Aquaculture 432 (2014) 321–326

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

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

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ARTICLE INFO

Article history:
Received 8 March 2014
Received in revised form 21 May 2014
Accepted 22 May 2014
Available online 2 June 2014

Keywords:
Feeding and nutrition
Starch
Chromium polynicotinate
Large yellow croaker
Larmichthys crocea

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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http://dx.doi.org/10.1016/j.aquaculture.2014.05.027
0044-8486/

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;

2.5. Calculations and statistical analysis

The following variables were calculated:

$$\text{Survival}(\%) = 100 \times N_t/N_0$$
$$\text{Specific growth rate}\left(\text{SGR}:\%\cdot\text{day}^{-1}\right) = 100 \times (\text{Ln}\,W_t - \text{Ln}\,W_0)/t$$
$$\text{Feed intake}\left(\text{FI, g 100 g}^{-1}\text{BW day}^{-1}\right) = D_f \times 100/((W_t + W_0)/2 \times t)$$
$$\text{Feed efficiency}(\text{FE}) = (W_t - W_0)/D_f$$
$$\text{Protein efficiency ratio}(\text{PER}) = (W_t - W_0)/\text{protein intake}$$

where BW is the wet body weight, W_t is the mean final body weight (g), W_0 is the mean initial body weight (g), and t is the experimental duration in d. N_0 and N_t represent initial and final numbers of fish in each cage, respectively. D_f is dry diet intake (g).

Results were analyzed by one-way analysis of variance (ANOVA). When the ANOVA identified differences among groups ($P < 0.05$), multiple comparisons among means were made with Duncan's multiple range test. Dietary chromium requirement for juvenile larger yellow croaker was estimated by the second-order polynomial regression, which is the better fitting model for growth and feed utilization data. The choice of estimated Cr requirement values was based on the higher coefficient of determination (R^2) and the smaller square error of regression. All tests were performed in Statistica 6.0 (Statsoft, Tulsa, USA).

3. Results

3.1. Survival, growth, and feed utilization

Survival rate was significantly ($P < 0.05$) higher in fish fed the diet supplemented with 5 mg kg^{-1} Cr-Nic (97.4%) than in fish fed the 42% CP-control diet (92.0%, lowest among all diets) and diets supplemented with ≥40 mg kg^{-1} Cr-Nic (Table 2).

Specific growth rate (SGR) was significantly ($P < 0.05$) higher in fish fed the diet supplemented with 5 mg kg^{-1} Cr-Nic than in fish fed any of the other diets. Feed intake (FI) was lowest for fish fed diets supplemented with 5 and 10 mg kg^{-1} Cr-Nic, followed by the 20 mg kg^{-1} Cr-Nic, 40 mg kg^{-1} Cr-Nic, and 45% CP-control diets. The highest FI was observed in fish fed the 42% CP-control diet and the diet supplemented with 80 mg kg^{-1} Cr-Nic. The differences in FI between the lowest and highest groups were significant ($P < 0.05$). Feed efficiency (FE) and protein efficiency ratio (PER) generally followed an inverse pattern with FI. Second-order polynomial regression analysis, based upon the observed SGR and FE, was used for estimation of the dietary requirement for Cr-Nic in *L. crocea* fed the starch-based diet (Fig. 1; dietary Cr-Nic concentrations were log transformed). The regression equations

Table 2
Survival and growth performance of large yellow croaker fed diets containing various concentrations of Cr-Nic for 10 weeks[a].

