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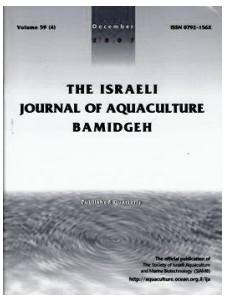

Effects of Dietary Chromium Methionine on Growth Performance, Hematological Characteristics and Carbohydrate Metabolic Enzyme Activities of Common Carp (*Cyprinus carpio*)

Pei Cui[1,2], Shuai Yin[2], Zhenyan Cheng[2], Xiuting Qiao[2], Qicun Zhou[1]*

[1] Laboratory of Fish Nutrition, School of Marine Sciences, Ningbo University, Ningbo 315211, China
[2] Tianjin Key Laboratory of Aqua-ecology and Aquaculture, Fisheries Science Department, Tianjin Agricultural University, Tianjin 300384, China

Keywords: chromium methionine; common carp; growth performance; hematological characteristics; carbohydrate metabolic enzyme

Abstract

A 60-day feeding trial was conducted to evaluate the effects of dietary chromium methionine (Cr-Met) supplementation on growth performance, digestive enzymes, hematological characteristics, and carbohydrate metabolic key enzyme activities in juvenile common carp (*Cyprinus carpio*). Seven diets (32.2% crude protein, 6% crude lipids of dry matter) were formulated to contain graded levels of Cr^{3+} (0.31, 0.43, 0.57, 0.73, 1.13, 1.90 and 3.64 mg/kg, respectively). Each diet was randomly assigned to triplicate groups of 60 juvenile common carp (approximately 40.95±4.80 g), which were stocked in plastic tanks. The results indicated that the highest weight gain (WG), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER), were observed in fish fed the diet containing 1.13 mg/kg Cr^{3+}. There were no significant differences in the wholebody compositions; however the glycogen levels in the muscle and hepatopancreas in fish fed the basal diet were lower than those fed the other diets. The insulin (INS) and glycogen (GC) concentrations in the serum were not significantly influenced by the dietary Cr-Met levels. However, fish fed the basal diet had higher glucose and cortisol concentrations, and lower insulin receptor (ISR) and LDH concentrations in the serum than those fed diets supplemented with Cr-Met. Fish fed the 1.13 mg/kg Cr^{3+} diet had significantly higher hexokinase (HK), 6-phosphofructo-1-kinase (6-PFK1), glucose-6-phosphate dehydrogenase (G6PDH), glycogen synthase (GS), and lower phosphoenolpyruvate carboxykinase (PEPCK) activities than those fed the basal diet. Based on the two-slope broken line model analysis between SGR and dietary $Cr3^{+}$ levels, the optimal dietary Cr^{3+} supplement level was estimated to be 1.09 mg/kg for juvenile common carp.

* Corresponding author. Qicun Zhou, e-mail: zhouqicun@nbu.edu.cn (Q. -C. Zhou)

Introduction

Carbohydrates are the most economical source of energy supplying nutrients (NRC, 2011). Using carbohydrates as a source of energy in formulated aquaculture diets could reduce feed costs, lower the catabolism of proteins and lipids used for energy, and reduce ammonia excretion by amino acid metabolism, thereby reducing environmental pollution (Moon, 2001). However, fish have a poor ability to digest, absorb, and metabolize carbohydrates and are generally considered to be glucose intolerant (Enes *et al*., 2009). Diets high in carbohydrates can also have detrimental effects on growth performance and may cause metabolic load and pathological functions in fish (Hemre, 2002), which might lead to suppressed immune functions and increased susceptibility to infectious diseases (Barton *et al*., 1991). Thus, it is essential to find a safe nutrient element that improves carbohydrate utilization of each major cultured fish species.

Trivalent chromium (Cr^{3+}) is an essential trace element that plays a key role in carbohydrate, protein, and lipid metabolism. It appears to potentiate the action of insulin and promote glucose tolerance (Hoffman, 2014). Numerous studies have shown that dietary Cr^{3+} supplementation has beneficial effects on growth performance and feed utilization of grass carp *Ctenopharyngodon idellus* (Liu *et al*., 2010), rainbow trout *Oncorhynchus mykiss* (Selcuk, *et al*., 2010), large yellow croaker *Larmichthys crocea* (Wang *et al*., 2014), and hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus* (Pan *et al*., 2002). It improves the immune status of rainbow trout *O. mykiss* (Gatta *et al*., 2001b), and promotes carbohydrate utilization of striped bass *Morone saxatilis*. Moreover, fish given diets supplemented with Cr^{3+} showed reduced plasma concentrations of cortisol to alleviate the stress response (Marcello *et al*., 2014).

Cyprinus carpio are economically valuable in many countries and also have the distinction of being omnivorous in nature, with a high capacity for carbohydrate assimilation. Some studies have been conducted on *Cyprinus carpio* with inorganic forms of chromium (Ahmed *et al*., 2013; Hertz, et al., 1989), rather than organic forms, which have higher bioactivity (NRC, 2011). However, the organic chromium safety levels and physiological function of *Cyprinus carpio* have not yet been reported. Thus, the objective of the present study was to evaluate the effects of dietary Cr-Met on growth performance, feed utilization, health, and key enzyme activities related to carbohydrate mechanisms.

