015), reproduction (Darias et al., 2011; Sarmento et al., 2018; Shahkar et al., 2015), cartilage and bone formation (Kraus et al., 2004), the absorption iron (Hsu & Shiau, 1998; Padayatty & Levine, 2001), response to stressors (Caxico Vieira et al., 2017; Chakrabarti, Singh, Sharma, & Mittal,

 © 2019 John Wiley & Sons Ltd

2019; Narra, Rajender, Reddy, Rao, & Begum, 2015; Yan et al., 2017), wound healing (Wahli, Verlhac, Girling, Gabaudan, & Aebischer, 2003), immune response (Barros et al., 2014; Dawood, Koshio, El-Sabagh, et al., 2016; Gabaudan & Verlhac, 2001; Zhou, Wang, Wang, Xie, & Wang, 2012) and inflammatory response (Abdel Rahman, Khalil, Abdallah, & ElHady, 2018; Ruiz et al., 2018; Xu et al., 2016). Most teleosts cannot synthesize vitamin C de novo due to the lack of the last enzyme (L-gulonolactone oxidase) required for vitamin C biosynthesis (Fracalossi, Allen, Yuyama, & Oftedal, 2001; Moreau, Dabrowski, & Sato, 1999; Xie & Niu, 2006). Therefore, most farmed fishes require dietary vitamin C from diets for optimum growth and other physiological functions (Ai et al., 2006; Dawood & Koshio, 2016; Fracalossi et al., 2001). Insufficient vitamin C in the diets for fish causes several disorders, such as high mortality, poor growth and reproduction, anorexia, anaemia, haemorrhagic exophthalmia, spinal deformity and depressed immunity (Liu et al., 2010; Sarmento et al., 2018; Shahkar et al., 2015; Zhou et al., 2012). Therefore, dietary vitamin C intake is required for normal physiological functions of fish body.

Dietary vitamin C requirements for growth and development have been reported in several cultured fish species. The optimum vitamin C levels have been determined to 13.6 mg/kg in cobia (*Rachycentron canadum*) (Zhou et al., 2012), 28.2 mg/kg in large yellow croaker (*Pseudosciaena crocea*) (Ai et al., 2006), range from 92.8 to 129.8 mg/kg in grass carp (*Ctenopharyngodon idella*) (Xu et al., 2016), 142.2 mg/kg in Yellow drum (*Nibea albiflora*) (Wang et al., 2017) and 700 mg/kg in Wuchang bream (*Megalobrama amblycephala Yih*) (Ming et al., 2012). However, the vitamin C requirements vary among fish species (NRC, 2011). The variations may due to the difference in fish species, development stage, size, vitamin C form, feed formulations, feeding behaviour and culture environment of the fish (Chen et al., 2015; Dawood & Koshio, 2016; Lin & Shiau, 2004; Xu et al., 2016; Zhou et al., 2012). Hence, research on every fish species is required to establish the optimum vitamin C requirements.

Immunity response is one of the important parameters influenced by dietary vitamin C supplementation through its antioxidant capacity in many animal species (Gao et al., 2013; Lee, Oe, & Blair, 2001; Roginsky & Stegmann, 1994). It has been shown that dietary vitamin C enhances the antioxidant capacity in various farmed fish, such as gibel carp (*Carassius auratus gibelio*) (Shao et al., 2018), cobia (Zhou et al., 2012), large yellow croaker (Ai et al., 2006), grouper (*Epinephelus malabaricus*) (Lin & Shiau, 2005) and Japanese sea bass (*Lateolabrax japonicas*) (Ai et al., 2004). Accordingly, dietary vitamin C enhanced significantly the immunological parameters (Khan et al., 2017), such as lysozyme (LZM) (Ai et al., 2006, 2004; Wang et al., 2017; Xu et al., 2016) and AKP (Ming et al., 2012; Wan et al., 2014). Furthermore, dietary vitamin C improved significantly SOD (Zhou et al., 2012), total antioxidant capacity (T-AOC) (Huang et al., 2015), catalase (CAT) (Chen et al., 2015) and ALT (Daniel et al., 2018) activities, and reduced MDA content in the liver of juvenile Chinese sucker (*Myxocyprinus asiaticus*) (Huang et al., 2015). Accordingly, an appropriate level of vitamin C is required in fish for optimum immunity response.

The Chu's croaker (*Nibea coibor*) is a predatory marine fish widely distributed in India, the Philippines and the coastal areas of China (Shen, Huang, & Huang, 2004). The Chu's croaker has been considered as a potential species for aquaculture in China due to its high tolerance to harsh conditions, large size, high meat yield, delicious meat and high market price (Chai, Ji, Han, Dai, & Wang, 2013; Huang et al., 2017; Huang, Wen, Li, Li, & Zhu, 2016a, 2016b; Lin et al., 2018; Shao, 2006). In recent years, the nutrient requirements of Chu's croaker have been studied widely, such as dietary palm oil substitution (Huang et al., 2016a), dietary lipid (Huang et al., 2016b), dietary protein (Huang et al., 2017) and the LC-PUFA biosynthesis pathway and mechanism (Huang et al., 2017; Lin et al., 2018). To our knowledge, information on vitamin C requirements of Chu's croaker is currently unavailable. Therefore, this study determined the optimum dietary vitamin C requirements and its effects on growth performance, body composition and biochemical parameters of juvenile Chu's croaker.

2 | MATERIALS AND METHODS

2.1 | Ethical consideration

This research was approved by the Animal Ethics Committee of Shantou University China, and all experiments were conducted according to the protocols and procedures for the Laboratory Animal Management Ordinance of China.

2.2 | Experimental diets

Six isoproteic (450 g/kg crude protein) and isolipidic (100 g/kg crude lipid) experimental diets were formulated to contain increasing vitamin C levels at 2.24 (basal diet), 39.03, 85.01, 171.16, 356.49 and 715.47 mg/kg referred to as ASA 2.24, ASA 39.03, ASA 85.01, ASA 171.16, ASA 356.49 and ASA 715.46, respectively (Table 1). The vitamin C was supplied in the form of L-ascorbyl-2-monophosphate (35% vitamin C equivalent, Hangzhou Dehong Biology, Zhejiang, China). The actual vitamin C levels in the diets were analysed by high-performance liquid chromatography (HPLC; Agilent 1260 Infinity II, USA) with a national standard method (GB/T 238882-2009). To produce the experimental diets, fishmeal (Coastal Villages Pollock, Anchorage, AK, USA) and soy protein concentrate (Yuequn, Jieyang, Guangdong, China) were used as the protein sources, while fish oil (Yuequn, Jieyang, Guangdong, China) was used as the main lipid source. All ingredients were finely ground, mixed and sieved through a 60-mesh sieve. Fresh dechlorinated water was then added to make a dough, which was subsequently pelleted (3 mm diameter) by using a laboratory pelleting mill (SLP-45; Fishery Mechanical Facility Research Institute, Shanghai, China). Diets were air-dried until the moisture content was reduced to less than 100 g/kg and sealed in vacuum-packed bags. All the diets were stored at −20°C until needed for use and analysis.

TABLE 1 Formulation and proximate composition of the experimental diets (dry matter basis, g/kg) and vitamin C level (mg/kg)

	Diet					
Ingredient composition	**ASA 2.24**	**ASA 39.03**	**ASA 85.01**	**ASA 171.16**	**ASA 356.49**	**ASA 715.46**
Fish meal[a]	300	300	300	300	300	300
Soy protein concentrate[b]	300	300	300	300	300	300
Fish oil[c]	70	70	70	70	70	70
Amino acid premix	46	46	46	46	46	46
Soy bean lecithin	10	10	10	10	10	10
Dextrin	30	30	30	30	30	30
Tapioca starch	180	180	180	180	180	180
Choline chloride	5	5	5	5	5	5
Vitamin premix (vit. C-free)[d]	10	10	10	10	10	10
Monocalcium phosphate	10	10	10	10	10	10
Mineral premix[e]	10	10	10	10	10	10
Cellulose microcrystalline	29	28.857	28.714	28.428	27.856	26.712
L-ascorbyl-2-monophosphate[f]	0	0.143	0.286	0.572	1.144	2.288
Proximate composition						
Moisture	132.7	132.7	153.3	164.5	164.4	143.3
Crude protein	459.5	458.4	462.9	460.1	456	456
Crude lipid	107.5	106.8	105.1	104.9	99.7	97.4
Ash	87.8	89.3	90.9	93.6	96.2	103.5
Vitamin C level	2.24	39.03	85.01	171.16	356.49	715.47

[a]Fish meal: crude protein 69% dry matter, crude lipid 10% dry matter; Coastal Villages Pollock LLC., AK, USA.
[b]Soy protein concentrate: Shangdong Wandefu Industrial Group Co., Ltd., Shangdong, China.
[c]Fish oil: Yuequn, Jieyang, Guangdong, China.
[d]Vitamin mixture (vit. C-free) (IU or g/kg mixture): A, 2,200,000 IU; D3, 320,000 IU; E, 9,200 IU; K3, 2 g; B1, 3g; B2, 5.6 g; B6, 2 g; B12, 16 mg; folic acid, 0.8 g; pantothenic acid, 4 g; nicotinic acid, 15.6 g; inositol, 25.6 g; biotin, 16 mg.
[e]Mineral premix (g/kg mixture): $MgSO_4$, 15 g; $FeSO_4$, 2.5 g; $CuSO_4$, 0.031 g; $MnSO_4$, 0.162; $ZnSO_4$, 0.353 g; KIO_3, 0.003 g; Na_2SeO_3, 0.003 g; $CoSO_4$, 0.001 g.
[f]L-ascorbyl-2-monophosphate: Hangzhou Dehong Biology Co., Ltd., Zhejiang, China.

