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Dietary Chromium Picolinate Supplementation Affects Growth, Whole-Body Composition, and Gene Expression Related to Glucose Metabolism and Lipogenesis in Juvenile Blunt Snout Bream, *Megalobrama amblycephala*

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Abstract
An 11-week feeding trial was carried out to investigate the effects of supplemented chromium picolinate (Cr-Pic) on the growth, whole-body composition, and relative mRNA expression related to lipogenesis and glucose metabolism in juvenile blunt snout bream. Seven isonitrogenous and isoenergetic diets with graded Cr supplementation levels were fed to triplicate groups. The final weight (FW), feed conversion ratio (FCR), and specific growth rate (SGR) were improved with increasing dietary Cr supplementation levels up to 0.4 mg/kg, and thereafter showed relatively constant. However, 12.0 mg/kg dietary Cr supplementation decreased growth and feed utilization. Based on SGR and FCR, the optimal dietary Cr supplementation level for the juvenile was estimated to be 0.28 mg/kg. Significantly higher plasma insulin levels were found in juvenile fed diets with 0.4 and 0.8 mg/kg Cr supplementation compared to those fed diet sans supplemented Cr. Plasma glucose levels decreased with increasing dietary Cr supplementation, and the lowest value was remarked in the group added 3.2 mg/kg of Cr. Adding 0.4–0.8 mg/kg Cr enhanced insulin receptor substrate 1 (IRS-1), phosphoinositide-3-kinase (PI3K), and pyruvate kinase (PK) and inhibited expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase), and glycogen synthase (GS) mRNA levels. High dietary Cr (12.0 mg/kg) supplementation resulted in high G6Pase and PEPCK expression. The highest content of whole-body lipid was remarked in fish fed with 0.4 mg/kg dietary Cr, which related to the enhanced gene expression related to lipogenesis; thereafter, mRNA levels showed a diminishing trend. These findings indicate that optimum dietary Cr-Pic supplementation has a positive effect on growth and blood glucose homeostasis by modifying the mRNA levels related to glucose metabolism and lipogenesis in juvenile blunt snout bream.

Keywords Blunt snout bream · *Megalobrama amblycephala* · Chromium picolinate · Glucose metabolism · Lipogenesis · Growth performance

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Introduction

Chromium III (Cr) has been accepted as an essential nutrient for animals for decades [1]. Although some studies show that Cr has not been an indispensable trace element for healthy mammals recently [2], supplemental Cr given to diabetics has increased the insulin sensitivity and interfered with general metabolism [3, 4]. In skeletal muscle cells of rat, chromium increased insulin and significantly enhanced the mRNA levels of the insulin receptor, glycogen synthase, and glucose transporter 4 [5]. Sundaram et al. [6] reported that Cr supplementation is beneficial for hyperglycemia correction by glucose metabolism modification in a diabetic rat. The Cr supplementation also can decrease proinflammatory cytokine blood

levels, oxidative stress, and lipid levels, and then diminishes the vascular inflammation risk in diabetes [7]. In different farmed livestock, the Cr also has been confirmed as a vital element involved in lipid and glucose metabolism and subsequently growth improvement [8]. The previous studies suggest the supplemental Cr form greatly influences Cr absorption and its effectiveness in animals [6]. Generally, the Cr bioavailability is higher in the organic forms compared with the inorganic forms. It is accepted that chromium picolinate (Cr-Pic) is a convenient form of Cr that is used more efficiently, slightly affected by nutritional and environmental conditions compared with other forms of Cr [9, 10].

The dietary carbohydrate utilization in fish is limited [11]. Precedent studies have provided evidence of the positive effect of chromium as a growth inducer in several fish species [12], such as in tilapia *Oreochromis niloticus* x *O. aureus* [13], grass carp *Ctenopharyngodon idellus* [14], common carp *Cyprinus carpio L.* [15], mirror carp *Cyprinus carpio L* [16], and yellow croaker *Larmichthys crocea* [17]. The supplementation of Cr in the diets significantly improved growth performance, immunity, and carbohydrate utilization. In contrast, no growth benefits have been remarked in channel catfish *Ictalurus punctatus* [18], gilthead seabream *Sparus aurata L.* [19], and rainbow trout *Oncorhynchus mykiss* [20] fed supplemented Cr in the diets. Although Liu et al. [14] stated that Cr supplementation could significantly improve the growth, plasma lipid, and carbohydrate profile at a level 0.8 mg/kg in grass carp fingerlings, the mechanism by which Cr effects growth, lipids, and glucose metabolism in fish is still unclear.

Blunt snout bream, *Megalobrama amblycephala*, is a mean reared freshwater fish species in China, and its production reached approximately 0.8 million tons in 2015 [21]. In our previous studies, we have investigated the effects of differences at dietary carbohydrate level [22] and kinds (wheat starch, corn starch, dextrin, maltose, glucose, and cellulose) [23] on growth performance, carbohydrate utilization, and immunity in this fish species, which indicated that high dietary carbohydrate level impaired growth performance and resulted in glucose metabolism disorder. This study was aimed to investigate the potential nutritional benefits of Cr-Pic supplementation in practical diets for juvenile blunt snout bream, and to reveal the mechanism by which Cr affects gene expression related to glucose metabolism and lipogenesis involving the insulin signaling pathway.

