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Effects of dietary citric acid on growth performance, mineral status and intestinal digestive enzyme activities of large yellow croaker *Larimichthys crocea* (Richardson, 1846) fed high plant protein diets

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ABSTRACT

The positive effects of citric acid (CA) on aquaculture species have been reported. However, extensive application of CA needs a comprehensive understanding of its nutritional functions. A 9-week feeding trial was conducted to determine the effect of dietary CA on growth performance, tissue mineral content, intestinal enzyme activities and oxidative status of large yellow croaker *Larimichthys crocea* fed high plant protein diets. Six isonitrogenous and isolipidic diets were formulated and fed to triplicated groups of fish. A high fish meal diet formulated with 45% fish meal and 11.5% soybean meal was set as the positive control diet, while a high plant protein diet formulated with 31.50% fish meal and 30.63% soybean meal was used as the negative control diet. The other four diets were supplemented with 0.4%, 0.8%, 1.6% and 3.0% of CA into the negative control diet, respectively. The results showed that the specific growth rate, feed efficiency, protein and phosphorus retention, phosphorus and zinc concentrations in whole body and intestine, activities of the leucine-aminopeptidase, alkaline phosphatase and Na^+, K^+-ATPase were significantly reduced after soybean meal replacement and recovered by dietary CA supplementation ($P < 0.05$). Data on oxidative stress and anti-oxidative responses of intestine showed that the content of malondialdehyde was significantly increased in soybean meal-enhanced diets, which decreased with supplementation of CA varying from 0.4% to 1.6% ($P < 0.05$). The total anti-oxidative capacity, activities of total superoxide dismutase and Cu–Zn superoxide dismutase were decreased by soybean meal replacement and increased as CA level increasing from 0.4% to 0.8% ($P < 0.05$). In conclusion, 0.8–1.6% of CA in diet is helpful for large yellow croaker fed high plant protein diets to get better growth performance. The improvement of growth performance could be partly due to the increased mineral bioavailability, enhanced intestinal antioxidant capacity and recovered intestinal function by dietary CA supplementation.
Statement of relevance: This study is not a test of commercial aquaculture.

1. Introduction

Large yellow croaker *Larimichthys crocea* is one of the most successful marine fish culture operations in terms of the number of juveniles produced and commercial size fish production annually in China (Liu et al., 2008). The production of this farmed species was more than 120,000 metric tons in 2014 (China Fishery Statistical Yearbook, 2015). However, chopped or minced trash fish is still the major diet for large yellow croaker, which brought about resource waste and water pollution. Increased demands and limited supply led to the expensiveness of fish meal (Hardy, 2010), which caused higher cost and less market share of formulated feed compared with trash fish. Therefore, replacing some of the fish meal by plant protein ingredients with lower cost could be a feasible way for sustainability of large yellow croaker culture.

Suppressed growth performance has been reported in various carnivorous fish species fed feed containing higher plant protein ingredients (Kaushik et al., 1995; Burrells et al., 1999), including large yellow croaker (Zhang et al., 2008a). The reduced growth performance is partially explained by the presence of anti-nutritional factors in plant protein sources (Francis et al., 2001). Moreover, differences of the nutritional composition and nutritional availability between plant protein ingredients and fish meal, including sulfur amino acid, hydroxyproline, phosphorus (P) and zinc (Zn) (Gatlin and Wilson, 1984; Savolainen and Gatlin, 2010; Vandenberg et al., 2011; Liu et al., 2014), also result in growth reduction. The adverse effects of fish meal replacement on digestive capacity, intestinal morphology and oxidative status have been widely published as well (Burrells et al., 1999; Krogdahl et al., 2003; Dong et al., 2013). Dietary organic acids have been shown to engender positive effects, such as enhanced growth (Pandey and Satoh, 2008;

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Goosen et al., 2011; Zhu et al., 2015), increased dietary nutrition availability and elevated activities of digestive enzymes (Vielma et al., 1999; Sarker et al., 2007; Castillo et al., 2014). In this context, organic acids may be helpful in overcoming some problems brought by fish meal replacement.