2.3 | Experimental fish and feeding trial

Juvenile Chu's croaker were obtained from local marine fish hatchery (Raoping, Guangdong) and transported alive to the Nan'Ao Marine Biology Station (NAMBS), Shantou University, Shantou, China. Prior to the feeding trial, all fish were fed the basal diet (unsupplemented with vitamin C diet) for two weeks to acclimatize them to the experimental conditions and diets. Twenty-four hours before the start of the experiment, fish were starved and anesthetized with eugenol (1:10,000; Shanghai Reagent, Shanghai, China) to reduce handling stress. A total of 540 visually healthy juvenile Chu's croaker (initial body weight: 14.17 g ± 0.1 g) were randomly stocked into 18 cages (1 m × 1 m × 1.5 m) each containing 30 fish in three replicates. Fish in each cage were fed on the respective test diets twice daily (07:30 and 16:30 hr) to satiation level for 56 days. Survival rate was monitored on daily basis, and dead fish were removed and weighed immediately. During the experimental period, water quality parameters were maintained at optimum levels for Chu's croak growth and survival. The water temperature and pH were 29 ± 0.5°C and 7.7 ± 0.6, respectively, whereas unionized ammonia nitrogen was lower than 0.05 mg/L, salinity ranged between 30 and 32 g/L and dissolved oxygen ranged from 5.58 to 5.68 mg/L.

2.4 | Data collection

After eight weeks, all fish from each cage were starved for 24 hr, collected and anaesthetized with eugenol (1:10,000; Shanghai Reagent) to reduce handling stress. They were counted to determine survival rate (SR) using the following formula:

$$\mathrm{SR}\,(\%) = 100 \times \frac{\text{Final fish numbers}}{\text{Initial fish numbers}}.$$

All fish in each cage were weighed for the determination of final weight (FW), while WG and SGR were calculated by using the following formulae:

$$\mathrm{WG}\,(\%) = 100 \times \left(\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}}\right)$$

$$\mathrm{SGR}\,(\%\ \mathrm{day}^{-1}) = 100 \times \left(\frac{\mathrm{Ln}(\text{Final body weight}) - \mathrm{Ln}(\text{Initial body weight})}{\text{Time (days)}}\right).$$

The weight of fish, feed intake and total protein intake were used to determine feed efficient (FE) and protein efficiency ratio (PER) as follows:

$$\text{FE (\%)} = 100 \times \left(\frac{\text{Wet weight gain (g)}}{\text{Feed intake (g)}} \right)$$

$$\text{PER (\%)} = 100 \times \left(\frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}} \right)$$

The viscera and liver were weighed for the determination of viscerosomatic index (VSI) and hepatosomatic index (HSI), respectively, using the following formulae:

$$\text{HSI (\%)} = 100 \times \left(\frac{\text{Liver weight (g)}}{\text{Whole body weight (g)}} \right)$$

$$\text{VSI (\%)} = 100 \times \left(\frac{\text{Viscera weight (g)}}{\text{Whole body weight (g)}} \right).$$

Five fish were randomly sampled from each cage and measured individually for body length and weight to determine to condition factor (CF) using the following formula:

$$\text{CF (\%)} = 100 \times \left(\frac{\text{Total body weight (g)}}{\text{Total body length}^3 (\text{cm}^3)} \right).$$

2.5 | Proximate composition analysis

An initial sample of eight fish was randomly collected and frozen (−20°C) for initial body proximate analysis. At the end of the feeding trial, three fish from each cage (nine per dietary treatment) were randomly collected and frozen (−20°C) for final whole-body composition analysis. Three samples of fish muscles from each cage were also used for proximate composition. Furthermore, about 20 g of each diet was used for proximate composition analysis. The proximate composition analysis of whole fish, muscle and diet samples was analysed in triplicate. Crude protein, crude lipid and ash contents of the diets and experimental samples were performed following standard methods (AOAC, 1995). Protein content (nitrogen × 6.25) was determined by Kjeldahl methods, following an acid digestion by using Kjeldahl Auto Sampler System 1035 Analyzer (Foss, Hoganas, Sweden). Crude lipid content was determined by ether extract using SoxtecTM 8000 extraction system (Foss). The ash content was analysed through combustion in a muffle furnace (Carbolite CWF 11/5, Hope Valley, UK) at 550°C for 6 hr. Moisture was determined by oven drying at 105°C to constant weight.

2.6 | Biochemical analysis

The blood from three fish in each cage was collected at the caudal vein by using a heparinized syringe. Serum was obtained by centrifugation at 5,000 *g* for 10 min at 4°C (centrifuge MR23i; Jouan, Saint-Herblain, France). The serum was stored at −80°C until needed for further analysis. The liver and muscle samples were collected from three bloodless fish from each cage. A part of the liver and muscle samples were quickly frozen in liquid nitrogen and then stored at −80°C. The serum and liver samples were used for biochemical analysis for enzymes activities. The liver and muscle samples were homogenized in nine volumes of cold saline water containing 0.65% NaCl, and then centrifuged at 3,500 *g* for 10 min at 4°C to fractionate supernatant. Finally, the supernatant was collected and stored at −80°C for subsequent analysis. The activities of T-AOC, SOD, CAT, AKP ALT and the MDA and LZM contents in the serum and liver were determined by using specific commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The MDA content was also determined in muscle using commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7 | Vitamin C content analysis

The vitamin C content was analysed in the muscle, serum, liver and diet samples. The vitamin C concentration in the diets was determined by using HPLC based on a national standard method (GB/T 238882-2009). Briefly, the phosphate buffer solution (PBS) and mobile phase were prepared. The PBS contained 0.054 g/ml KH_2PO_4 at ph 3.0, and the mobile phase was prepared by using KH_2PO_4 (6.8 g), tetrabutyl hydroamine sulphate (0.5 g) and methanol (75 ml), and then fixed with distilled water into a 1,000-ml volumetric flask. The solution was shacked with hand for 5 min, then filtered through a 0.45-μm filter and finally degassed. The diet samples (3–5 g) were ground and extracted in 50 ml of PBS, degassed for 15 min in the dark and then centrifuged at 3,500 *g* at a temperature of 4°C for 5 min. The supernatant was filtered through a 0.45-μm syringe filter. Standard solution of tris-(cyclohexyl-ammonium)-ascorbic acid-2-phosphate was prepared in PBS and stored at 4°C in the dark. The flow rate was 1.0 ml/min. The absorbance was recorded with an UV detector at 250 nm. Tissue vitamin C concentration was determined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) according to a spectrophotometric method (Dabrowski & Hinterleitner, 1989).

2.8 | Statistical analyses

Data are presented as mean ± standard error (*SE*). All data were tested for normality by using the Kolmogorov–Smirnov test and homogeneity of variances by using Levene's test before statistical analyses. Differences in measured parameters among the dietary treatments were compared by using one-way analysis of variance (ANOVA). When significant differences were detected, the means were further compared by using Duncan's multiple range tests. The optimum vitamin C concentrations were estimated by using broken-line polynomial regression analysis. All statistical analyses were performed by using SPSS 20.0 (SPSS, Chicago, IL, USA). Results with $p \leq 0.05$ were considered statistically significant.

TABLE 2 The growth performance, feed utilization, survival and organ indices of juvenile Chu's croaker *Nibea coibor* fed different dietary vitamin C diets for 56 days

	Diet					
Parameters	**ASA 2.24**	**ASA 39.03**	**ASA 85.01**	**ASA 171.16**	**ASA 356.49**	**ASA 715.46**
IW (g)	14.32 ± 0.09	14.21 ± 0.10	14.25 ± 0.09	14.18 ± 0.43	13.98 ± 0.11	14.05 ± 0.21
FW (g)	62.86 ± 4.05^{a}	72.09 ± 3.22^{ab}	89.95 ± 5.23^{c}	94.04 ± 7.19^{c}	90.09 ± 3.27^{c}	84.91 ± 3.68^{bc}
WG (%)	348.98 ± 28.95^{a}	414.9 ± 23.01^{ab}	542.52 ± 37.39^{c}	571.74 ± 51.39^{c}	543.49 ± 23.4^{c}	506.5 ± 26.3^{bc}
SGR (% day^{-1})	2.65 ± 0.12^{a}	2.91 ± 0.08^{ab}	3.3 ± 0.10^{c}	3.36 ± 0.13^{c}	3.32 ± 0.06^{c}	3.18 ± 0.06^{bc}
SR (%)	67.78 ± 8.89^{a}	75.56 ± 4.84^{ab}	85.56 ± 1.11^{b}	85.56 ± 2.22^{b}	83.33 ± 1.93^{b}	88.89 ± 1.11^{b}
FE (%)	61.65 ± 3.55^{a}	69.67 ± 1.36^{ab}	70.21 ± 3.69^{ab}	72.9 ± 1.84^{b}	72.45 ± 3.27^{ab}	63.25 ± 4.79^{ab}
PER (%)	1.33 ± 0.08^{a}	1.51 ± 0.03^{ab}	1.53 ± 0.08^{ab}	1.59 ± 0.04^{b}	1.59 ± 0.07^{b}	1.40 ± 0.11^{ab}
CF (g/cm^{3})	1.23 ± 0.02	1.30 ± 0.03	1.25 ± 0.06	1.28 ± 0.04	1.24 ± 0.03	1.23 ± 0.03
HSI (%)	2.84 ± 0.09^{a}	3.1 ± 0.89^{ab}	3.33 ± 0.12^{b}	3.38 ± 0.20^{b}	3.16 ± 0.56^{ab}	3.06 ± 0.12^{ab}
VSI (%)	5.08 ± 0.16^{a}	5.71 ± 0.12^{b}	5.85 ± 0.12^{b}	5.77 ± 0.22^{b}	5.46 ± 0.12^{ab}	5.83 ± 0.25^{b}

Note: Values are means ± *SE* (n = 3). Values with different superscript letters within a row are significant different (p < 0.05).
Abbreviations: CF, condition factor; FE, feed efficiency; FW, final weight; HSI, hepatosomatic index; IW, initial weight; PER, protein efficiency ratio; SGR, specific growth rate; SR, survival rate; VIS, viscerosomatic index; WG, weight gain.