Materials and Methods

Diet Preparation

The experimental diets are shown in Table 1. Fish meal, canola meal, soybean meal, and cotton seed meal were used as protein sources. Soybean oil and lecithin were used as lipid sources. The basal diet was supplemented with 0, 0.2, 0.4, 0.8; 1.6, 3.2, and 12.0 mg/kg Cr in the form of Cr-Pic (Sigma-Aldrich (Shanghai) Trading Co., Ltd., Shanghai, China). The analyzed Cr concentrations of dry diet were 0.84, 1.13, 1.42, 1.94, 2.46, 4.24, and 12.6 mg/kg, respectively. Ingredients were crushed and crumbled into powder and passed via mesh sieve, afterwards entirely mixed with soybean oil, lecithin, and water for stiff dough production; the pelletizer (F-26 (II), South China University of Technology, China) was used to transfer this dough and produce pellets; the latter was dried in an aerated furnace overnight. After drying, the diets were broken up and sieved into 1.5 × 2.0 mm pellet size. All diets were sealed in bags and kept at − 10 °C until used.

Experimental Procedure

The present study was approved by the Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China). Juvenile blunt snout breams were caught from the Freshwater Fisheries Research Center (Wuxi, China). Fish were reared and acclimatized to the experimental conditions in floating cages (located in a 2000 m^3 culture pond) for 2 weeks and fed a commercial diet containing 6% lipid and 30% protein (Wuxi Tongwei feedstuffs Co., Ltd., Wuxi, China). Prior to the feeding trial, the fish were starved for 24 h, caught and weighed, and then randomly placed into floating cages (1 m × 1 m × 1 m) with a group of 20 fish in each cage. Each group was haphazardly allotted to triplicate cages. Fish were hand-fed three times a day at 8:00, 12:00, and 16:00 until apparent satisfaction based on the visual observation. During the 11 weeks of the feeding trial, the feed consumption and dead fish were weighed and recorded daily. The water temperature varied from 24 to 30 °C and dissolved oxygen was about 6 mg L^{-1} throughout the feeding trial, while the Cr level in the water was less than 6 μg/L.

Sample Collection and Chemical Analysis

At the experiment termination, the fish were harvested from the cages, anesthetized with 100 mg/L MS-222, and steadily weighed and counted after the last feeding time. Five fish were randomly taken up from each cage and separately weighed, and then blood sampling operation has been carried out from the caudal vein followed by the centrifugation process to separate the plasma. Liver and plasma samples were stored at − 80 °C for subsequent measurements of glycogen contents and relative genes mRNA levels. Meanwhile, five others from each cage were sampled and kept at similar temperature degree for body composition analysis.

Crude protein, lipid, dry matter, and ash contents in the diets and whole body were found out in accordance with the Association of Official Analytical Chemists (AOAC) methods [24]. The diets and whole body of dry matter was obtained after

Table 1 Ingredient and nutrient composition of experimental diets

Ingredients (% of dry basis)	Chromium added (mg/kg)						
	0	0.2	0.4	0.8	1.6	3.2	12.0
Fish meal	5	5	5	5	5	5	5
Soybean meal	24	24	24	24	24	24	24
Canola meal	17.6	17.6	17.6	17.6	17.6	17.6	17.6
Cotton seed meal	8	8	8	8	8	8	8
Soybean oil	2	2	2	2	2	2	2
Soybean lecithin	2	2	2	2	2	2	2
Vitamin and mineral premix †	1	1	1	1	1	1	1
Monocalcium phosphate	3	3	3	3	3	3	3
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Wheat meal	29.25	29.25	29.25	29.25	29.25	29.25	29.25
Rice bran	8	8	8	8	8	8	8
Chromium picolinate (mg/kg)	0	1.6	3.2	6.4	12.8	25.6	102.4
Proximate analysis by dry diet							
Crude protein (%)	30.2 ± 0.23	30.1 ± 0.18	29.8 ± 0.36	29.9 ± 0.38	29.9 ± 0.41	30.4 ± 0.23	29.3 ± 0.31
Crude lipid (%)	6.9 ± 0.11	6.9 ± 0.13	6.8 ± 0.11	6.9 ± 0.15	7.0 ± 0.12	7.0 ± 0.13	7.1 ± 0.16
Ash (%)	7.5 ± 0.15	7.5 ± 0.11	7.4 ± 0.16	7.5 ± 0.11	7.5 ± 0.12	7.5 ± 0.11	7.5 ± 0.16
Cr (mg/kg)	0.84 ± 0.05	1.13 ± 0.06	1.42 ± 0.04	1.94 ± 0.06	2.46 ± 0.08	4.24 ± 0.08	12.6 ± 0.12

† Vitamin and mineral premix (IU or mg/kg of diet): vitamin A, 25,000 IU; vitamin D3, 20,000 IU; vitamin E, 200 mg; vitamin K3, 20 mg; thiamin, 40 mg; riboflavin, 50 mg; calcium pantothenate, 100 mg; pyridoxine HCl, 40 mg; cyanocobalamin, 0.2 mg; biotin, 6 mg; folic acid, 20 mg; niacin, 200 mg; inositol, 1000 mg; vitamin C, 2000 mg; choline, 2000 mg; calcium biphosphate, 20 g; sodium chloride, 2.6; potassium chloride, 5 g; magnesium sulfate, 2 g; ferrous sulfate, 0.9 g; zinc sulfate, 0.06 g; cupric sulfate, 0.02; manganese sulfate, 0.03 g; sodium selenate, 0.02 g; cobalt chloride, 0.05 g; potassium iodide, 0.004; and zeolite was used as a carrier

