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Dietary chromium polynicotinate enhanced growth performance, feed utilization, and resistance to *Cryptocaryon irritans* in juvenile large yellow croaker (*Larmichthys crocea*)

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ABSTRACT

A feeding trial was conducted to determine the effects of dietary chromium polynicotinate (Cr-Nic) on growth, feed utilization, and resistance to *Cryptocaryon irritans* in juvenile large yellow croaker (*Larimichthys crocea*). Six diets containing 42% crude protein and graded levels of Cr-Nic (0, 5, 10, 20, 40 and 80 mg kg^{-1} diet) were fed to croaker juveniles initially averaging 8.74 ± 0.48 g for 10 weeks. Another diet containing 45% crude protein without Cr-Nic supplementation was also fed and served as a control. Wheat starch was used as the carbohydrate source for all of the experimental diets. Fish fed the diet supplemented with 5 mg kg^{-1} Cr-Nic had significantly higher rates of survival, specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER), but lower feed intake than fish fed diets with 0 and 80 mg kg^{-1} Cr-Nic. Fish fed the diet containing 5 mg kg^{-1} Cr-Nic had significantly higher SGR and PER than fish fed the 45% crude protein control diet. Analysis of SGR, FE, and PER by second-order regression indicated that the optimal dietary level of Cr-Nic for juvenile large yellow croaker was estimated to be 6.70–7.10 mg kg^{-1} of diet. The 3-week cumulative mortality rate following natural infection of the parasite *C. irritans* was lowest in fish fed the diet containing 5 mg kg^{-1} Cr-Nic, which was significantly lower than in fish fed the diet without Cr-Nic addition. It is suggested that Cr-Nic supplementation protects against *C. irritans* infection prior to parasitic outbreak to alleviate mortality.

1. Introduction

Chromium (Cr) is an essential nutrient which can potentiate the action of insulin (Anderson and Mertz, 1977), is required to promote glucose tolerance, and plays key roles in carbohydrate, protein, and lipid metabolism (Jeejeebhoy et al., 1977). However, high dietary Cr has detrimental effects on growth and feed utilization (Shiau and Liang, 1995). Dietary Cr supplementation has also been demonstrated to exert some beneficial effects on both the nonspecific and specific immune systems of animals so as to alleviate the stress response (e.g. in pigs, Heugten and Spears, 1997; in cows and calves, Chang et al., 1995; Kegley et al., 1996; Mallard and Borgs, 1997). However, very little is known about the effects of dietary Cr on the immune response of fish (Gatta et al., 2001). At present, knowledge of the nutritional effects of dietary Cr in aquatic animals is relatively limited. The dietary requirement for chromium has only been demonstrated in a few species such as tilapia *Oreochromis niloticus* × *O. aureus* and channel catfish *Ictalurus punctatus* (Mehrim, 2012; Ng and Wilson, 1997; Pan et al., 2003; Shiau and Lin, 1993; Shiau and Shy, 1998). In addition, inorganic forms of chromium have generally been used in previous studies, rather than organic forms which have a higher bioactivity (NRC, 1997).

The large yellow croaker (*Larmichthys crocea*) is an important marine fish species that has been widely cultured in southeast China (Shen and Heino, 2014). Like most other carnivorous species, the large yellow croaker is unable to utilize dietary carbohydrate efficiently as an energy source. Since Cr is able to promote glucose tolerance and carbohydrate utilization, dietary Cr supplementation may allow for the partial substitution of dietary protein with carbohydrate. This has significant implications in the context of a globally decreasing supply of marine fish meals and oils. Several studies have investigated the nutritional requirements and immunological characteristics of the large yellow croaker (Ai et al., 2011; Li et al., 2013a, 2013b; Yu et al., 2012; Zhao et al., 2013; Zuo et al., 2013). However, no information is available on the nutritional value of Cr in this species. Moreover, due to the high-density culture of marine fish in floating sea cages, white spot disease caused by infections of the ciliate *Cryptocaryon irritans* can be problematic.

The purpose of this study was to determine if dietary chromium polynicotinate (Cr-Nic) supplementation using starch as the main carbohydrate source would affect the growth and feed utilization of the large yellow croaker. The preventative effect of Cr supplementation on susceptibility to *C. irritans* infection following a natural infection was

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also evaluated to determine whether mortality could be reduced through nutritional means.

2. Materials and methods

2.1. Diet preparation

The ingredient formulation and proximate composition of the basal diet were similar to that of Wang et al. (2010), which has been shown to be adequate for the large yellow croaker (Table 1). Wheat starch was used as the carbohydrate source. Seven diets were prepared to contain graded levels of Cr-Nic (Sigma, USA). Six diets containing 42% crude protein (CP) were supplemented with 0 (42% CP-control), 5, 10, 20, 40, and 80 mg kg^{-1} Cr-Nic. Another diet containing 45% CP and no Cr-Nic addition (45% CP-control) was designed for comparison with treatment diets supplemented with Cr-Nic and lower dietary protein. The aim was to assess the possibility of decreasing the dietary protein by Cr supplemental without negative impacts on growth in *L. crocea*. Diets were prepared and handled as previously described (Wang et al., 2010). The Cr content of the diets and fish samples was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Vista-MPX, Varian, USA). The Cr detected in the two control diets (Table 1) may be due to the presence of Cr in the raw feed ingredients (e.g. fishmeal usually contains Cr) or contamination from stainless-steel equipment used in the manufacture of the raw ingredients and/or during the diet preparation.