3 | RESULTS

3.1 | Growth performance, feed utilization, survival rate and organ indices

The growth performance, feed utilization survival rate and organ indices of juvenile Chu's croaker were significantly affected by vitamin C supplementation (p < 0.05; Table 2). The FW, WG and SGR of the fish fed on the basal diet (2.24 mg/kg) were significantly lower than those fed on the diets supplemented with vitamin C levels starting from 85.01 to 715.46 mg/kg (p < 0.05). The optimum dietary vitamin C requirements of juvenile Chu's croaker based on WG and SGR were estimated as 102.28 and 98.21 mg/kg, respectively (Figure 1).

Similarly, FE and PER were significantly higher in fish fed on 171.16 mg/kg vitamin C than those fed on the basal diet (p < 0.05; Table 2). The fish fed on 85.01, 171.16, 356.49 and 715.46 mg/kg vitamin C diets had significantly higher survival rate than those fed on the basal diet (p < 0.05). The HSI and VSI were significantly influenced by the dietary vitamin C levels, displaying the lowest value for fish fed on the basal diet, and the maximum values were observed in fish fed on the diets supplemented with 85.01 and 171.16 mg/kg (p < 0.05; Table 2). However, CF was not affected by dietary vitamin C levels (p > 0.05).

3.2 | Body composition

The results of whole-body and muscle proximate compositions are shown in Table 3. Fish fed on the diets supplemented with 85.01 and 171.16 mg/kg vitamin C had significantly higher protein contents in the whole body than other groups (p < 0.05). The muscle crude protein content of fish fed on 356.49 mg/kg vitamin C diet was significantly higher than those fed the other diets (p < 0.05). Fish fed on the basal diet had the lowest crude lipid contents in whole body and muscle than those fed on 715.46 mg/kg vitamin C diet (p < 0.05). However, vitamin C did not affect the moisture and ash contents in the whole body and muscle (p > 0.05).

The vitamin C content in the serum increased in a dose-dependent manner (Table 4). Similarly, vitamin C concentration in both liver and muscle significantly increased with increasing vitamin C supply from 2.24 to 171.16 mg/kg (p < 0.05) and remained fairly constant afterwards (Table 4). The optimum vitamin C requirement of juvenile Chu's croaker was 150.26 mg/kg in the liver and in the muscle (164.38 mg/kg; Figure 2).

3.3 | Biochemical parameters

The MDA content in the liver and muscle decreased markedly with increasing dietary vitamin C supply from 2.24 to 39.03 mg/kg and then reached a plateau (Figure 3a). The liver and muscle MDA content for fish fed on basal diet was significantly higher than those fed on vitamin C diets (p < 0.05). Moreover, the muscle MDA content for fish fed on 715.46 mg/kg was significantly lower than those fed on other vitamin C diets (p < 0.05). The serum MDA content decreased with increasing vitamin C levels from 39.03 to 715.46 mg/kg. However, vitamin C did not significantly affect the serum MDA content (p > 0.05; Figure 3b). The ALT activity in the liver of fish fed on the basal diet was lower than those fed on vitamin C diets. Fish fed on diet supplemented with 171.16 mg/kg vitamin C had the highest value (p < 0.05; Figure 3c) of all the other diets, except 356.49 mg/kg vitamin C diet (p > 0.05). In the serum, the ALT activity significantly decreased with increasing dietary vitamin C supply from 2.24 to 85.01 mg/kg (p < 0.05; Figure 3d), except 39.03 mg/kg (p > 0.05), and then remained constant. The optimum vitamin C requirements for juvenile Chu's croaker were 71.46 and 176.19 mg/kg based on liver MDA content (Figure 3e) and liver ALT activity (Figure 3f), respectively.

The liver and serum antioxidant capacity are shown in Figure 4. Fish fed on basal diet had significantly lower SOD (Figure 4a) and CAT activity (Figure 4e) in the liver than those fed on other vitamin C supplement diets ($p < 0.05$), except 39.03 mg/kg ($p > 0.05$). Moreover, fish fed on 171.16 and 356.49 mg/kg vitamin C diets had the highest SOD and CAT activities, respectively. The serum SOD activity of fish fed on basal diet was significantly lower than those fed on other diets ($p < 0.05$; Figure 4b), except for 39.03 mg/kg ($p > 0.05$). When dietary vitamin C level exceeded 85.01 mg/kg, the serum SOD activity reached a plateau. The hepatic T-AOC activity of fish fed on basal diet was significantly lower than those fed on 85.01, 171.16 and 356.49 mg/kg vitamin C diets ($p < 0.05$; Figure 4c). Furthermore, fish fed on 715.46 mg/kg had significantly lower T-AOC activity than those fed on 85.01 and 171.16 mg/kg vitamin C diets in the liver ($p < 0.05$). However, the T-AOC activity in the serum was not significantly influenced by dietary vitamin C diets ($p > 0.05$; Figure 4d), except fish fed on 715.46 mg/kg diet ($p < 0.05$). The optimum vitamin C requirement in Chu's croaker was estimated as 103.78 mg/kg based on liver SOD activity (Figure 4f).

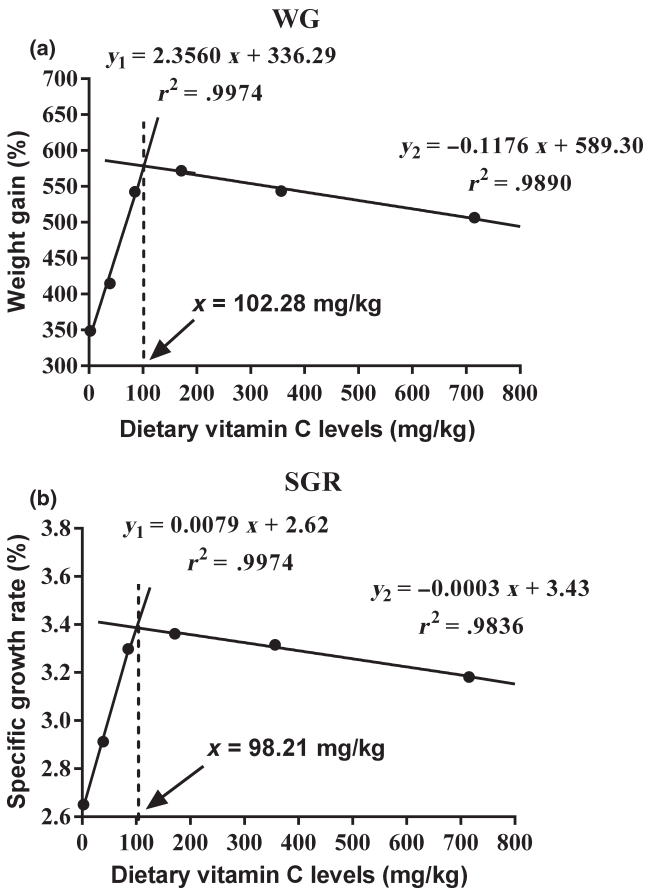


FIGURE 1 Broken-line regression analysis of weight gain (WG) (a), and specific growth rate (SGR) (b) of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet)

Dietary vitamin C supplementation significantly affected LZM and AKP activities in the liver and serum (Figure 5). The LZM activity in the liver (Figure 5a) and serum (Figure 5b) was lower for fish fed on basal diet than those fed on vitamin C diets ($p < 0.05$), except 39.03 mg/kg ($p > 0.05$). Moreover, fish fed on 715.46 mg/kg vitamin C diets had the highest LZM activities. The liver AKP activity increased significantly with vitamin C from 2.24 to 171.16 mg/kg ($p < 0.05$; Figure 5c) and then decreased. However, the AKP activity in the serum decreased markedly with increasing dietary vitamin C supply from 2.24 to 85.01 mg/kg ($p < 0.05$; Figure 5d), except 39.03 mg/kg ($p > 0.05$), and then reached a plateau. The optimum vitamin C requirement for Chu's croaker was 84.84 mg/kg based on liver AKP activity (Figure 5e).

4 | DISCUSSION

The aim of this study was to evaluate the vitamin C requirements and its effects on growth performance, body composition and biochemical parameters of juvenile Chu's croaker. Our data depicted highest WG, SGR, FE and PER for fish fed on 171.16 mg/kg vitamin C diet

TABLE 3 The compositions of whole body and muscle in juvenile Chu's croaker *Nibea coibor* fed different dietary vitamin C diets for 56 days (g/kg wet weight)

	Diet					
Composition	**ASA 2.24**	**ASA 39.03**	**ASA 85.01**	**ASA 171.16**	**ASA 356.49**	**ASA 715.46**
Whole body						
Moisture	694.6 ± 5.68	694.6 ± 8.04	680.2 ± 5.07	680.9 ± 6.49	690.7 ± 9.26	679.0 ± 8.49
Protein	162.7 ± 0.61^{a}	166.0 ± 1.56^{a}	182.2 ± 1.38^{c}	181.3 ± 0.37^{c}	175.3 ± 0.75^{b}	175.6 ± 1.69^{b}
Lipid	84.2 ± 3.71^{a}	93.6 ± 1.26^{ab}	95.3 ± 1.23^{ab}	94.1 ± 2.25^{ab}	87.6 ± 0.23^{ab}	97.4 ± 8.09^{b}
Ash	41.2 ± 1.07	39.6 ± 1.19	38.8 ± 1.21	40.5 ± 2.57	39.0 ± 1.03	42.1 ± 1.10
Muscle						
Moisture	760.8 ± 1.72	762.4 ± 2.86	767.1 ± 2.17	745.3 ± 1.43	737.7 ± 0.93	748.5 ± 1.02
Protein	177.2 ± 0.14^{ab}	175.4 ± 0.79^{a}	181.4 ± 0.63^{b}	181.5 ± 1.51^{b}	189.1 ± 0.75^{c}	181.0 ± 0.51^{b}
Lipid	28.5 ± 1.38^{a}	33.1 ± 2.24^{ab}	30.8 ± 2.63^{ab}	33.8 ± 0.84^{ab}	30.0 ± 1.67^{ab}	34.8 ± 0.55^{b}
Ash	12.6 ± 0.79	13.7 ± 0.26	13.8 ± 0.23	12.9 ± 1.02	12.5 ± 0.87	13.4 ± 0.53

Note: Values are means ± *SE* ($n = 3$). Values with different superscript letters within a row are significant different ($p < 0.05$).