drying in a furnace at 105 °C until constant weight. While crude protein was determined after acid digestion by the Kjeldahl method, crude percent lipid was determined by the Soxhlet method and ash was obtained after combustion during 5 h at 550 °C. The diet Cr content was analyzed in a professional laboratory (Jiangnan University, Wuxi, China) using the method of graphite furnace atomic absorption spectrometric. Glucose analyses, protein and triglyceride in the plasma were measured by an automatic biochemical analyzer (Mindray BS-400, Mindray International Ltd., Shenzhen, China) with these three methods, respectively; glucose oxidase, biuret and glycerine phosphate oxidase-peroxidase coupling (GPO-POD). Plasma insulin levels were determined using an automatic chemiluminescence analyzer (MAGLUMI 1000, Snibe Diagnostic Ltd., Shenzhen, China) according to the chemiluminescence method. The hepatic glycogen content was determined by a glycogen assay kit (JianCheng Bioengineering Institute, Nanjing, China).

A real-time PCR was used to determine levels of mRNA using PrimerScript TM RT reagent kit and analyze the relative gene expressions as mentioned in our prior study [25]. Concisely, an RNAiso plus kit (Takara, Dalian, China) was used for a total RNA extraction from the fish liver. After purification, quality and quantity were assessed and a PrimeScript TM RT reagent kit (Takara, Dalian, China) was used to synthesize a complementary DNA. Specific primers (Table 2) were designed by the *M. amblycephala* transcriptome analysis [26] according to the partial cDNA sequences of the related genes. The levels of relative gene expression were normalized to the β-actin expression level. The method of $2^{-\Delta\Delta CT}$ has been used to analyze the expression findings after confirmation that the amplification efficiency primers is nearly 100% [27].

Statistical Analysis

After assumptions' evaluation of normality, outliers, and variances equality, the software SPSS 13.0 for Windows was used to subject them to one-way ANOVA, where the significant difference between treatments was evaluated by Turkey's multiple comparison tests. Meanwhile, the estimation of the ideal dietary Cr supplementation level in the practical diet has been carried out by the linear-broken-line regression analysis [28, 29] according to FCR and SGR after the comparison of the estimated coefficient (R^2) using the second-order polynomial regression model.

Table 2 Primer sequences for qRT-PCR analysis

Primer	Sequence information	
	Forward primer (5′-3′)	Reverse primer (5′-3′)
β-actin	TCGTCCACCGCAAATGCTTCTA	CCGTCACCTTCACCGTTCCAGT
IRS-1	AACCTGGTTGGCATCTACCG	ATCAGCTGGAGCACGATAGC
PI3K	GGCGTAACATCCAGCTTTGC	GCTCCTGGAAGCTGGGTAAC
PK	CGAGATTGAGAACGGAGGCA	GTCCTTCTCAGACACTGCGG
PEPCK	TCGCCTGGATGAAGTTCGAC	GTCTTGGTGGAGGTTCCTGG
G6Pase	TTCAGTGTCACGCTGTTCCT	TCTGGACTGACGCACCATTT
GS	TTACACGGTCATTGCGTCCA	GACACAGCTCAGTCGGTGAA
SREBP1	ACAACAGTAGCGACACCCTG	AGGAGCGGTAGCGTTTTTCA
FAS	GTTTGCCAACCGCTTGTCTT	GGCCATGGCGAATAGCATTG

IRS-1 insulin receptor substrate 1, *PI3K* phosphatidylinositol-3–kinases, *PK* pyruvate kinase, *PEPCK* phosphoenol pyruvate carboxykinase, *G6Pase* glucose 6-phosphatase, *GS* glycogensynthase, *SREBP1* element-binding protein 1, *FAS* fatty acid synthase

Results

Growth Performance

The growth performance results for the juvenile blunt snout bream are shown in Table 3. The survival rate was not significantly affected by dietary Cr (Cr as a form of Cr-Pic) supplementation ($P > 0.05$), but dietary Cr supplementation significantly affected the growth performance of juvenile blunt snout bream ($P < 0.05$). The highest values of the final weight (FW) and specific growth rate (SGR) were remarked in fish fed with 0.4 mg/kg of supplemented Cr in the diet, thereafter showed a relatively constant trend. In the same diet (0.4 mg/kg of Cr supplementation), the lowest value of feed conversion ratio (FCR) has been recorded. However, high dietary Cr (12.0 mg/kg) supplementation level has decreased the growth and feed utilization of juvenile fish ($P < 0.05$). Based on SGR (Fig. 1) and FCR (Fig. 2) using the broken-line regression model, the optimum level of Cr supplementation in the practical diet was estimated at 2.8 mg/kg.

Table 4 presented whole-body compositions. The contents of whole-body moisture, protein, and ash significantly were not affected by dietary treatments. In fish fed diet supplemented with 0.4 mg/kg of Cr, the highest content of whole-body lipid was found compared to other fed diets supplemented with 0, 0.2, 0.8, and 12.0 mg/kg Cr ($P < 0.05$). As shown in Fig. 3, the addition of 0.2–1.6 mg/kg of Cr in the diets significantly reduced the content of hepatic glycogen in juvenile blunt snout bream in comparison to the fish fed without Cr supplementation in the diets.