Materials and Methods

Diet preparation

Seven isonitrogenous and isolipidic purified diets (32.2% crude protein, 6% lipid) were formulated to contain seven graded levels of Cr (ZINPRO, USA); the levels of Cr were analyzed by Graphite Furnace Atomic Absorption Spectrometry (modelAA240FS, USA) to be 0.31 (control), 0.43, 0.57, 0.73, 1.13, 1.90, and 3.64 mg/kg dry diet (see Table 1). Dextrin (Xiwang Sugar Industry Co., LTD, Shandong, China) was used as a source of carbohydrates in all experimental diets. The dietary ingredients were ground through 80-mesh. All the dry ingredients were thoroughly mixed to homogeneity in a Hobart-type mixer (M-256, South China University of Technology, Guangzhou, China). Lipids and water were added and thoroughly mixed. Cold-extruded (F-26 (II), South China University of Technology, Guangzhou, China) pellets (Φ = 1.0 mm and 1.5 mm) were produced, air-dried to approximately 10% moisture, sealed in vacuum-packed bags and stored at -20°C until use.

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

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*	*Diet 6*	*Diet 7*
Casein	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Soybean oil	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Dextrin	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calcium biphosphate	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Sodium carboxymethylcellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Y_2O_3	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Microcrystalline cellulose	19.00	18.90	18.80	18.60	18.20	17.40	15.80
Cr-Met (mg/kg dry matter)	0.00	0.10	0.20	0.40	0.80	1.60	3.20
Analyzed dietary Cr (mg/kg)	0.31	0.43	0.57	0.73	1.13	1.90	3.64
Proximate composition (%)							
Dry matter	91.23	91.54	91.62	91.46	91.75	91.22	91.43
Crude protein	32.21	32.06	32.10	32.11	32.00	32.29	32.10
Crude lipid	5.41	5.39	5.68	5.73	5.89	5.61	5.76
Ash	3.07	2.79	2.88	2.98	2.90	3.03	2.98

[1] premix(mg/kg premix): vitamin A ,60000IU; vitamin D3, 20000IU; vitamin E, 6000; vitamin K3, 1000; vitamin B1, 900.0; vitamin B2, 900.0; vitamin B6, 750.0; vitamin B12, 3; D-biotin, 15;D- pantothenic acid, 3000; folic acid, 300; nicotinamide,4500; vitamin C,9000; inositol, 8000; Fe,14000; Cu, 350.0; Mn,1500; Zn, 4000; Mg, 10000; Co, 25.0; I, 50;Se,30; Ethoxyquin,500

Fish and experimental conditions

The experiment was conducted at the facility of Tianjin Chenhui Feed Co., LTD, Tianjin, China. Common carp juveniles, *Cyprinus carpi*o, were obtained from the Tianjin Chenhui Feed Co., LTD. Prior to the feeding trial, fish were fed with a control diet (32.2% crude protein, 6% crude lipid, no chromium supplementation) for 2 weeks to acclimatize to the experimental conditions. At the end of the acclimation, triplicates of 60 fish per treatment of a similar size (initial weight 40.95±4.80 g) were stocked randomly and sorted in a rearing system consisting of 21 plastic tanks (800 L). The fish were handfed twice (8:00 and 17:00 h) a day initially at a rate of 6% body weight, and then gradually reduced to 4%. Fish were weighed biweekly, and the daily ration was adjusted accordingly. During the experimental period, the temperature ranged from 28-30°C and pH ranged from 7.6-7.8. Ammonia nitrogen content was lower than 0.05 mg/L, and the dissolved oxygen content was not less than 6.0 mg/L. The duration of the feeding trial was 60 days.

Sample collection techniques and analyses

At the end of the feeding trial, all experimental test fish were fasted for 24 h and then anesthetized with 100 mg/L MS-222 for weighing, counting, and measurement. Six fish per tank were collected and stored at -20°C to analyze the proximate composition of the whole body. In addition, blood, intestine, muscle, and hepatopancreas samples were taken from ten fish in each tank. Blood samples were collected from the caudal vein and centrifuged at 4°C, 5000 rpm for 10 min, then the supernatant was collected. Intestine, muscle, and hepatopancreas samples were collected using a scalpel on ice. Muscle and hepatopancreas samples were divided into two parts for enzyme and glycogen assays. All samples were packaged and stored at -80°C until analysis.

Proximate composition

Proximate composition analysis of diets and wholebody was performed by standard methods of the Association of Official Analytical Chemists (AOAC, 1995). Moisture was determined by oven drying at 105°C to a constant weight. Crude protein (N × 6.25) was determined by measuring nitrogen using the Kjeldahl method (Thermo Scientific Flash 2000 CHNS/O). Crude lipid content was determined by ether extraction using Soxhlet apparatus. Ash was measured by combustion at 550°C in a muffle furnace for 8 h.