2.2. Experimental procedures

The experiment was conducted in Xiangshan Bay, Zhejiang Province, Southern China. Juvenile *L. crocea* were obtained from a local commercial hatchery. Upon arrival, they were acclimated to experimental conditions for 2 weeks in floating sea cages (3 m × 3 m × 3 m) and fed the 45% CP-control diet. At the end of the acclimation, the fish were fasted for 24 h, anesthetized with eugenol (1:10,000; Shanghai Reagent, China), and weighed. Fish of similar size (8.74 ± 0.48 g) were randomly distributed into 21 sea cages ($n = 60$ fish per cage: 1.0 m × 1.0 m × 1.5 m). The 21 cages were randomly divided into seven treatment groups ($n = 3$ cages per treatment). The fish were hand-fed to apparent satiation twice daily (04:30 and 16:30). Fish were considered satiated when they did not exhibit a feeding behavior towards the pellets. Daily consumption of feed was recorded for each cage and the feeding trial lasted 10 weeks. Water column characteristics were monitored weekly in the morning using a YSI model 556 (Yellow Spring Instrument Co. Inc., Yellow Spring, Ohio, USA). During the trial, water temperature ranged from 26.5 to 30.5 °C, the salinity ranged from 25 to 28 g l^{-1} and dissolved oxygen content was approximately 7 mg l^{-1}.

2.3. Natural infection by C. irritans

At 10 weeks into the feeding trial, a significant decrease in appetite and the appearance of visible white spots scattered on the body were observed in many of the experimental fish. Similar events were observed by many local farmers in the area at almost the same time. Experimental fish were confirmed to be infected with *C. irritans* according to morphological and molecular identification following the methods of Sun et al. (2006). Shortly after the clinical signs were observed, experimental fish in each cage were weighed and counted to determine the survival rate during the 10-week feeding experiment. At this time, 10 fish per cage were sampled. To determine the effect of Cr supplementation on the cumulative mortality and resistance to *C. irritans* over the next 3 weeks, 40 fish were left in each cage.

2.4. Analysis and measurement

The fish were fasted for 24 h at the end of the feeding trial prior to sampling. The total number and mean body weight of the fish in each cage were measured. Ten fish were randomly sampled from each cage for individual proximate composition analysis. Proximate composition analysis of feed ingredients, experimental diets, and fish was performed by the standard methods of Association of Official Analytical Chemists (AOAC, 1995). Samples of diets and fish were dried to a constant weight at 105 °C to determine moisture content. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method. Lipid levels were quantified by ether extraction using a Soxhlet apparatus. The level of ash was measured by combustion at 550 °C. The Cr content in the diet and fish samples was determined by ICP-OES (Vista-MPX, Varian, USA).

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

	Diet (Cr-Nic supplementation level, mg kg^{-1} diet)						
Formulation	0 (45% CP-control)	0 (42% CP-control)	5	10	20	40	80
Fishmeal[a]	40.00	36.00	36.00	36.00	36.00	36.00	36.00
Soybean meal[a]	19.00	17.00	17.00	17.00	17.00	17.00	17.00
Wheat starch	23.29	29.29	29.28	29.28	29.28	29.28	29.28
Premix[b]	13.80	13.80	13.80	13.80	13.80	13.80	13.80
Vitamin mixture[c]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mixture[d]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromium polynicotinate (mg kg^{-1})	0	0	5	10	20	40	80
Analyzed dietary Cr (mg kg^{-1})	0.94	0.50	5.07	9.12	20.12	37.68	78.18
Proximate composition (%)							
Dry matter	92.93	92.59	94.17	93.90	94.27	94.08	93.93
Crude protein	44.98	41.90	41.68	41.67	41.75	41.47	41.30
Crude lipid	12.23	11.46	11.14	11.32	11.70	11.28	11.82
Ash	10.63	9.79	10.75	10.90	11.72	11.97	12.27

[a] Fishmeal, obtained from Russia. AKROS Fishing Co., Ltd (Russia), crude protein, 69.70% dry matter, crude lipid 7.08% dry matter; soybean meal, obtained from Liulu Oli Lit. (Heilongjiang, China), crude protein 53.29% dry matter, crude lipid 1.93% dry matter.

[b] Premix contained (%): beer yeast, 4.0; lecithin, 2.5; fish oil, 5.0; soybean oil, 2.0; attractant (glycine and betaine), 0.2; mold inhibitor (contained 50% calcium propionic acid and 50% fumaric acid), 0.1.

[c] Vitamin mixture (mg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 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; a-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg, ethoxyquin, 150 mg, wheat middling 18.52 g.

[d] Mineral mixture (mg 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; $Ca(H_2PO_3)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; zoelite, 5.45 g;