Table 3 Growth performance and feed utilization of juvenile blunt snout bream fed with the experimental diets

Cr added (mg/kg)	IW (g)[†]	FW(g) [‡]	Survival (%)	SGR (% day^{-1})[§]	FCR (%)[¶]
0	20.0 ± 0.04	94.7 ± 1.76^{a}	93.3 ± 4.41	2.05 ± 0.03^{a}	1.46 ± 0.03^{bc}
0.2	20.0 ± 0.02	113.0 ± 3.78^{c}	91.7 ± 1.67	2.28 ± 0.05^{c}	1.34 ± 0.04^{ab}
0.4	20.0 ± 0.04	115.0 ± 2.64^{c}	95.0 ± 5.00	2.30 ± 0.06^{c}	1.30 ± 0.05^{a}
0.8	20.0 ± 0.04	110.3 ± 1.20^{bc}	93.3 ± 4.41	2.25 ± 0.01^{bc}	1.35 ± 0.02^{abc}
1.6	20.0 ± 0.07	110.0 ± 2.89^{bc}	96.7 ± 1.67	2.24 ± 0.03^{bc}	1.35 ± 0.03^{abc}
3.2	20.0 ± 0.04	108.3 ± 2.19^{bc}	100 ± 0.00	2.22 ± 0.03^{bc}	1.35 ± 0.05^{abc}
12.0	20.0 ± 0.04	100.0 ± 1.53^{ab}	93.3 ± 1.24	2.12 ± 0.03^{ab}	1.48 ± 0.04^{c}

Mean values and standard error (±SE) are presented for each parameter. Significant differences within the diets are indicated by different superscripts ($P < 0.05$)

[†] IW (g): initial weight

[‡] FW (g): final weight

[§] SGR (% day^{-1}): specific growth rate = 100 × ((ln FBW − ln IBW)/76 days)

[¶] FCR (%): feed conversion ratio = 100 × dry feed intake/wet weight gain

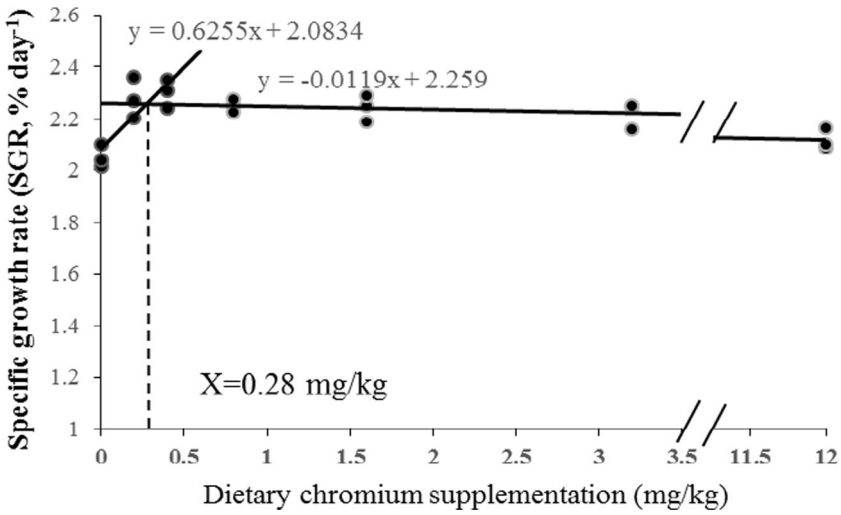

Fig. 1 The relationship between specific growth rate (SGR, % day^{-1}) and dietary chromium supplementation (mg/kg) of juvenile blunt snout bream fed with the experimental diets for 11 weeks

Plasma Parameters

The results are shown in Table 5, where total protein concentration in the plasma was not significantly affected by Cr supplementation ($P > 0.05$). Likewise, the high levels of triglyceride were remarked in fish fed with supplements of 0.4 and 0.8 mg/kg Cr in the diets and were significantly higher than those fed other diets. Glucose concentrations decreased with rising dietary Cr supplementation and reached the lowest level when the juveniles were fed with 3.2 mg/kg of supplemented Cr in the diets ($P < 0.05$). Significantly higher plasma insulin levels were found in juvenile fed diets with 0.4 and 0.8 mg/kg Cr supplementation compared to those fed diet sans supplemented Cr.

Relative Gene Expression

Relative mRNA levels of IRS-1, PI3K (insulin signaling pathway-related genes), and plasma insulin levels were significantly affected by supplemented Cr in the diets. The highest IRS-1 expression was observed in juvenile fed with 0.8 mg/kg Cr supplementation in the diet, and thereafter showed a decreased trend ($P < 0.05$). Significantly higher PI3K expressions were found in juvenile fed diets with 0.4, 0.8, and 1.6 mg/kg Cr supplementation compared to those fed diets with 3.2 and 12.0 mg/kg Cr supplementation ($P < 0.05$) (Fig. 4).