Dietary Cr-Nic (mg kg^{-1})	Survival	SGR[b]	FI[b]	FE[b]	PER[b]
0 (45% CP-control)	95.3^{ab}	1.98^{b}	1.59^{ab}	1.12^{ab}	2.50^{a}
0 (42% CP-control)	92.0^{c}	1.90^{b}	1.75^{a}	1.01^{a}	2.41^{a}
5	97.4^{a}	2.02^{a}	1.52^{b}	1.17^{b}	2.81^{b}
10	95.3^{ab}	1.93^{b}	1.53^{b}	1.18^{b}	2.83^{b}
20	94.8^{abc}	1.93^{b}	1.62^{ab}	1.11^{ab}	2.67^{ab}
40	92.9^{bc}	1.86^{b}	1.60^{ab}	1.11^{ab}	2.68^{ab}
80	92.8^{bc}	1.83^{b}	1.74^{a}	1.03^{a}	2.49^{a}
Pooled SEM	0.90	0.04	0.05	0.04	0.09
ANOVA					
F value	3.680	5.242	2.932	2.887	3.275
P value	0.021	0.005	0.046	0.048	0.000

[a] Data represent the mean of triplicate groups, with 60 fish per group. Values in the same row with the same letters are not significantly different ($P > 0.05$, Duncan's test); SEM: standard error of means.

[b] SGR: specific growth rate; FI: feed intake; FE: feed efficiency; PER: protein efficiency ratio.

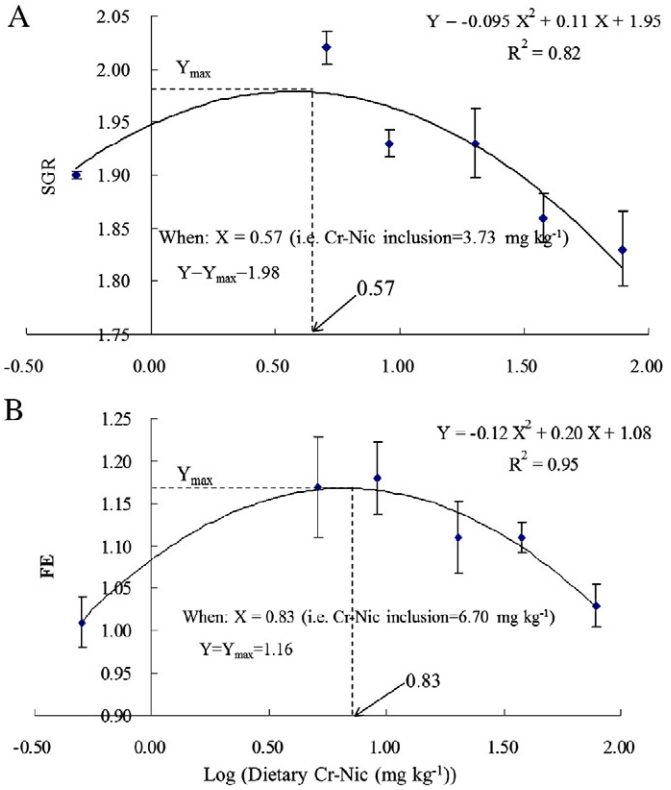

Fig. 1. The effect of dietary Cr-Nic on (A) specific growth rate (SGR) and (B) feed efficiency (FE) in juvenile large yellow croaker (*Larimichthys crocea*). Each point represents the mean (±SEM) of three groups of fish ($n = 3$), with 60 fish per group. The analyzed dietary Cr-Nic concentrations were log transformed for a better visualizing. Requirements derived with the linear regression method for SGR and FE were 3.73 and 6.70 mg kg^{-1} Cr-Nic, respectively.

obtained for SGR and FE were as follows: $Y = -0.095X^2 + 0.11X + 1.95$ ($R^2 = 0.82$) and $Y = -0.12X^2 + 0.20X + 1.08$ ($R^2 = 0.95$), respectively. Dietary requirements derived with the polynomial regression method for SGR and FE were 3.73 and 6.70 mg kg^{-1} Cr-Nic, respectively.

3.2. Body composition

Whole body protein and ash content were significantly different between dietary treatments (Table 3). Protein content was significantly ($P < 0.05$) higher in fish fed diets supplemented with 10, 20, and 40 mg kg^{-1} Cr-Nic compared with fish fed the other diets, except for fish fed 45% CP-control diet. The ash content was higher in fish fed

Table 3
Body composition (% wet weight) of large yellow croaker fed diets containing various concentrations of Cr-Nic for 10 weeks[*].