TABLE 4 Vitamin C concentrations (mg/kg) in liver, serum and muscle of juvenile Chu's croaker (*Nibea coibor*) fed the experimental diets with different VC levels for 56 days

	Diet					
Tissue	ASA 2.24	ASA 39.03	ASA 85.01	ASA 171.16	ASA 356.49	ASA 715.46
Liver	38.29 ± 1.33^{a}	45.26 ± 2.10^{b}	51.83 ± 1.40^{c}	61.25 ± 1.92^{d}	58.94 ± 1.00^{d}	58.38 ± 1.84^{d}
Muscle	25.26 ± 0.47^{a}	31.64 ± 0.33^{b}	34.39 ± 0.76^{bc}	37.09 ± 0.17^{c}	37.88 ± 0.20^{c}	37.93 ± 0.12^{c}
Serum	11.89 ± 0.82^{a}	13.03 ± 0.40^{ab}	14.86 ± 0.60^{b}	15.09 ± 0.40^{bc}	17.83 ± 0.40^{c}	25.6 ± 1.87^{cd}

Note: Values are means ± *SE* (n = 3). Values with different superscript letters within a row are significant different (p < 0.05).

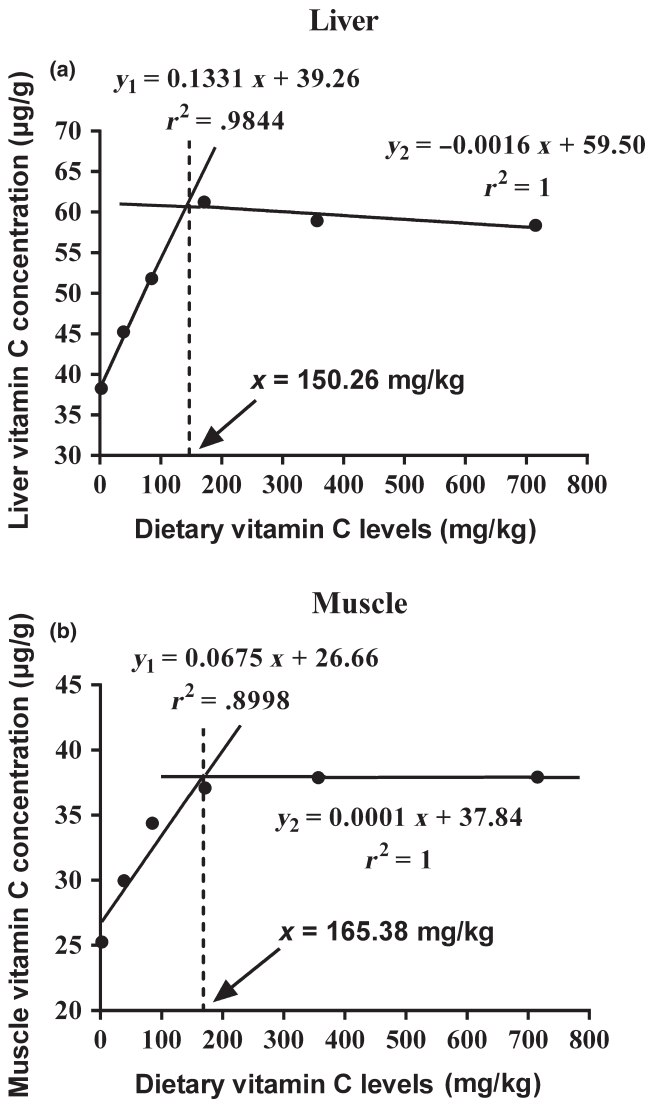


FIGURE 2 The vitamin C concentration in the liver (a) and muscle vitamin C concentration (b) of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet)

than those fed on basal vitamin C diet, similar to previous results in yellow croaker, juvenile largemouth bass (Ai et al., 2006), (*Micropterus salmoides*), juvenile hybrid tilapia (Chen et al., 2015), mahseer fish (*Tor putitora*) (Khan et al., 2017), *Oreochromis niloticus* × *Oreochromis aureus* (Shiau & Hsu, 1999) and juvenile cobia (Zhou et al., 2012). The optimum dietary vitamin C requirements of juvenile Chu's croaker based on WG and SGR were estimated as 102.28 and 98.21 mg/kg, respectively. This amount is comparable to those obtained in other fish species such as largemouth bass (102.6–109.5 mg/kg, Chen et al., 2015) and grass carp (92.8–129.8 mg/kg, Xu et al., 2016), but lower than some other fish species such as yellow drum (142.2 mg/kg, Wang et al., 2017) and Wuchang bream (700 mg/kg, Ming et al., 2012), and higher than large yellow croaker (28.2 mg/kg, Ai et al., 2006), cobia (13.6 mg/kg, Zhou et al., 2012) and striped catfish (*Pangasianodon hypophthalmus*) (46 mg/kg, Daniel et al., 2018). The differences in vitamin C requirement between Chu's croaker and those species may be attributed to specific requirements of different fish species due to variations in metabolic activities (Dabrowski, 1991) and their sizes (Chen et al., 2015). For example, the optimum dietary vitamin C requirement of cobia (25.85 g) was estimated as 75 mg/kg (Zhao et al., 2008), while it was only 44.7 mg/kg for the same species weighing 4.59 g (Xiao et al., 2010). Furthermore, the use of various forms of vitamin C, which vary in efficiency (Lin & Shiau, 2005) and bioavailability (Dabrowski, 1994), may also account for the observed variations. For instance, by using higher graded levels of vitamin C, Wang, Xie, Wu, and Yu (2006) obtained optimum vitamin C requirement as 750 mg/kg for cobia, while it was only 75 mg/kg (Zhao et al., 2008) when lower graded levels (0–300 mg/kg) of vitamin C were used. Moreover, the differences in nutrient contents in the formulated feeds used (Chen et al., 2015) also may cause variations in vitamin C requirements among fish species.

The increased growth performance may be related to the increased organ indices, nutrient body composition and feed utilization after feeding Chu's croaker with different levels of vitamin C. In the present study, HSI and VSI were higher for the fish fed on 85.01 and 171.16 mg/kg vitamin C diets than those fish fed on the basal vitamin C diet, similar to results in obtained previously in golden shiners (*Notemigonus crysoleucas*) (Chen et al., 2003) and stripped catfish (Daniel et al., 2018). The HSI and VSI are important body indices, which are often used to assess the nutritional status and physiological condition of fish (Dawood, Koshio, El-Sabagh, et al., 2016; Goede & Barton, 1990; Lee, Jeon, & Lee, 2002). Their higher levels indicated that fish fed the diets supplemented with vitamin C had superior nutritional and physiological conditions and thus grew better than those fed on the basal diet. Similarly, results were reported in catfish (Daniel et al., 2018), cobia (Zhou et al., 2012), largemouth bass (Chen et al., 2015) and golden shiners (*Notemigonus crysoleucas*) (Chen et al., 2003). Evidently, the survival rate of the fish fed on the basal diet was the lowest (67.78%) compared with those fed on the vitamin C

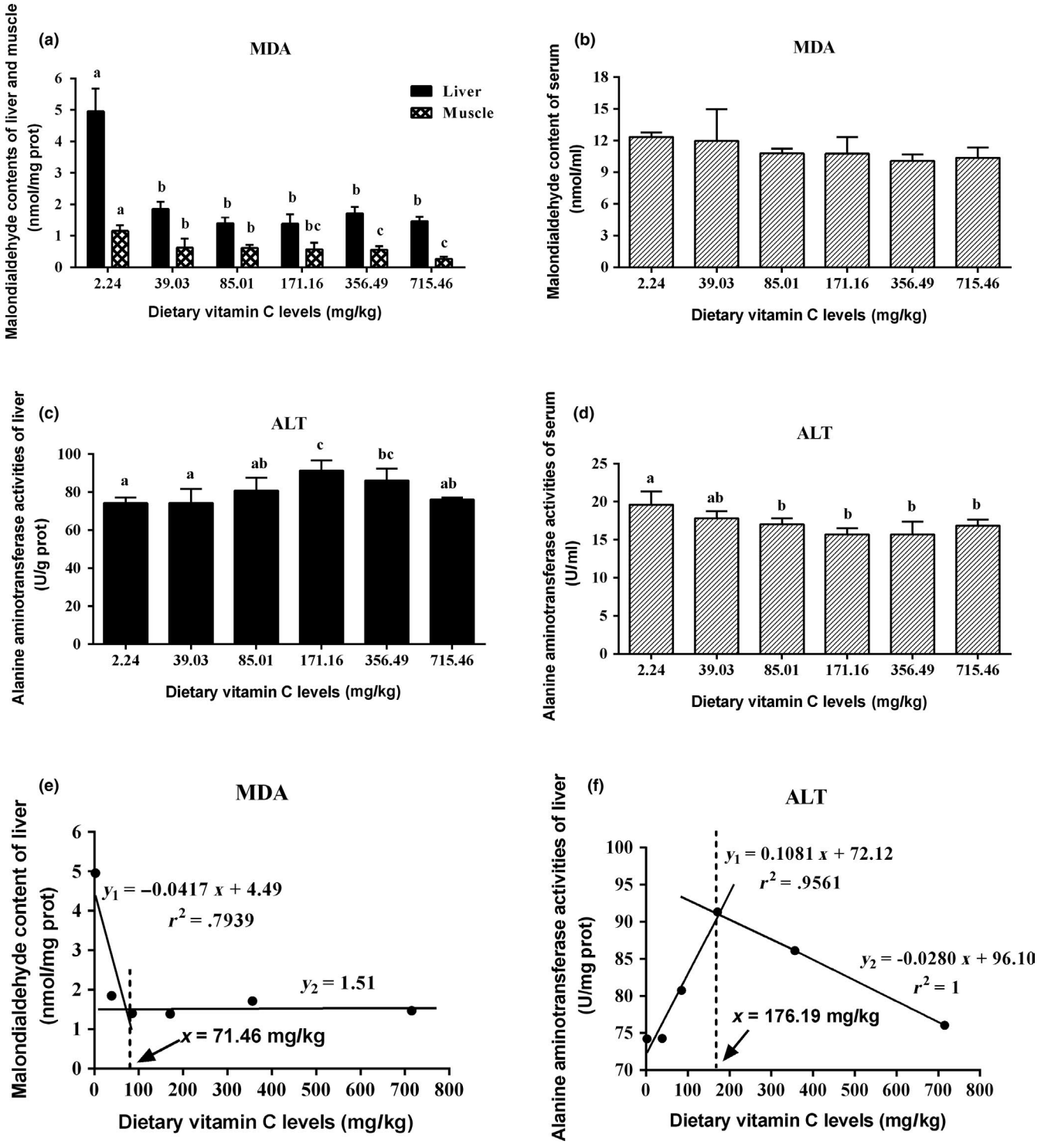


FIGURE 3 The malondialdehyde (MDA) contents in the liver and muscle (a), serum (b) and activities of alanine aminotransferase (ALT) in the liver (c), serum (d) of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet). Results are presented as the mean ± *SE* (n = 3). Different letters (a, b, c) on column indicated significant differences ($p < 0.05$). The optimum vitamin C requirement in the liver of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet) based on MDA (e) and ALT activity (f)

