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Effects of dietary chlorogenic acid on growth performance, antioxidant capacity of white shrimp *Litopenaeus vannamei* under normal condition and combined stress of low-salinity and nitrite

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ABSTRACT

An eight-week feeding trial followed by an acute combined stress test of low-salinity and nitrite were performed to evaluate effects of chlorogenic acid (CGA) on growth performance and antioxidant capacity of white shrimp *Litopenaeus vannamei*. Shrimp were randomly allocated in 12 tanks (30 shrimp per tank) and triplicate tanks were fed with a control diet or diets containing different levels of CGA (100, 200 and 400 mg kg^{-1} feed) as treatment groups. Growth performance including weight gain (WG), biomass gain (BG), feed conversion ratio (FCR), and feed intake were determined after feeding for 56 days. Antioxidant capacity were evaluated by determining the activity of total antioxidant status (TAS), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) as well as the gene expression of GSH-Px and CAT in the hepatopancreas of shrimp at the end of feeding trial and again at the end of the combined stress test. The results indicated that supplemention of CGA had no significant effects on the growth performance and the activities of TAS, SOD, GSH-Px and CAT in hepatopancreas of shrimp cultured under normal conditions for 56 days. However, compared with the control group, CGA (200, 400 mg kg^{-1} feed) significantly improved the resistance of *L. vannamei* against the combined stress of low-salinity and nitrite, as indicated by the significant ($P < 0.05$) higher survival, higher activities of TAS, GSH-Px and CAT, as well as higher transcript levels of *GPx* and *CAT* gene in shrimp treated with CGA in the combined tress test. Our findings suggested that CGA possessed dual-modulatory effects on antioxidant capacity of *L. vannamei* and could be a potential feed additive that can enhance shrimp resistance against environmental stresses. The recommended application dosage is 200 mg kg^{-1} and further studies are needed to clarify the action model of CGA efficiency.

1. Introduction

Litopenaeus vannamei is one of the most important economic shrimp species worldwide, especially in the Southeast Asian region [1]. In recent years, the frequent outbreak of diseases has led to sharp drop of *L. vannamei* production and huge economic losses [2]. Besides the direct infection by virus and bacterial pathogens [3,4], environmental stress is also considered a key inducing factor for various diseases [5–7]. For example, salinity and nitrite stress weaked the shrimp immune responses and increased the susceptibility of *L. vannamei to Vibrio alginolyticus* infection [8–10]. The changes of environmental factors such as nitrite and salinity triggered the generation of reactive oxygen species (ROS) [11,12]. A certain concentration of ROS is essential for host defense against microbial infection; however, over production of ROS and the residual ROS can also result in severely cellular damage. To maintain the balance of ROS in organisms, the animal employs the

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antioxidant system to remove the excess ROS and protects cells against the deleterious effects of endogenous ROS [13,14]. The antioxidant system includes non-enzyme small molecules (i.e, ascorbic acid, glutathione and vitamin E) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). SOD scavenges the superoxide anions by converting them to hydrogen peroxide and oxygen. Then hydrogen peroxide is transformed to water and oxygen via CAT or GSH-Px [15]. Therefore, improvement of antioxidant capacity is crucial for aquatic animals to overcome the environmental stresses and sustain aquatic animal production.

Unlike vertebrates, the cultured shrimp are generally exposed to an environment that is easily affected by physicochemical changes. Among these physicochemical changes, salinity has been shown to have great impact on the metabolism, growth, molting and survival of farmed shrimp [16,17]. Although this species is generally believed to be able to tolerate a wide range of salinity from 2‰ to 40‰ [18], much attention has been focused on "oxidative stress" caused by salinity on *L. vannamei*. Liu et al. found that acute salinity changes (from 35‰ to 5‰ and 50‰) significantly decreased the enzymatic activity of SOD, GPX, but showed no effects on CAT activity; and dietary supplementation of vitamin E enhanced the white shrimp growth performance under acute salinity stress [19]. Changes of salinity also affect nitrate toxicity which increases with the decrease of salinity [20]. Nitrite is an intermediate product of nitrification of ammonia or denitrification of nitrate by microorganisms. It has become a serious problem in the aquaculture system as it can be easily accumulated in an intensive culture system and reaches a level of 20 mg L^{-1} [21,22]. For shrimp and prawn farming, nitrite stress has been documented to reduce the growth, molting and survival as well as to decrease the antioxidant enzymes activities and immunity [23–25]. However, most of these studies on salinity and nitrite stress were only towards the effects of single environmental factor, in reality aquatic animals are always exposed to a variety of environmental stressors. As a result, it remains to be investigated as how *L. vannamei* responds to a combined stress of low-salinity and nitrite.