Citric acid (CA), as one kind of organic acid, has been reported to increase the availability of dietary minerals in aquaculture species when used as feed additive (Sugiura et al., 1998; Sarker et al., 2005), in some cases, supplementation of CA could trigger beneficial effects on growth performance (Sarker et al., 2007; Castillo et al., 2014). Based on an analysis of published data, the growth-promoting effect of CA is partly due to the gastrointestinal acidification and antimicrobial effects in pig (Partanen and Mroz, 1999). It is generally considered that lower gastric pH induced by CA result in increased activity of digestive enzymes, which lead to higher nutrients availability (Castillo et al., 2014; Márquez et al., 2012). In addition, the chelation and complex formation of the minerals can be affected by CA, resulting in increased bioavailability of dietary minerals (Khajepour and Hosseini, 2012a; Zhu et al., 2015). Many studies on effects of CA on growth, immune responses and mineral utilization in aquatic animals had been published (e.g., Baruah et al., 2005; Baruah et al., 2007; Ng and Koh, 2011). However, successful application of CA in aquafeeds requires further understanding of its mode of action.

Minerals are essential for growth and health in fish. Some kinds of mineral serve as a cofactor or activator for enzyme systems (NRC, 2011), such as Mn superoxide dismutase (Mn SOD) and Cu–Zn SOD, which are both capable of ameliorating oxidative stress and show positive correlation with mineral status respectively (De Rosa et al., 1980; Okado-Matsumoto and Fridovich, 2001; Ma et al., 2014; Zhang et al., 2016). Considering that replacement of fish meal could suppress growth through dysfunction of intestinal digestion and absorption which was in part attributed to oxidative damage (Zhang et al., 2013), alleviated intestinal oxidative damage brought by increased mineral status may be a mode of action of CA for promoting growth. However, the effects of CA on intestinal health and function have not been investigated in detail.

Thus, the purpose of the present study was to investigate the effect of CA supplementation in diet on mineral status, intestinal oxidative status and intestinal absorption in large yellow croaker fed high plant protein diet. The study would contribute to dietary fish meal replacement also.

Table 1
Composition and proximate analysis of the experimental diets (% dry matter).

Ingredient	FM	SBM	0.4% CA	0.8% CA	1.6% CA	3.0% CA
Fish meal[a]	45.0	31.5	31.5	31.5	31.5	31.5
Soybean meal[a]	11.50	30.63	30.63	30.63	30.63	30.63
Wheat meal[a]	24.0	24.0	24.0	24.0	24.0	24.0
Fish oil[a]	2.7	3.7	3.7	3.7	3.7	3.7
Lecithin[a]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[b]	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix[c]	2.0	2.0	2.0	2.0	2.0	2.0
Attractant[d]	0.3	0.3	0.3	0.3	0.3	0.3
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Mold inhibitor[e]	0.1	0.1	0.1	0.1	0.1	0.1
Citric acid (CA)	0.0	0.0	0.4	0.8	1.6	3.0
Microcrystalline cellulose	9.85	3.22	2.82	2.42	1.62	0.22
Proximate composition						
Moisture (%)	6.5	6.6	6.7	6.7	6.7	6.7
Crude protein (%)	47.5	47.9	47.6	47.6	47.5	47.7
Crude lipid (%)	8.8	8.5	8.4	8.8	8.9	9.0
Ash (%)	9.1	8.2	8.2	8.4	8.3	8.3
P (g/kg diet)[f]	10.0	9.0	9.5	8.9	10.1	10.1
Available P (g/kg diet)[g]	7.7	6.2	6.2	6.2	6.2	6.2
Mn (mg/kg diet)	37.1	38.4	39.1	38.9	39.9	40.6
Cu (mg/kg diet)	13.2	14.2	11.6	14.3	12.8	12.0
Zn (mg/kg diet)	102.1	101.2	97.6	103.1	104.0	110.3
pH	6.24	6.26	6.02	5.85	5.53	5.22