The levels of relative mRNA expression are presented in Fig. 5. The highest pyruvate kinase (PK) was remarked in the fish fed with 0.4 of supplemented Cr in the diets ($P < 0.05$). Phosphoenolpyruvate carboxykinase (PEPCK), glycogen synthase (GS), and glucose-6-phosphatase (G6pase) mRNA expressions showed a downward trend with increasing of the Cr supplementation level in the diets, and the lowest value was recorded in the dietary treatments supplemented with 0.4 or 0.8 mg/kg of Cr, respectively. Subsequently, these three gene expressions showed an increased trend. The mRNA levels of lipogenesis-related genes are shown in Fig. 6. The sterol regulatory element binding protein-1 (SREBP1) and fatty

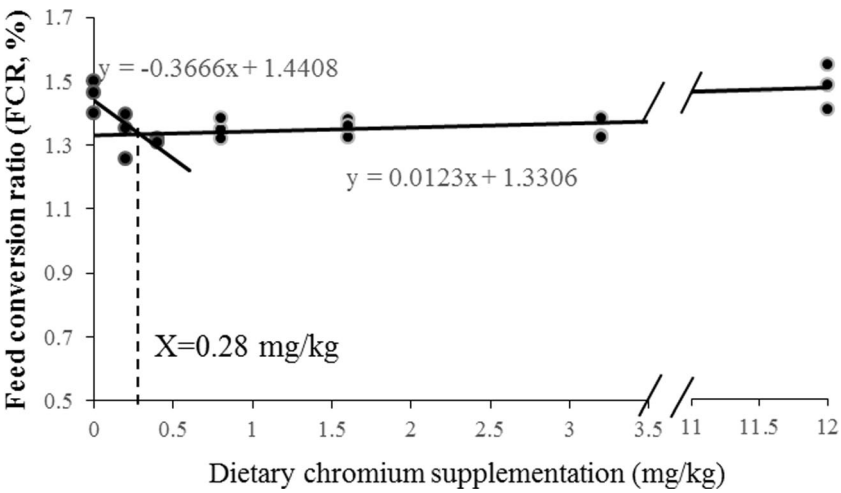

Fig. 2 The relationship between feed conversion ratio (FCR, %) and dietary chromium supplementation (mg/kg) of juvenile blunt snout bream fed with the experimental diets for 11 weeks

Table 4 Whole-body composition (wet basis) of juvenile blunt snout bream fed with the experimental diets

Cr added (mg/kg)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
0	70.90 ± 0.23	17.24 ± 0.09	8.63 ± 0.19^{a}	3.65 ± 0.07
0.2	71.03 ± 1.10	17.22 ± 0.33	8.48 ± 0.54^{a}	3.53 ± 0.08
0.4	69.45 ± 1.13	17.79 ± 0.50	10.29 ± 0.36^{b}	3.66 ± 0.03
0.8	71.48 ± 0.09	17.32 ± 0.63	8.63 ± 0.19^{a}	3.61 ± 0.21
1.6	70.81 ± 0.17	17.15 ± 0.09	8.82 ± 0.21^{ab}	3.68 ± 0.04
3.2	70.70 ± 0.56	17.11 ± 0.17	8.89 ± 0.39^{ab}	3.91 ± 0.42
12.0	72.20 ± 0.39	16.74 ± 0.20	7.90 ± 0.11^{a}	3.56 ± 0.19

Mean values and standard error (±SE) are presented for each parameter. Significant differences within the diets are indicated by different superscripts ($P < 0.05$)

synthase (FAS) augmented with the supplementation of dietary Cr up to 0.8 mg/kg, and thereafter showed a decreased trend ($P < 0.05$).

Discussion

In the current study, the survival rate of the fish was high and independent of dietary Cr supplementation, which is in agreement with previous reports in the mirror and common carp [15, 16], while adding 5.0 mg/kg of chromium polynicotinate in the yellow croaker diet has significantly improved its survival rate [17]. Dietary 0.2–3.2 mg/kg Cr supplementation has improved growth performance of the juvenile, and optimal Cr supplementation level in the practical diet for the fish was assessed to be 0.28 mg/kg. Similar benefits on other farmed animals were detailed and explained in the scientific literature [12]. Many studies have reported that the Cr supplementation in fish diets improves the growth performance. Supplemental 0.5 mg/kg organic Cr in a starch-based diet revealed positive

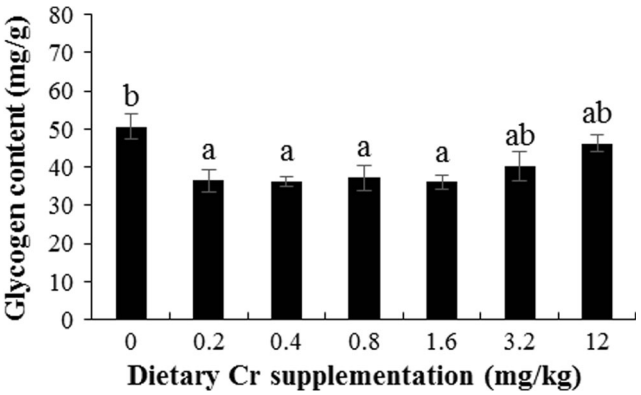

Fig. 3 Glycogen content in the liver of juvenile blunt snout bream fed experimental diets for 11 weeks. Values are means for nine fish per treatment, with standard errors represented by vertical bars. Significant differences within the diets are indicated by different superscripts ($P < 0.05$)

effects on growth performance for the common carp [15]. Adding 0.8 mg/kg of Cr in the diet could increase growth and change serum lipid and carbohydrate profiles in the grass carp [14]. However, trivalent chromium supplementation has not improved growth performance for the channel catfish and rainbow trout [18, 20, 30]. Based on the previous studies, the beneficial advantage of supplemented Cr in fish diets might be species-specific and dependent on the dose and form of the used Cr.