Previous studies suggested that increased antioxidant enzymes induced by dietary exogenous antioxidants such as selenium, astaxanth protected aquatic animals against environmental stresses [26,27]. Here, it is hypothesized that chlorogenic acid (CGA) possesses a similar function like other antioxidants. As one of the most abundant polyphenols isolated from natural plants such as coffee, apples and beans, CGA is also well known for its antioxidant activity, anti-inflammatory and anti-cancer effects [28–31]. *In vitro* studies have indicated that CGA exhibited better or equal efficacy in scavenging superoxide radical compared with vitamin C, E [32,33]. Wen et al. reported that dietary supplementation of CGA (200, 400 mg kg^{-1} feed) improved the antioxidant enzyme activity of turtle *Trionyx sinensis* [34]. A separate study demonstrated that dietary CGA at a dosage of 200, 400 mg kg^{-1} in feed enhanced non-specific immunity, serum antioxidant capacity and growth performance of *Cyprinus carpio var. Jian* [35]. Taken together, CGA is effective in improving the antioxidant functions of these tested animals under regular conditions. However, it remains unknown whether dietary CGA has any positive effects on the growth performance, antioxidant capacity of *L. vannamei* under normal conditions, and especially under the combined stress of salinity and nitrite.

Therefore, the present study is designed to investigate the effects of CGA 1) on the growth performance and feed efficiency of *L. vannamei*; 2) on antioxidant capacity of *L. vannamei* including the activity of TAS, SOD, CAT and GSH-Px as well as gene expression of above mentioned antioxidant enzymes when shrimp were cultured under normal conditions and under combined stress of low-salinity and nitrite.

2. Materials and methods

2.1. Diet preparation

CGA (CAS 3878) was purchased from Sigma–Aldrich (St. Louis, USA). The formulation and composition of the experimental diets are shown in Table 1. Four diets (control, CGA-100, CGA-200, CGA-400) containing 0, 100, 200, and 400 mg CGA kg^{-1} feed were prepared. The method of diet preparation was similar to that described by Niu et al. [36]. All ingredients were ground in a laboratory grinder and sifted out using a 60 μm sieve. The dry ingredients for each experimental diet were weighed, combined and thoroughly mixed to homogeneity in a Hobart-type mixer. Oil was then added and thoroughly mixed for 5 min. Deionized water (30% weight of dry ingredient mixture) was added and mixed to achieve homogeneity for pelleting. The wet mixture was pelleted in a monoscrew pelleter (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) through a 1.5 mm die, at a temperature of 70–80 °C. The pellets were then dried at 25 °C with the aid of an air conditioner and electrical fan. All the diets as described were stored at −20 °C until use. Moisture, crude protein, crude lipid and ash of the experimental diets were determined using a standard methods [37].

2.2. Shrimp and culture conditions

Shrimp were purchased from a local hatchery and reared in a semi-intensive culture pond at Shenzhen Base, South China Sea Fisheries Research Institute of Chinese Academy of Fishery Sciences

Table 1
Formulation and proximate composition of experimental diets.

Group	Control	CGA-100	CGA-200	CGA-400
Ingredients (% dry matter)				
White fish meal[a]	25	25	25	25
Soybean meal[b]	18	18	18	18
Peanut bran[b]	16.4	16.4	16.4	16.4
Wheat flour[b]	23	22.99	22.98	22.96
Beer yeast[b]	5	5	5	5
Krill meal[b]	5	5	5	5
Soybean lecithin[c]	1	1	1	1
Fish oil[d]	1	1	1	1
Soybean oil[b]	1	1	1	1
Choline chloride (50%)	0.5	0.5	0.5	0.5
$Ca(H_2PO_4)_2$	1	1	1	1
V_C-phosphate ester[e]	0.1	0.1	0.1	0.1
Vitamin premix[f]	1	1	1	1
Mineral premix[g]	1	1	1	1
Chlorogenic acid[h]	0	0.01	0.02	0.04
Sodium alginate	1	1	1	1
Total	100	100	100	100
Nutrient value (% dry weight)[i]				
Crude protein	39.74	39.74	39.64	39.7
Lipid	7.31	7.38	7.30	7.40
Ash	11.21	11.17	11.25	11.33
Moisture	9.65	9.8	9.75	9.61

[a] Imported from N.E.L.T.O. Australia Pty Ltd.
[b] Zhuhai Shihai Feed Corporation Ltd., Zhuhai, China.
[c] Kemin Industries (Zhuhai) Ltd., Zhuhai, China.
[d] Imported from New Zealand (Bakels Edible Oils Ltd, Mt Macnganui).
[e] Guangzhou Chengyi Company Ltd., Guangzhou, China.
[f] Vitamin premix (g kg^{-1}): retinyl acetate, 2.5; cholecalciferol, 6.25; all-rac-a-tocopheryl acetate, 75; menadione, 2.5; thiamin, 0.25; riboflavin, 1; D-calcium pantothenate, 5; pyridoxine HCl, 0.75; cyanocobalamin, 2.5; niacin, 2.5; folic acid 0.25; biotin 2.5; meso-inositol, 379; cellulose, 500 (Niu et al., 2008).
[g] Mineral premix (g kg^{-1}): KCl, 90; KI, 0.04; NaCl, 40; $CuSO_4$–$5H_2O$, 3; $ZnSO_4$–$7H_2O$, 4; $CoSO_4$–$7H_2O$, 0.02; $FeSO_4$–$7H_2O$, 20; $MnSO_4$–H_2O, 3; $MgSO_4$–$7H_2O$, 124; $CaHPO_4$–$2H_2O$, 500; $CaCO_3$, 215 (Cahu et al., 1999).
[h] Chlorogenic acid was purchased from Sigma–Aldrich (St. Louis, USA).
[i] Measured values.