[a] Those ingredients were supplied by Qingdao Great-Seven Bio-Tech, Co., Ltd. Shandong Province, China.
[b] Vitamin premix (mg/kg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine·HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; 50% alpha-tocopheryl acetate, 240 mg; 35% ascorbic acid polyphosphate, 2000 mg; choline chloride, 2500 mg; ethoxyquin, 150 mg; microcrystalline cellulose, 13.892 g. All those ingredients were supplied by Qingdao Master Bio-Tech, Co., Ltd. Shandong Province, China.
[c] Mineral premix (mg/kg or g/kg diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; Ca $(H_2PO_4)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; microcrystalline cellulose, 15.447 g. All those ingredients were supplied by Qingdao Master Bio-Tech, Co., Ltd. Shandong Province, China.
[d] Attractant: contained 50% glycine and 50% betaine. All those ingredients were supplied by Qingdao Master Bio-Tech, Co., Ltd. Shandong Province, China.
[e] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid. All those ingredients were supplied by Qingdao Master Bio-Tech, Co., Ltd. Shandong Province, China.
[f] Analyzed data.
[g] Calculated data.

2. Materials and methods

2.1. Diet preparation

The formulation and proximate composition of the six experimental diets are shown in Table 1. Fish meal and soybean meal were used as the dietary protein sources. Fish oil and lecithin were used as the lipid sources. The positive control diet (FM) consisted of 45% fish meal and 11.5% soybean meal. The negative control diet (SBM) was formulated to have 31.50% of fish meal and 30.63% of soybean meal. Citric acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) with 99.5% purity was added into the SBM diet to formulate experimental diets with 4 graded levels of CA (0.4%, 0.8%, 1.6% and 3.0%, respectively). In addition, all six diets were supplemented with 0.05% of fumaric acid and 0.05% of calcium propionate as mold inhibitors. As shown in Table 1, the pH values of the experimental diets were decreased by the addition of CA.

2.2. Feeding trial

Large yellow croaker juveniles were obtained from Fufa hatchery (Ningde, Fujian Province, China). Juveniles were stocked in a large sea cage (4 × 4 × 4 m) to acclimate to the experimental condition and fed the FM diet for 2 weeks. Prior to the start of the feeding trial, the fish were not fed for 24 h, and then weighed after being anesthetized with eugenol (1:10,000) (Sinopharm Chemical Reagent Co., Ltd., SCR, Shanghai, China). Fish with similar size (initial mean weight 7.71 ± 0.02 g) were distributed to 18 sea cages (1.5 × 1.5 × 2.0 m) at density of 70 fish per cage. Each diet was hand-fed to triplicate groups of fish twice daily (05:00 and 17:00) to apparent satiation for 9 weeks. During the feeding trial, the water temperature ranged from 27.4 to 33.2 °C, salinity varied between 30 and 33‰ and dissolved oxygen was higher than 7 mg/l. Fish were reared under natural light conditions (about 14 L:10D) throughout the feeding trial. And the total amount of feed offered to each replicate (cage) was 1.2 kg during the feeding trial.

2.3. Sample collection and analysis

At the beginning of the feeding trial, twenty fish were sampled and stored frozen (−20 °C) for the analysis of the initial whole-body composition. At the end of the feeding trial, animals were not fed for 24 h and anesthetized with eugenol. They were counted and weighted to calculate the survival rate, specific growth rate (SGR) and feed efficiency (FE). Five fish were randomly selected from each cage to measure the weight and length individually and stored frozen (−20 °C) to determine the whole-body proximate composition and mineral composition. Another five fish were randomly selected from each cage and dissected to obtain intestine for intestine somatic index calculation, meanwhile, intestines were stored frozen (−20 °C) for mineral analysis. Nine fish