In this study, 0.4 mg/kg of supplemented Cr has improved feed utilization of juvenile blunt snout bream versus to those fed diet sans supplemented Cr, which might be due to the increase of dietary nutrient utilization [17]. It has been confirmed that in mammals, the IRS-PI3k-Akt insulin pathway plays a key role in glucose and lipid utilization and metabolism [31].The findings of this study indicated that highest mRNA expression values of IRS-1 and PI3K were observed in fish diet supplemented with 0.4–0.8 mg/kg of Cr and were significantly higher than those sans Cr supplementation. Furthermore, in our study, we found that plasma insulin content shows a positive relationship with IRS-1 and PI3K. Identical findings have been found in higher animals, where the chromium has been proved as motivator for the insulin binding to cells, enhancer for insulin receptor number, and stimulator for IRS-1 phosphorylation and PI3K activity in mice [32–34], the latter associated with the blood glucose homeostasis and used by the cells [35]. In agreement with aforementioned findings in mammals, our results indicated that Cr supplementation in the diets has decreased plasma glucose concentrations in juvenile blunt snout bream, and we suggest that the optimal Cr supplementation has a beneficial effect on blood glucose homeostasis which might be involved in insulin signaling molecules and insulin production. Similarly, Hertz et al. [36] reported that chromium chloride supplementation decreased serum glucose level in the common carp.

Likewise, the glycolysis is the route of glucose catabolism in the animals including fish, and the regulation of

Table 5 Plasma parameters of juvenile blunt snout bream fed with the experimental diets

Cr added (mg/kg)	Total protein (g/L)	Triglyceride (mmol/L)	Glucose (mmol/L)	Insulin (μIU/mL)
0	27.55 ± 0.93	2.42 ± 0.19^{a}	9.00 ± 0.54^{b}	3.68 ± 0.13^{a}
0.2	27.89 ± 0.79	2.42 ± 0.14^{a}	8.20 ± 0.70^{b}	4.14 ± 0.10^{ab}
0.4	30.37 ± 1.57	3.85 ± 0.33^{b}	7.89 ± 0.55^{ab}	4.46 ± 0.17^{b}
0.8	31.14 ± 2.22	3.56 ± 0.29^{b}	7.24 ± 0.98^{ab}	4.59 ± 0.14^{b}
1.6	30.25 ± 1.62	2.55 ± 0.23^{a}	7.50 ± 0.96^{ab}	4.21 ± 0.02^{ab}
3.2	32.41 ± 1.27	1.93 ± 0.12^{a}	4.86 ± 0.91^{a}	3.80 ± 0.10^{a}
12.0	30.79 ± 1.22	2.08 ± 0.12^{a}	6.46 ± 0.49^{ab}	4.06 ± 0.14^{ab}

Mean values and standard error (±SE) are presented for each parameter. Significant differences within the diets are indicated by different superscripts ($P < 0.05$)

gluconeogenesis is depends on key enzymes' activities such as PK [37]. In our research, the highest level of relative mRNA expression of PK was detected in the diet supplemented with 0.8 mg/kg of Cr. Similar results have been obtained in dietary treatments for the common carp where the high activities of hexokinase (HK) have been recorded in the fish fed starch-based supplemented with 0.5–1.0 mg/kg of Cr in the diet [15]. The present study showed that 0.4 mg/kg dietary Cr supplementation significantly lowered the relative mRNA expression of PEPCK and G6Pase. Similarly, Hertz et al. [36] reported that chromium chloride supplementation inhibited gluconeogenesis from amino acids in the common carp. In this study, we suggested that optimal Cr supplementation in juvenile blunt snout bream commercial diet could regulate mRNA expression related to glycolysis and gluconeogenesis. Similarly, in streptozotocin-induced experimental diabetes, there is an ameliorating effect of chromium administration on hepatic glucose metabolism by improving glycolysis and suppressing gluconeogenesis [6]. In fish, glycogen plays a key role in the metabolism and homeostasis of glucose, and a synthesis of the glycogen is catalyzed by GS as the same procedure in mammals [37]. In the current study, the content of hepatic glycogen and GS relative mRNA level was regulated by dietary Cr supplementation and presented alike trend. In a like manner, a positive relationship between total GS activity and hepatocyte glycogen content was reported in rainbow trout [38]. Liu et al. [14] reported although the higher hepatic glycogen level was indicated in grass carp fed a diet with low chromium level, glycogen content showed a downward trend with the augmentation of dietary chromium supplementation (above than 0.4 mg/kg), which may be considered as partially in agreement with our results. The findings from this research about GS hepatic mRNA level and content of glycogen showed negative relation with IRS-1 and PI3K expression, which suggested glycogen synthesis might be sensitive to insulin signaling regulated by supplemented Cr in the diets. In agreement with the current trial, after insulin injection in the teleost *Pimelodus maculatus*, some abnormities have been recorded such as the depletion of hepatic glycogen content and has led to the diminution of glucose level in the plasma [39]. In contrast, Cr has proven as an insulin activator and stimulator for glycogen synthesis by increasing or stabilizing GS mRNA in higher animals [40]. The mechanism that dietary Cr regulates hepatic glycogen synthesis in juvenile blunt snout bream is still unclear and needs to be further investigated.