Dietary Cr-Nic (mg kg^{-1})	Moisture	Crude protein	Crude lipid	Ash
0 (45% CP-control)	74.3	15.1^{a}	7.1	3.7^{ab}
0 (42% CP-control)	76.8	13.4^{cd}	6.9	3.3^{c}
5	76.6	13.2^{d}	7.2	3.3^{c}
10	74.5	14.4^{b}	7.0	3.9^{a}
20	74.5	14.6^{b}	7.4	3.5^{bc}
40	74.6	14.8^{b}	7.4	4.0^{a}
80	75.4	13.8^{c}	7.7	3.5^{bc}
Pooled SEM	1.41	0.09	0.31	0.82
ANOVA				
F value	0.326	43.8	0.56	2.922
P value	0.921	0.000	0.75	0.020

[*] Data represent the mean of triplicate groups, with 60 fish per group. Values in the same row with the same letters are not significantly different ($P < 0.05$, Duncan's test); SEM: standard error of means.

diets supplemented with ≥10 mg kg^{-1} Cr-Nic compared to fish fed the 10 mg kg^{-1} Cr-Nic and 42% CP-control diets. Lipid content generally increased with increasing dietary Cr-Nic, although no statistically significant difference was detected. The dietary treatments did not affect moisture content in fish.

3.3. Cumulative mortality rate

The cumulative mortality following parasitic *C. irritans* infection was significantly affected by Cr-Nic supplementation (Fig. 2). The cumulative mortality (43.4–67.5%) was lowest for fish fed diets supplemented with 5 and 10 mg kg^{-1} Cr-Nic, intermediate in fish fed diets supplemented with 20 mg kg^{-1} Cr-Nic and the 42% CP-control group, and highest in fish fed the 40 mg kg^{-1} Cr-Nic, 80 mg kg^{-1} Cr-Nic, and the 45% CP-control diets. The difference among the lowest and highest groups was significant ($P < 0.05$).

4. Discussion

The present study clearly demonstrated that Cr-Nic supplementation markedly improved growth performance and probably enhanced resistance to *C. irritans* in *L. crocea* fed a high-starch diet. This was particularly evidenced by the improved performance of fish fed diets with a reduced dietary protein content and Cr-Nic inclusion, compared with fish fed the diet with a higher protein content (45%) without Cr-Nic supplementation. Significantly lower growth and survival of fish were observed in the 42% CP-control group compared to fish fed the diet supplemented with 5 mg kg^{-1} Cr-Nic. This result was somewhat consistent with the findings of Mertz and Roginski (1969), who reported that Cr deficiency resulted in growth retardation of rats fed low-protein diets, as the nutritional stress of a suboptimal protein supply increased Cr requirements. In addition, these authors demonstrated that supplemental Cr reduced mortality and morbidity of rats stressed by a low protein diet (Mertz and Roginski, 1969).

As the fish fed the diet supplemented with 5 mg kg^{-1} Cr-Nic had the lowest feed intake, their improved performance was mainly due to an improved FE and a significantly higher PER (Table 2). These results are in accordance with previous findings by Shiau and Shy (1998) in tilapia and Liu et al. (2010) in grass carp (*Ctenopharyngodon idellus*). Chromium can potentiate enzyme activities related to carbohydrate utilization (Shiau and Chen, 1993), enhance glucose tolerance, facilitate the glycolysis pathway, and eventually improve carbohydrate utilization (Anderson, 1981; Mertz et al., 1974; Sahin et al., 2001). Therefore, in the present study, Cr supplementation may have improved PER by increasing carbohydrate utilization and inhibiting gluconeogenesis from amino acids, thereby having a protein-sparing effect. Similar mechanisms have been reported in common carp (*Cyprinus carpio*) (Hertz et al., 1989) and in rats (Roginski and Mertz, 1969; Schroeder et al., 1965) in which Cr in combination with insulin enhanced the incorporation of amino acids into protein. This effect resulted in increased carbohydrate catabolism for energetic purposes, while protein or lipid was spared for deposition into tissues. The higher body protein and lipid content of *L. crocea* fed diets with Cr-Nic supplementation may reflect this general pattern of dietary ingredients utilization. Similarly, Shiau and Lin (1993) also observed a delayed plasma glucose plateau and significantly increased body lipid content in fish fed diets supplemented with Cr.