diets, which is similar to results reported by Ai et al. (2006) and Wang et al. (2017). Moreover, higher FE and PER were observed in fish fed on 171.16 mg/kg vitamin C diet than those fed on the basal vitamin C diet. The FE represents the ability of fish to consume efficiently the feeds given. A higher FE indicates feeds with vitamin C were utilized and the amount of protein was absorbed efficiently as indicated by higher PER. Indeed, the nutrients contained in the feeds fed were efficiently used, which led to higher protein and lipid contents in the fish fed on diets supplemented with vitamin C. In the present study, the crude protein contents of whole body and muscle were enhanced

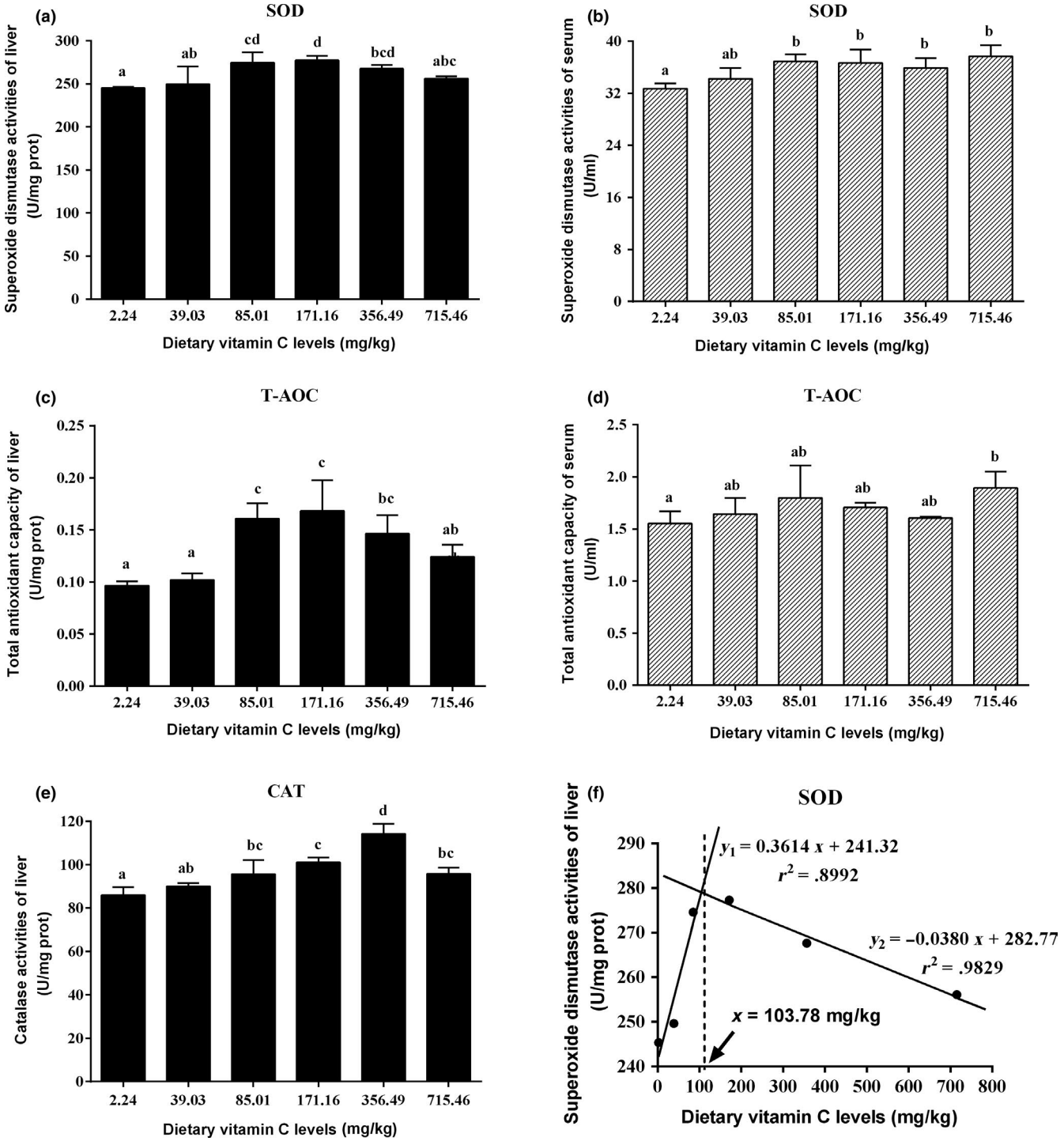


FIGURE 4 Activities of superoxide dismutase (SOD) (a), total antioxidant capacity (T-AOC) (c) and catalase (CAT) (e) in the liver and activities of SOD (b), T-AOC (d) in the serum of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet). Results are presented as the mean ± *SE* (*n* = 3). Different letters (a, b, c, d) on column indicated significant differences ($p < 0.05$). The optimum vitamin C requirement in the liver of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet) based on SOD activity (f)

with increasing dietary vitamin C levels from 85.01 to 715.46 mg/kg, which indicated that vitamin C as a cofactor promoted protein synthesis of juvenile Chu's croaker (Biswas, Biswas, Junichi, Kim, & Takii, 2013; Chagas & Val, 2003; Chen et al., 2015). Furthermore, the fish fed on the basal diet had lower crude lipid contents in whole body and muscle than those fed on vitamin C diets, consistent with results obtained in largemouth bass (Chen et al., 2015) and Chinese sucker (Huang et al., 2015). The higher protein and lipid contents in whole body and muscle were used to build muscle and supply energy, respectively, which resulted in higher growth performance. Therefore, we advise to supplement diets with 102.28 and 98.21 mg/kg vitamin C to improve growth performance of the Chu's croaker fish.

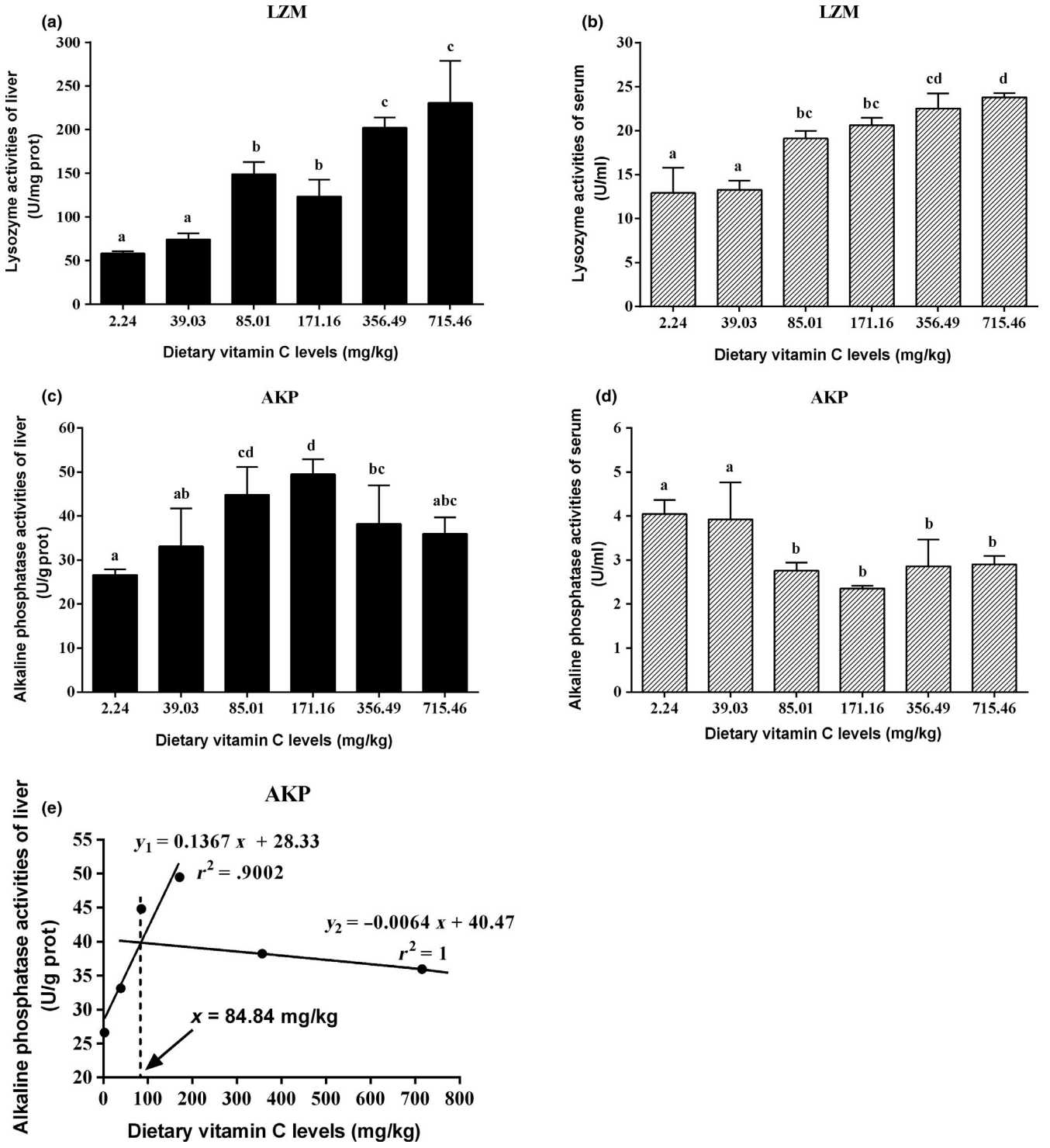


FIGURE 5 Activities of lysozyme (LZM) in the liver (a), serum (b) and activities of alkaline phosphatase (AKP) in the liver (c), serum (d) of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet). Results are presented as the mean ± *SE* (n = 3). Different letters (a, b, c, d) on column indicated significant differences ($p < 0.05$). The optimum vitamin C requirement in the liver of Chu's croaker fed diets containing graded levels of vitamin C (mg/kg diet) based on AKP activity (e)