In the current study, the supplementation of Cr in the diets has not changed the moisture, protein, and ash content of

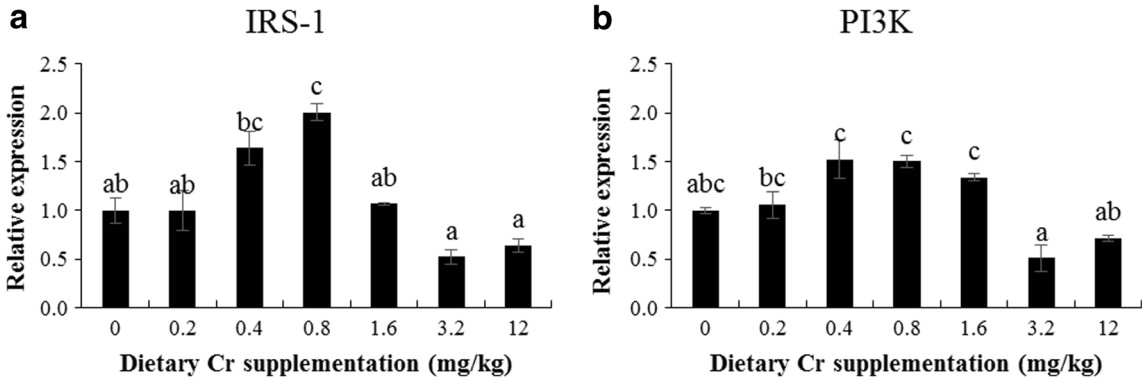

Fig. 4 Relative expression of insulin signaling pathway-related genes including insulin receptor substrate 1 (IRS-1) (**a**) and phosphoinositide-3-kinase (PI3K) (**b**) genes in the liver of juvenile blunt snout bream fed experimental diets for 11 weeks. Values are means for nine fish per treatment, with standard errors represented by vertical bars. Relative mRNA levels of IRS-1 and PI3K were normalized, and significant differences within the diets are indicated by different superscripts ($P < 0.05$)

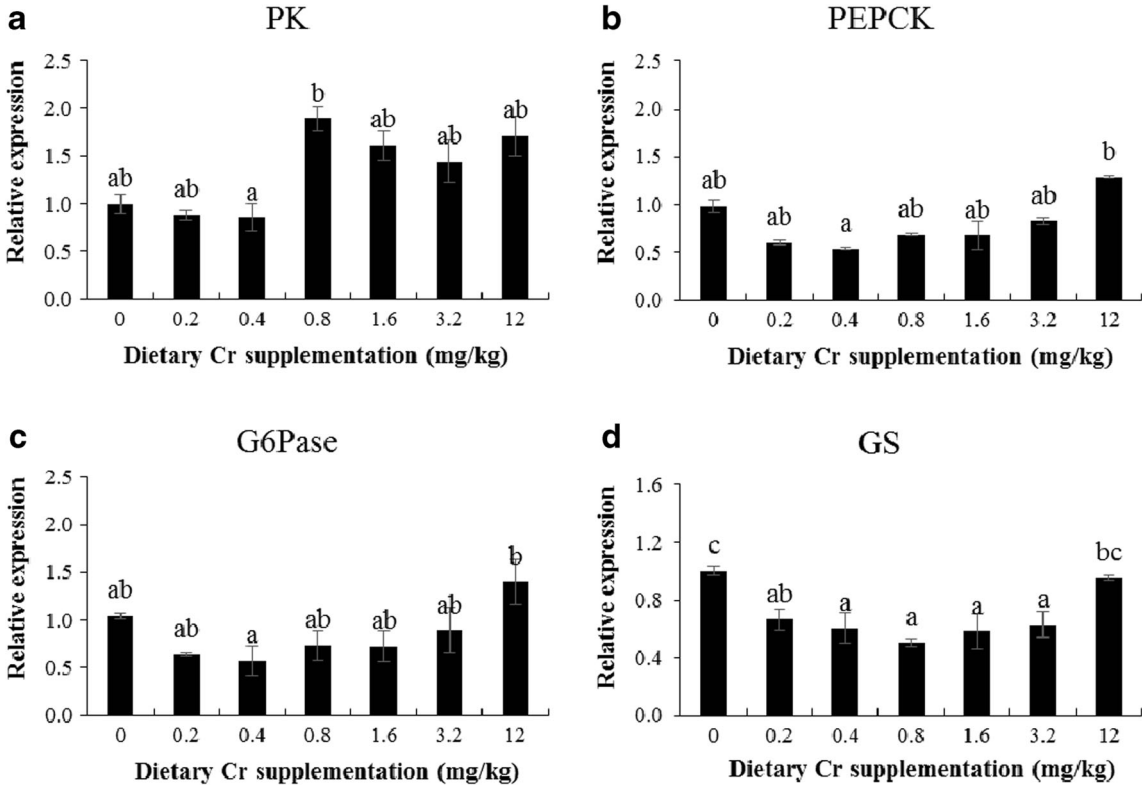

Fig. 5 Relative expression of glucose metabolism-related genes including pyruvate kinase (PK) (**a**), phosphoenolpyruvate carboxykinase (PEPCK) (**b**), glucose-6-phosphatase (G6Pase) (**c**), and glycogen synthase (GS) (**d**) in the liver of juvenile blunt snout bream fed experimental diets for 11 weeks. Values are means for nine fish per treatment, with standard errors represented by vertical bars. Relative mRNA levels of PK, PEPCK, G6Pase, and GS were normalized, and significant differences within the diets are indicated by different superscripts ($P < 0.05$)

whole-body composition, which is in agreement with the reports in other carp species [16]. Though the fish fed diet with milligram/kilogram Cr supplementation showed highest whole-body lipid content among the dietary treatments. Similar results have been recorded in other fish species such as in grass carp [14] and mirror carp nourished with maize starch [16], also in higher animals such as in beef cows [41]. The same trends were shown in plasma triacylglyceride level and relative expression of lipogenesis-related genes such as SREBP1 and FAS. It is worth noting that whole-body lipid, plasma triacylglyceride level, and the relative mRNA expression levels of lipogenesis-related genes declined with the increasing dietary Cr supplementation, when the adding levels of Cr in the diets were higher than 0.4 mg/kg, suggesting that