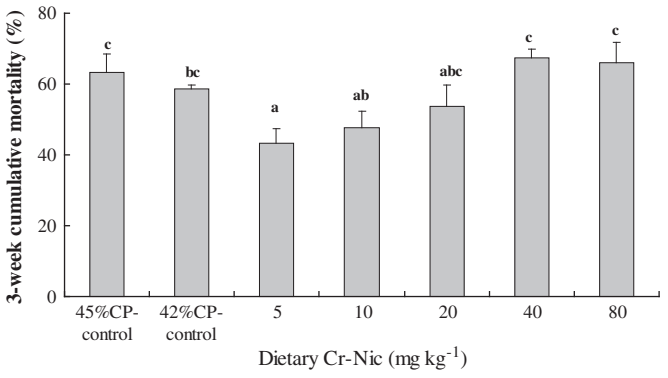

Fig. 2. Cumulative mortality following natural infection by *Cryptocaryon irritans* of large yellow croaker (*Larimichthys crocea*) fed diets containing various concentrations of Cr-Nic. Values (mean + SEM) that do not have the same letter are significantly different ($P < 0.05$) between treatments.

It is noteworthy that the efficiency of the Cr-mediated enhancement of carbohydrate utilization, and consequently the growth of fish, may be influenced by the source of the dietary carbohydrate. Schroeder et al. (1971) noted a difference in the effect of chromium on carbohydrate utilization when rats were fed diets containing sucrose or starch. In fish, chromium seems to be more efficient at improving the utilization of dietary glucose than starch. For example, Shiau and Lin (1993) demonstrated that $CrCl_3$ supplementation markedly improved the utilization of glucose, but not starch, in tilapia. Similarly, many other experiments did not find any significant effect of Cr on growth or feed utilization in fish fed starch-based diets (Pan et al., 2002; Shiau and Chen, 1993; Shiau and Liang, 1995). However, in the present study, wheat starch was used as the carbohydrate source and significantly higher growth rate and feed utilization were observed in *L. crocea* fed the diets supplemented with Cr-Nic (Table 2). This has important implications, since starch is a good binder and almost always the major source of carbohydrate in practical aquatic feeds (Hertrampf and Piedad-Pascal, 2000). The differences between the present experiment and previous studies may be due to the different forms of chromium employed (organic Cr-Nic versus inorganic Cr_2O_3, $CrCl_3$, Na_2CrO_4, or chromium picolinate). Studies in humans, pigs, rats, and poultry have demonstrated that the combination of Cr^{3+} with polynicotinate results in a much higher rate of absorption than inorganic Cr (David et al., 1999; Kornegay, 1996; Olin et al., 1994). Organic forms of Cr are generally accepted to have a higher bioavailability than inorganic forms (NRC, 1997). Also, species-specific differences in carbohydrate utilization (Rawles and Gatlin, 1998) and the dietary formulation may influence the effects of Cr. Our results demonstrate that there are evident benefits to the supplementation of Cr-Nic in the practical starch based diet of *L. crocea*.

It is noteworthy, however, that growth was depressed when Cr-Nic supplementation was equal to, or higher, than 40 mg kg^{-1}. Growth retardation and decreased feed utilization in fish fed diets supplemented with relatively high levels of Cr have been observed in tilapia (Liu et al., 2010; Shiau and Liang, 1995) and rainbow trout (Tacon and Beveridge, 1982). These decreases in growth and feed efficiency were probably due to a toxic effect of Cr that occurred at very high levels. Adverse effects of trivalent Cr in humans (Lança et al., 2002), rabbits (Tandon et al., 1979), and mice (Kamboj and Kar, 1964) have been observed. In fish, gill damage, increased mucus secretion, and increased blood lactate were observed in *Colisa fasciatus* following Cr exposure (Nath and Kumar, 1987). These findings may provide an explanation for the depressed growth performance of *L. crocea* fed diets supplemented with ≥40 mg kg^{-1} Cr-Nic in the present study.