Hepatic and muscular vitamin C concentrations are usually considered as indicators of the vitamin C status for fish (Ai et al., 2006, 2004). Liver was an important organ in catabolism and storage of vitamin C (Dabrowski, 1994; Gábor, László, Miklós, Ferenc, & József, 1997; Xiao et al., 2010). In the present study, the vitamin C optimum requirements of liver and muscle were 150.26 and 165.38 mg/kg, respectively, which are much higher than the values based on WG and SGR (102.28 and 98.21 mg/kg) in juvenile Chu's croaker. These results are similar to the findings of Ai et al. (2004), Wu et al. (2015) and Xiao et al. (2010). These results indicated that the optimum vitamin

C required to maintain normal physiological functions is higher than the level needed for maximum growth (Xiao et al., 2010).

Our results further indicated that the vitamin C requirements of juvenile Chu's croaker were lower (150.26 mg/kg) in the liver than in muscle (164.38 mg/kg), which is similar to previous results (Ai et al., 2004; Wu et al., 2015; Xiao et al., 2010). Logically, two reasons may ascribe for the obtained results: (a) the dietary vitamin C absorbed in the intestine was first transported to liver to satisfy the requirements of physiological functions and the excessive amount was transported from liver to muscle; (b) the liver is a metabolically active organ and thus its vitamin C requirements are lower than muscle (Xiao et al., 2010). Taken together, Chu's croaker farmers are suggested to supplement diets with 150.26–164.38 mg/kg vitamin C for proper physiological functions of the fish.

The health status of the liver should also be considered during the determination of the optimum vitamin C requirements, because the suboptimal dietary vitamin C may damage the proper functioning of liver (Daniel et al., 2018; Huang et al., 2015). The MDA is an indicator of lipid peroxidation, which can damage the cell structure and function (Dawood, Koshio, El-Sabagh, et al., 2016; Freeman & Crapo, 1982; Sies, 1997). The ALT is another important indicator of liver function and animal health status (Lin, Mai, & Tan, 2007), with higher activity signifying high-stress conditions (Dawood & Koshio, 2016; Menezes et al., 2006). In the present study, the MDA content of tissue in juvenile Chu's croaker was significantly decreased with dietary vitamin C levels, expect in the serum, similar to results in previous studies (Huang et al., 2015; Wu et al., 2015). Furthermore, the ALT activity in the serum for fish fed on the basal diet was significantly higher than those fed on other diets. The optimum vitamin C requirements for the health status of the liver in juvenile Chu's croaker were 71.46 and 176.19 mg/kg based on liver MDA content and liver ALT activity, respectively.

We reasoned that the decreased MDA content and ALT activity are due to the increased antioxidant capacity after vitamin C supplementation. The SOD, T-AOC and CAT are all critical antioxidant enzymes in the body, which can scavenge free radicals and participate in protective mechanisms in tissue injury (Fontagne-Dicharry et al., 2014). In the present study, the hepatic SOD, T-AOC and CAT activities increased with vitamin C levels from 2.24 to 171.16 mg/kg, beyond which they decreased. These data suggested that, dietary vitamin C enhanced the hepatic SOD, T-AOC and CAT activities (Cheng, Liang, & Guo, 2017), but higher doses did not have any beneficial effects on antioxidant capacity, similar to previous results reported in cobia (Zhou et al., 2012), Chinese sucker (Huang et al., 2015) and gibel carp (Shao et al., 2018). The optimum vitamin C requirement for proper functioning of antioxidant capacity in Chu's croaker was estimated as 103.78 mg/kg based on liver SOD activity. Therefore, the observed decrease in MDA content and ALT activity is due to increased activities of antioxidants, which cleared the free radicals and thus reduced lipid peroxidation and lipotoxicity (Caxico Vieira et al., 2017; Lee et al., 2001). These results indicate that dietary vitamin C improves the health status of liver in juvenile Chu's croaker. Fish farmers culturing Chu's croaker may supplement the diet with 103.78 mg/kg vitamin C to increased antioxidant capacity and reduce hepatic lipid peroxidation and lipotoxicity of the fish.

The innate immune system of fish is considered to be the vital first line of defence against a broad spectrum of pathogens for fish (Zhou et al., 2012). The LZM activity is often used to evaluate the innate immune capacity of fish as it is ubiquitously distributed among living organisms (Saurabh & Sahoo, 2008). Furthermore, AKP is a polyfunctional hydrolase enzyme involved in the immune defence system of some fishes (Shao et al., 2018; Yan, Tian, Dong, Fang, & Yang, 2014). The AKP plays an important role in improving the recognition ability and phagocytosis by changing the surface structure of the pathogens and strengthening the disease resistance (Lazado, Lund, Pedersen, & Nguyen, 2015). In the present study, fish fed on vitamin C diets had higher LZM activities in the serum and liver than those fed on the basal diet, expect those fed on 39.03 mg/kg, similar to results reported in previous studies (Ai et al., 2006, 2004; Wang et al., 2017; Zhou et al., 2012). Moreover, the dietary vitamin C could enhance the hepatic AKP activity, but higher dose did not offer further beneficial effect on immunity of Chu's croaker as previously reported in cobia (Zhou et al., 2012) and Atlantic halibut (Lee et al., 2002). The optimum vitamin C requirement for proper functioning of innate immune system for Chu's croaker was 84.84 mg/kg based on liver AKP activity. Surprisingly, the serum AKP activity of Chu's croaker decreased with dietary vitamin C supply from 2.24 to 85.01 mg/kg and then levelled off. The contrasting results in the serum were also observed in previous studies (Ming et al., 2012; Wan et al., 2014). High serum AKP activity is related to inflammation, atherosclerosis and metabolic syndrome (Kang, Hong, Park, & Ju, 2015).

5 | CONCLUSION

This study determined the optimal dietary vitamin C requirements of juvenile Chu's croaker (ranging from 84.84 to 150.26 mg/kg diet). This amount is essential to improve growth performance, reduce hepatotoxicity, increase antioxidant capacity and maintain innate immunity in juvenile Chu's croaker. We recommend that Chu's croaker farmers may supplement the diets of fish with 84.84–150.26 mg/kg vitamin C for normal fish growth, physiology and diseases protection in aquaculture.

ACKNOWLEDGEMENTS

This study was financially supported by the National Nature Science Foundation of China (Grant No. A201005D06-1) from Guangdong Oceanic and Fishery Science and Technology Foundation and Major Scientific Research Projects of Guangdong University Foundation (Grant No. 2016KQNCX058).

ORCID

Zhideng Lin https://orcid.org/0000-0002-4828-9806
Xiaobo Wen https://orcid.org/0000-0001-9244-6269

REFERENCES

Abdel Rahman, A. N., Khalil, A. A., Abdallah, H. M., & ElHady, M. (2018). The effects of the dietary supplementation of *Echinacea purpurea* extract and/or vitamin C on the intestinal histomorphology, phagocytic activity, and gene expression of the Nile tilapia. *Fish & Shellfish Immunology*, *482*, 96–102.

Ai, Q. H., Mai, K. S., Tan, B. P., Xu, W., Zhang, W. B., Ma, H. M., & Liufu, Z. G. (2006). Effects of dietary vitamin C on survival, growth, and immunity of large yellow croaker, *Pseudosciaena crocea*. *Aquaculture*, *261*, 327–336. https://doi.org/10.1016/j.aquaculture.2006.07.027

Ai, Q. H., Mai, K. S., Zhang, C. X., Xu, W., Duan, Q. Y., Tan, B. P., & Liufu, Z. G. (2004). Effects of dietary vitamin C on growth and immune response of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture*, *242*, 489–500. https://doi.org/10.1016/j.aquaculture.2004.08.016

AOAC (1995). *Official methods of analysis of AOAC international* (P. A. Cunniff Ed.), (Vol. *1*, 14th ed., p. 1102). Arlington, VA: Association of Official Analytical Chemists.

Barros, M. M., Falcon, D. R., Orsi, R. D., Pezzato, L. E., Fernandes, A. C., Guimaraes, I. G., … Sartori, M. M. P. (2014). Non-specific immune parameters and physiological response of Nile tilapia fed beta-glucan and vitamin C for different periods and submitted to stress and bacterial challenge. *Fish & Shellfish Immunology*, *39*, 188–195.

Biswas, B. K., Biswas, A., Junichi, I., Kim, Y. S., & Takii, K. (2013). The optimal dietary level of ascorbic acid for juvenile Pacific bluefin tuna, *Thunnus orientalis*. *Aquaculture International*, *21*, 327–336. https://doi.org/10.1007/s10499-012-9555-z

Caxico Vieira, C. A. S., Vieira, J. S., Bastos, M. S., Zancanela, V., Barbosa, L. T., Gasparino, E., & Del Vesco, A. P. (2017). Expression of genes related to antioxidant activity in Nile tilapia kept under salinity stress and fed diets containing different levels of vitamin C. *Journal of Toxicology and Environmental Health, Part A*, *81*, 20–30.