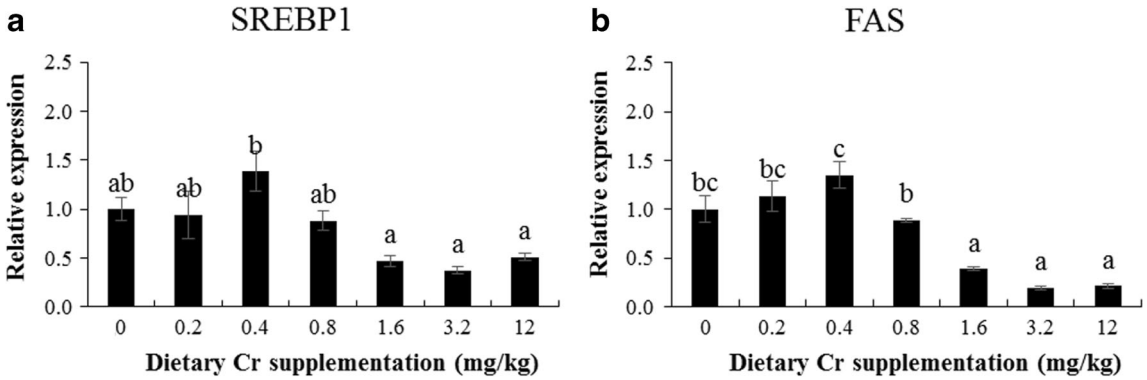

Fig. 6 Relative expression of lipogenesis-related genes including sterol regulatory element binding protein-1 (SREBP1) (**a**) and fatty acid synthase (FAS) (**b**) in the liver of juvenile blunt snout bream fed experimental diets for 11 weeks. Values are means for nine fish per treatment, with standard errors represented by vertical bars. Relative mRNA levels of SREBP1 and FAS were normalized and significant differences within the diets are indicated by different superscripts ($P < 0.05$)

high supplemented level in the diet might inhibit the synthesis of fatty acids at least in the level of mRNA of juvenile blunt snout bream. In pigs, dietary Cr supplementation might also inhibit the synthesis of fatty acids, which supports our result [42]. Recently, a new understanding into the insulin signaling pathway that controls SREBP1 has been revealed in mammals [43]. SREBP1 can be responsible for the biosynthesis of fatty acids by regulating the FAS gene; the latter plays a fault-finding role in lipogenesis metabolic actions [44–46]. Regardless of mammals, SREBP1 and FAS mRNA have indicated a positive relation to the expression of the insulin signaling pathway-related gene (IRS-1 and PI3K) in this research, suggesting that the lipogenesis gene expression upregulation by dietary Cr could be related to the improved insulin signaling molecules. However, this study reports the Cr effect on the gene expression related to lipogenesis for the first time in fish. Whether Cr supplementation affects the aforementioned enzymes' activities or genes in fish needs more investigation and clarification.

In this study, significant depressions of feed utilization and growth were observed in the fish fed diet with 12.0 mg/kg (extremely high) Cr supplementation, whereas reflect the same results reported in fish and other animals [12]. In the previous studies, the decreases in feed utilization and growth were attributed to Cr toxic effect with a high supplemented level. This extreme level as a form of chromium chloride in the common carp diet had an adverse impact on the hepatic structural level and midsection of the intestine which negatively affected the growth performance and fish health [15]. The exposure of goldfish to the chromium also results in an oxidative stress in the liver and kidney [47]. Several experiments in cell culture have been undertaken to grasp the effect of high level of Cr and led to suggest that the possibility of increasing DNA damage and caused toxicity when the chromium taken in excess [48]. However, Komorowski et al. [49] reported that Cr-Pic did not provoke damage of chromosomes in the cell of the bone marrow even with a dose of 2000 mg/kg body weight and consequently any Cr-Pic toxicity has been recorded. In the current study, the poor growth performance and feed utilization seem to be due to depressed utilization and metabolism of glucose by the fish fed diet with high Cr level. We clearly observed a high level of blood glucose and relative expression of gluconeogenesis-related gene (PEPCK and G6Pase) which might be affected by depressed gene expression related to the insulin signaling pathway (IRS-1 and PI3K) in the fish given diet supplemented with 12.0 mg/kg of Cr compared to those fed other diets.

Conclusions

The findings of this study suggested that Cr (as the form of Cr-Pic) supplementation in the practical diet of juvenile blunt snout bream has positive effects on growth and blood glucose homeostasis by modifying glucose metabolism, and the optimal dietary Cr supplementation level is estimated to be 0.28 mg/kg. However, excess dietary Cr (12.0 mg/kg) supplementation results in poor growth performance and enhancement of gene expression related to gluconeogenesis.

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Compliance with Ethical Standards The present study was approved by the Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China).

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