Dietary Cr-Nic supplementation markedly decreased the mortality of large yellow croaker, indicating that the resistance to parasitic *C. irritans* infection was enhanced in juvenile *L. crocea*. This is the first time that dietary Cr-Nic supplementation has been demonstrated to improve parasite resistance in fish. This information is of particular economic importance for croaker mariculture as it will allow the prevention of heavy mortality by nutritional means prior to *C. irritans* outbreak. Supplementation with Cr may directly influence parasite resistance as it is an essential nutrient for animals to sustain normal metabolic and immune responses. An inadequate supply of Cr can affect an animal's immunity (Lindell et al., 1994; Mowat et al., 1993). Under normal circumstances, animals can acquire enough Cr through the diet.

However, disease stress increases urinary excretion of Cr which may exacerbate a marginal Cr deficiency (Borel et al., 1984; Mertz, 1992). Moreover, disease or nutritional stress often leads to elevated glucose metabolism, which results in increased mobilization and excretion of Cr in the urine (Anderson, 1990; Mertz and Roginski, 1969). Consequently, the Cr requirement of animals under stress is elevated by these combined effects (Chang and Mowat, 1992). Following infection with *C. irritans*, the experimental *L. crocea* may have suffered a Cr deficiency, which increased susceptibility to the infection. It is reasonable that the lower cumulative mortality of the croaker receiving low or moderate supplemental Cr was likely due to a compensatory effect on Cr deficiency. Modulation of the immune response by Cr may be achieved through enzyme activation, maintenance of the structure of nucleic acids, or alterations in protein synthesis (Anderson and Mertz, 1977; Anderson, 1987). Supplementation with Cr may also influence resistance to *C. irritans* in the croaker indirectly. Immunoglobulin production is regulated by specific enzymes that have a trace element at their core, with the most common being copper (Cu) and zinc (Zn) (Fielden and Rotilio, 1984). It is possible that Cr may be another element that is incorporated into certain enzymes that increase immunoglobulin synthesis. Also, Cr may influence Cu and/or Zn metabolism, thus indirectly affecting immunoglobulin production. Chang and Mowat (1992) demonstrated that Cr increased serum alkaline phosphatase in steers fed soybean meal, suggesting that Cr supplementation may improve Zn status. Schrauzer et al. (1986) found that Cr supplementation offered protection against stress-induced losses of several trace elements (Zn, Cu, Fe and Mn) in mice. Increased urinary excretion of Zn and Cu was observed in calves undergoing the stress of inoculation with infectious bovine rhinotracheitis (Nockels, 1990; Orr et al., 1990). The susceptibility to *C. irritans* infection in the croaker is possibly aggravated by the synergistic effects of deficiencies in several trace elements. Dietary supplementation with Cr may be helpful in counteracting such detrimental synergistic effects of trace element deficiency on the immune response. Further research is needed to investigate the mechanisms by which Cr improves the immunocompetence of croaker challenged with a *C. irritans* infection. Studies are also needed to determine whether Cr-Nic supplementation would provide protection against other important parasites.

In conclusion, the present study clearly demonstrated that growth and feed utilization efficiency of juvenile *L. crocea* fed a high wheat starch diet could be improved by Cr-Nic supplementation. Dietary supplementation with Cr-Nic allows for a reduction in dietary protein content of at least 3% without negative impacts on growth in *L. crocea*. Further studies are needed to investigate whether more drastic reductions in dietary protein could be achieved through the use of Cr supplementation in *L. crocea*. The dietary Cr-Nic concentration for optimal growth and feed utilization in juvenile *L. crocea* is estimated to be 3.7–6.7 mg kg^{-1} diet.

Acknowledgements

The study was supported by the National Natural Science Foundation of China (31302200), the National Department Public Benefit Research Foundation (Ministry of Agriculture, 201003020) and the Special Fund of Basic Research for Central Non-Profit Scientific Research Institute (2012TS24).

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