Chagas, E. C., & Val, A. L. (2003). Effect of vitamin C on weight and hematology of tambaqui. *Pesquisa Agropecuaria Brasileira*, *38*, 397–402.

Chai, X. J., Ji, W. X., Han, H., Dai, Y. X., & Wang, Y. (2013). Growth, feed utilization, body composition and swimming performance of giant croaker, *Nibea japonica* Temminck and Schlegel, fed at different dietary protein and lipid levels. *Aquaculture Nutrition*, *19*, 928–935.

Chakrabarti, R., Singh, M. K., Sharma, J. G., & Mittal, P. (2019). Dietary supplementation of vitamin C: An effective measure for protection against UV-B irradiation using fish as a model organism. *Photochemical & Photobiological Sciences*, *18*, 224–231. https://doi.org/10.1039/C8PP00481A

Chen, R. G., Lochmann, R., Goodwin, A., Praveen, K., Dabrowski, K., & Lee, K. J. (2003). Alternative complement activity and resistance to heat stress in golden shiners (*Notemigonus crysoleucas*) are increased by dietary vitamin C levels in excess of requirements for prevention of deficiency signs. *Journal of Nutrition*, *133*, 2281–2286. https://doi.org/10.1093/jn/133.7.2281

Chen, Y. J., Yuan, R. M., Liu, Y. J., Yang, H. J., Liang, G. Y., & Tian, L. X. (2015). Dietary vitamin C requirement and its effects on tissue antioxidant capacity of juvenile largemouth bass, *Micropterus salmoides*. *Aquaculture*, *435*, 431–436. https://doi.org/10.1016/j.aquaculture.2014.10.013

Cheng, C. H., Liang, H. Y., Guo, Z. X., Wang, A. L., & Ye, C. X. (2017). Effect of dietary vitamin C on growth performance, antioxidant status and innate immunity of juvenile Pufferfish (*Takifugu obscurus*). *Israeli Journal of Aquaculture-Bamidgeh*, *69*, 1434–1446.

Dabrowski, K. (1991). Administration of gulonolactone does not evoke ascorbic acid synthesis in teleost fish. *Fish Physiology & Biochemistry*, *9*, 215–221. https://doi.org/10.1007/BF02265142

Dabrowski, K. (1994). Primitive actimoterigian fishes can synthesize ascorbic acid. *Cellular and Molecular Life Sciences*, *50*, 745–748. https://doi.org/10.1007/BF01919376

Dabrowski, K., & Hinterleitner, S. (1989). Applications of a simultaneous assay of ascorbic acid, dehydroascorbic acid and ascorbic sulphate in biological materials. *Analyst*, *114*, 83–87. https://doi.org/10.1039/an9891400083

Daniel, N., Muralidhar, A. P., Srivastava, P. P., Jain, K. K., Pani Prasad, K., Manish, J., & Sivaramakrishnan, T. (2018). Dietary ascorbic acid requirement for growth of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) juveniles. *Aquaculture Nutrition*, *24*, 616–624.

Darias, M. J., Mazurais, D., Koumoundouros, G., Cahu, C. L., & Zambonino-Infante, J. L. (2011). Overview of vitamin D and C requirements in fish and their influence on the skeletal system. *Aquaculture*, *315*, 49–60. https://doi.org/10.1016/j.aquaculture.2010.12.030

Dawood, M. A. O., & Koshio, S. (2016). Vitamin C supplementation to optimize growth, health and stress resistance in aquatic animals. *Reviews in Aquaculture*, *10*, 334–350. https://doi.org/10.1111/raq.12163

Dawood, M. A. O., Koshio, S., El-Sabagh, M., Billah, M. M., Zaineldin, A. I., Zayed, M. M., & Omar, A. A. E. D. (2016). Changes in the growth, humoral and mucosal immune responses following β-glucan and vitamin C administration in red sea bream, *Pagrus major*. *Aquaculture*, *470*, 214–222. https://doi.org/10.1016/j.aquaculture.2016.12.036

Fontagne-Dicharry, S., Lataillade, E., Surget, A., Larroquet, L., Cluzeaud, M., & Kaushik, S. (2014). Antioxidant defense system is altered by dietary oxidized lipid in first-feeding rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *424*, 220–227. https://doi.org/10.1016/j.aquaculture.2014.01.009

Fracalossi, D. M., Allen, M. E., Yuyama, L. K., & Oftedal, O. T. (2001). Ascorbic acid biosynthesis in Amazonian fishes. *Aquaculture*, *192*, 321–332. https://doi.org/10.1016/S0044-8486(00)00455-5

Freeman, B. A., & Crapo, J. D. (1982). Biology of disease: Free radicals and tissue injury. *Laboratory Investigation; A Journal of Technical Methods and Pathology*, *47*, 412–426.

Gabaudan, J., & Verlhac, V. (2001). Critical review of the requirements of ascorbic acid in cold and cool water fishes (salmonids, percids, plecoglossids, and flatfishes). In K. Dabrowski (Ed.), *Ascorbic acid in aquatic animals: Status and perspectives* (pp. 33–48). Boca Raton, FL: CRC Press.

Gábor, B., László, B., Miklós, C., Ferenc, P., & József, M. (1997). Ascorbate metabolism and its regulation in animals. *Free Radical Biology and Medicine*, *23*, 793–803. https://doi.org/10.1016/S0891-5849(97)00062-2

Gao, J., Koshio, S., Ishikawa, M., Yokoyama, S., Nguyen, B. T., & Mamauag, R. E. (2013). Effect of dietary oxidized fish oil and vitamin C supplementation on growth performance and reduction of oxidative stress in Red seabream *Pagrus major*. *Aquaculture Nutrition*, *19*, 35–44.

Goede, R. W., & Barton, B. A. (1990). Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *American Fisheries Society Symposium*, *8*, 93–108.

Hsu, T. S., & Shiau, S. Y. (1998). Comparison of vitamin C requirement for maximum growth of grass shrimp, *Penaeus monodon*, with l-ascorbyl-2-monophosphate-Na and l-ascorbyl-2-monophosphate-Mg. *Aquaculture*, *163*, 203–213. https://doi.org/10.1016/S0044-8486(98)00230-0

Huang, F., Wu, F., Zhang, S., Jiang, M., Liu, W., Tian, J., … Wen, H. (2015). Dietary vitamin C requirement of juvenile Chinese sucker (*Myxocyprinus asiaticus*). *Aquaculture Research*, *48*, 37–46.

Huang, Y. S., Lin, Z. D., Rong, H., Hao, M. L., Zou, W. G., Li, S. K., & Wen, X. B. (2017). Cloning, tissue distribution, functional characterization and nutritional regulation of delta 6 fatty acyl desaturase in Chu's croaker, *Nibea coibor*. *Aquaculture*, *479*, 208–216.

Huang, Y. S., Wen, X. B., Li, S. K., Li, W. J., & Zhu, D. S. (2016a). Effects of dietary fish oil replacement with palm oil on the growth, feed utilization, biochemical composition, and antioxidant status of Juvenile Chu's croaker, *Nibea coibor*. *Journal of the World Aquaculture Society*, *47*, 786–797.

Huang, Y. S., Wen, X. B., Li, S. K., Li, W. J., & Zhu, D. S. (2016b). Effects of dietary lipid levels on growth, feed utilization, body composition, fatty acid profiles and antioxidant parameters of juvenile Chu's croaker, *Nibea coibor*. *Aquaculture International*, *24*, 1229–1245. https://doi.org/10.1007/s10499-016-9980-5

Kang, K. Y., Hong, Y. S., Park, S. H., & Ju, J. H. (2015). Increased serum alkaline phosphatase levels correlate with high disease activity and low bone mineral density in patients with axial spondyloarthritis. *Seminars in Arthritis and Rheumatism*, *45*, 202–207. https://doi.org/10.1016/j.semarthrit.2015.03.002

Khan, K. U., Zuberi, A., Nazir, S., Ullah, I., Jamil, Z., & Sarwar, H. (2017). Synergistic effects of dietary nano selenium and vitamin C on growth, feeding, and physiological parameters of mahseer fish (*Tor putitora*). *Aquaculture Reports*, *5*, 70–75. https://doi.org/10.1016/j.aqrep.2017.01.002

Kraus, V. B., Huebner, J. L., Stabler, T., Flahiff, C. M., Setton, L. A., Fink, C., … Clark, A. G. (2004). Ascorbic acid increases the severity of spontaneous knee osteoarthritis in a guinea pig model. *Arthritis and Rheumatism*, *50*, 1822–1831. https://doi.org/10.1002/art.20291

Lazado, C. C., Lund, I., Pedersen, P. B., & Nguyen, H. Q. (2015). Humoral and mucosal defense molecules rhythmically oscillate during a light-dark cycle in permit, *Trachinotus falcatus*. *Fish & Shellfish Immunology*, *47*, 902–912. https://doi.org/10.1016/j.fsi.2015.10.037

Lee, S. M., Jeon, I. G., & Lee, J. Y. (2002). Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). *Aquaculture*, *211*, 227–239. https://doi.org/10.1016/S0044-8486(01)00880-8

Lee, S. H., Oe, T., & Blair, I. A. (2001). Vitamin C induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science*, *292*, 2083–2086. https://doi.org/10.1126/science.1059501

Lim, C., & Lovell, R. T. (1978). Pathology of the vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). *Journal of Nutrition*, *108*, 1137–1141. https://doi.org/10.1093/jn/108.7.1137

Lin, M. F., & Shiau, S. Y. (2004). Requirements of vitamin C (L-ascorbyl-2-monophosphate-Mg and L-ascorbyl-2-monophosphate-Na) and its effects on immune responses of grouper, *Epinephelus malabaricus*. *Aquaculture Nutrition*, *10*, 327–333. https://doi.org/10.1111/j.1365-2095.2004.00307.x

Lin, M. F., & Shiau, S. Y. (2005). Dietary L-ascorbic acid affects growth, nonspecific immune responses and disease resistance in juvenile grouper, *Epinephelus malabaricus*. *Aquaculture*, *244*, 215–221. https://doi.org/10.1016/j.aquaculture.2004.10.026

Lin, S., Mai, K., & Tan, B. (2007). Effect of soybean meal replacement by rapeseed cottonseed compound on growth, body composition and immunity of Tilapia *Oreochromis niloticus* x *O. aureus*. *Oceanologia et Limnologia Sinica*, *38*, 168–173.

Lin, Z. D., Huang, Y. S., Zou, W. G., Rong, H., Hao, M. L., & Wen, X. B. (2018). Cloning, tissue distribution, functional characterization and nutritional regulation of a fatty acyl Elovl5 elongase in Chu's croaker, *Nibea coibor*. *Gene*, *659*, 11–21. https://doi.org/10.1016/j.gene.2018.03.046

Liu, Y., Chi, L., Feng, L., Jiang, J., Jiang, W. D., Hu, K., … Zhou, X. Q. (2010). Effects of graded levels of dietary vitamin C on the growth, digestive capacity and intestinal microflora of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Research*, *42*, 534–548. https://doi.org/10.1111/j.1365-2109.2010.02649.x

Menezes, G. C. D., Tavares-Dias, M., Ono, E. A., Andrade, J. I. A. D., Brasil, E. M., Roubach, R., … Affonso, E. G. (2006). The influence of dietary vitamin C and E supplementation on the physiological response of pirarucu, *Arapaima gigas* in net culture. *Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology*, *145*, 274–279. https://doi.org/10.1016/j.cbpa.2006.06.035

Ming, J. H., Xie, J., Xu, P., Ge, X. P., Liu, W. B., & Ye, J. Y. (2012). Effects of emodin and vitamin C on growth performance, biochemical parameters and two HSP70s mRNA expression of Wuchang bream (*Megalobrama amblycephala* Yih) under high temperature stress. *Fish & Shellfish Immunology*, *32*, 651–661. https://doi.org/10.1016/j.fsi.2012.01.008

Moreau, R., Dabrowski, K., & Sato, P. H. (1999). Renal L-gulono-1, 4-lactone oxidase activity as affected by dietary ascorbic acid in lake sturgeon, *Acipenser fulvescens*. *Aquaculture*, *180*, 359–372. https://doi.org/10.1016/S0044-8486(99)00211-2

Narra, M. R., Rajender, K., Reddy, R. R., Rao, J. V., & Begum, G. (2015). The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. *Chemosphere*, *132*, 172–178. https://doi.org/10.1016/j.chemosphere.2015.03.006

NRC (2011). *Nutrient requirements of fish and shrimp* (pp. 68–77). Washington, DC: National Research Council, National Academy Press.

Padayatty, S. J., & Levine, M. (2001). New insights into the physiology and pharmacology of vitamin C. *Canadian Medical Association Journal*, *164*, 353–355.

Roginsky, V. A., & Stegmann, H. B. (1994). Ascorbyl radical as natural indicator of oxidative stress: Quantitative regularities. *Free Radical Biology & Medicine*, *17*, 93. https://doi.org/10.1016/0891-5849(94)90107-4

Ruiz, M. A., Betancor, M. B., Robaina, L., Montero, D., Hernández-Cruz, C. M., Izquierdo, M. S., … Caballero, M. J. (2018). Dietary combination of vitamin E, C and K affects growth, antioxidant activity, and the incidence of systemic granulomatosis in meagre (*Argyrosomus regius*). *Aquaculture*, *498*, 606–620. https://doi.org/10.1016/j.aquaculture.2018.08.078

Sarmento, N. L. A. F., Martins, E. F. F., Costa, D. C., Mattioli, C. C., da Costa Julio, G. S., Figueiredo, L. G., … Luz, R. K. (2018). Reproductive efficiency and egg and larvae quality of Nile tilapia fed different levels of vitamin C. *Aquaculture*, *482*, 96–102. https://doi.org/10.1016/j.aquaculture.2017.08.035

Saurabh, S., & Sahoo, P. K. (2008). Lysozyme: An important defence molecule of fish innate immune system. *Aquaculture Research*, *39*, 223–239. https://doi.org/10.1111/j.1365-2109.2007.01883.x

Shahkar, E., Yun, H., Kim, D. J., Kim, S. K., Lee, B. I., & Bai, S. C. (2015). Effects of dietary vitamin C levels on tissue ascorbic acid concentration, hematology, non-specific immune response and gonad histology in broodstock Japanese eel, *Anguilla japonica*. *Aquaculture*, *438*, 115–121. https://doi.org/10.1016/j.aquaculture.2015.01.001

Shao, L. Y., Han, D., Yang, Y. X., Jin, J. Y., Liu, H. K., Zhu, X. M., & Xie, S. Q. (2018). Effects of dietary vitamin C on growth, gonad development and antioxidant ability of on-growing gibel carp (*Carassius auratus gibelio* var. *CAS III*). *Aquaculture Research*, *49*, 1242–1249.

Shao, Y. Q. (2006). The construction of pituitary gland cDNA library of *Nibea coibor*. *South China Fisheries Science*, *2*, 8–12.

Shen, Z., Huang, W., & Huang, P. (2004). The artificial propagation and breeding technique of *Nibea coibor*. *Aquaculture*, *25*, 6–7.

Shiau, S. Y., & Hsu, T. S. (1999). Quantification of vitamin C requirement for juvenile hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus*, with l-ascorbyl-2-monophosphate-Na and l-ascorbyl-2-monophosphate-Mg. *Aquaculture*, *175*, 317–326. https://doi.org/10.1016/S0044-8486(99)00103-9

Sies, H. (1997). Oxidative stress: Oxidants and antioxidants. *Experimental Physiology*, *82*, 291–295. https://doi.org/10.1113/expphysiol.1997.sp004024

Wahli, T., Verlhac, V., Girling, P., Gabaudan, J., & Aebischer, C. (2003). Influence of dietary vitamin C on the wound healing process in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *225*, 371–386. https://doi.org/10.1016/S0044-8486(03)00302-8

Wan, J. J., Ge, X. P., Liu, B., Xie, J., Cui, S. L., Zhou, M., … Chen, R. L. (2014). Effect of dietary vitamin C on non-specific immunity and mRNA expression of three heat shock proteins (HSPs) in juvenile *Megalobrama amblycephala* under pH stress. *Aquaculture*, *434*, 325–333. https://doi.org/10.1016/j.aquaculture.2014.08.043

Wang, G. J., Xie, J., Wu, R. Q., & Yu, D. G. (2006). Optimal supplementations of vitamin E, vitamin C, choline and inositol in feed of *Rachycentron canadum*. *Journal of Zhejiang Ocean University*, *25*, 10–144.

Wang, L. G., Chen, D. X., Lou, B., Zhan, W., Chen, R. Y., Liu, F., & Mao, G. M. (2017). The effects of dietary vitamin C on growth performance, serum enzymes activities and resistance to *Vibrio alginolyticus* challenge of yellow drum *Nibea albiflora*. *Aquaculture Research*, *48*, 4684–4695.

Wu, F., Wen, H., Jiang, M., Liu, W., Tian, J., Yang, C. G., & Huang, F. (2015). Effect of dietary vitamin C on growth performance, flesh quality and antioxidant function in genetically improved farmed tilapia. *Journal of Fishery Sciences of China*, *22*, 79–87.

Xiao, L. D., Mai, K. S., Ai, Q. H., Xu, W., Wang, X. J., Zhang, W. B., & Liufu, Z. G. (2010). Dietary ascorbic acid requirement of cobia, *Rachycentron canadum Linneaus*. *Aquaculture Nutrition*, *16*, 582–589. https://doi.org/10.1111/j.1365-2095.2009.00695.x

Xie, Z., & Niu, C. (2006). Dietary ascorbic acid requirement of juvenile ayu (*Plecoglossus altivelis*). *Aquaculture Nutrition*, *12*, 151–156. https://doi.org/10.1111/j.1365-2095.2006.00395.x

Xu, H. J., Jiang, W. D., Feng, L., Liu, Y., Wu, P., Jiang, J., … Zhou, X. Q. (2016). Dietary vitamin C deficiency depresses the growth, head kidney and spleen immunity and structural integrity by regulating NF-kappa B, TOR, Nrf2, apoptosis and MLCK signaling in young grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology*, *52*, 111–138.

Yan, F. J., Tian, X. L., Dong, S. L., Fang, Z. H., & Yang, G. (2014). Growth performance, immune response, and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus* fed a supplementary diet of the potential probiotic *Paracoccus marcusii* DB11. *Aquaculture*, *420*, 105–111. https://doi.org/10.1016/j.aquaculture.2013.10.045

Yan, J., Liang, X., Zhang, Y., Li, Y., Cao, X., & Gao, J. (2017). Cloning of three heat shock protein genes (HSP70, HSP90α and HSP90β) and their expressions in response to thermal stress in loach (*Misgurnus anguillicaudatus*) fed with different levels of vitamin C. *Fish & Shellfish Immunology*, *66*, 103–111. https://doi.org/10.1016/j.fsi.2017.05.023

Zhao, H. X., Cao, J. M., Tan, Y. G., Liang, H. O., Wu, J. K., & Yang, D. W. (2008). Study on vitamin C requirement of juvenile cobia (*Rachycentron canadum* L.). *Chinese Journal of Animal Nutrition*, *20*, 435–441.

Zhou, Q. C., Wang, L. G., Wang, H. L., Xie, F. J., & Wang, T. (2012). Effect of dietary vitamin C on the growth performance and innate immunity of juvenile cobia (*Rachycentron canadum*). *Fish & Shellfish Immunology*, *32*, 969–975. https://doi.org/10.1016/j.fsi.2012.01.024

How to cite this article: Zou W, Lin Z, Huang Y, et al. Effect of dietary vitamin C on growth performance, body composition and biochemical parameters of juvenile Chu's croaker (*Nibea coibor*). *Aquacult Nutr.* 2019;00:1–14. https://doi.org/10.1111/anu.12967