Hematology and glycogen analysis

Serum was collected by centrifuging hemolymph samples at 4°C, 4000 rpm for 10 min. Glucose (GLU) in the blood was assayed using an automatic blood analyzer (Roche C311, German) from a clinical laboratory. The concentrations of insulin (INS), glucagon (GC), insulin receptor (ISR), growth hormone (GH), and cortisol (COR) were measured by validated radioimmunoassay (RIA) methods using microalbuminuria enzyme-linked immunosorbent assay (ELISA) kits specific for fish. INS, GC, and LDH kits were purchased from Jiancheng Bioengineering, Ltd. (Nanjing, China). ISR, GH and COR kits were purchased from the Meilian Biotechnology Co., Ltd. (Shanghai, China). Muscle and hepatic glycogen contents were determined spectrophotometrically at 620 nm using assay kits (Jiancheng Bioengineering, Ltd., Nanjing, China).

Hepatopancreas enzyme activities analysis

Activity of hepatic hexokinase (HK), pyruvate kinase (PK), and succinic acid dehydrogenase (SDH), was determined using assay kits (Jiancheng Bioengineering, Ltd., Nanjing, China).

Hepatopancreas phosphoenolpyruvate carboxykinase (PEPCK), phosphofructokinase (6-PFK1), glucose 6-phosphatase dehydrogenase (G6PDH), glycogen synthase (GS) activities were measured by the double antibody sandwich method using ELISA assay kits (Assay Designs Ltd., USA).

Calculations and statistical analysis

Parameters were calculated as follows:

Weight gain (WG, %) = 100 × [(final weight − initial weight) / initial weight

Specific growth rate (SGR, % day^{-1}) = 100 × [(Ln final weight − Ln initial weight) / duration]

Survival (%) = 100 × (final number of fish) / (initial number of fish)

Feed efficiency (FE) = weight gain (g, wet weight) / feed consumed (g, dry weight)

Protein efficiency ratio (PER) = weight gain (g, wet weight) / protein intake (g, dry weight)

All statistical analyses were performed using SPSS 19.0 (SPSS, IL USA). The results are presented as the means ± S.D. Differences between means were analyzed using one-way ANOVA. If there were significant differences detected ($P<0.05$), subsequent comparisons of the group means were performed using Tukey's test at a significance level of 5%. A two-slope broken line model was conducted to estimate the requirement of Cr^{3+} based on SGR in common carp (Fig. 1).

Results

Growth performance and feed utilization

Survival rate ranged from 91.11%-95.00%, and there were no significant differences among all treatments. Results of growth performance and feed utilization of common carp fed different levels of dietary Cr-Met are presented in Table 2. WG, SGR and PER significantly increased with the dietary Cr^{3+} levels increasing from 0.31-1.13 mg/kg ($P<0.05$); however, with a further increase of the dietary Cr^{3+} levels from 1.90-3.64 mg/kg, WG, SGR and PER decreased significantly ($P < 0.05$). A two-slope broken line model and an exponential model were employed to estimate the Cr^{3+} requirement of common carp, and the optimal dietary Cr^{3+} level based on the observed SGR was determined to be 1.09 mg/kg (Fig. 1). Fish fed the deficient Cr^{3+} diet had a significantly lower FE than those fed the diet containing 1.13 mg/kg Cr^{3+} ($P < 0.05$), but no significant differences were observed among those fed the Cr-Met diets ($P > 0.05$).

Table 2. Growth performance, survival and feed utilization of the juvenile *Cyprinus carpio* fed with different dietary CrMet levels

Item	Cr^{3+} level (mg/kg)						
	0.31	0.43	0.57	0.73	1.13	1.9	3.64
Initial weight (g)	40.56±3.58	40.56±3.52	36.82±3.03	39.28±1.41	41.96±0.52	45.89±9.92	40.52±0.86
Final weight (g)	93.00±7.62	97.41±15.33	91.01±5.90	91.01±5.90	114.47±2.89	118.66±23.75	95.02±1.72
Survival (%)	91.11±0.96	92.22±2.55	91.11±3.85	94.44±3.47	93.33±2.89	91.67±1.67	95.00±4.41
Weight gain (%)	129.56±10.98^{a}	133.97±3.70^{ab}	147.80±17.67^{abc}	157.10±7.22^{bc}	172.79±4.25^{c}	172.79±4.25^{c}	134.57±7.18^{ab}
SGR[1] (%/day)	1.38±0.08^{a}	1.42±0.03^{ab}	1.51±0.12^{abc}	1.57±0.05^{bc}	1.67±0.03^{c}	1.59±0.03^{bc}	1.42±0.05^{ab}
FE[2]	0.54±0.02^{a}	0.56±0.09^{ab}	0.56±0.05^{ab}	0.64±0.01^{ab}	0.74±0.02^{b}	0.71±0.15^{ab}	0.54±0.06^{ab}
PER[3]	1.66±0.33^{a}	1.78±0.43^{a}	1.75±0.16^{a}	1.98±0.04^{a}	2.53±0.07^{b}	2.31±0.44^{ab}	1.68±0.05^{a}

Data represent mean ± S.D. of three replicates. Values in the same line with different superscripts are significantly different (P<0.05)

[1]SGR:Specific growth rate; FE: Feed efficiency; PER: Protein efficiency ratio

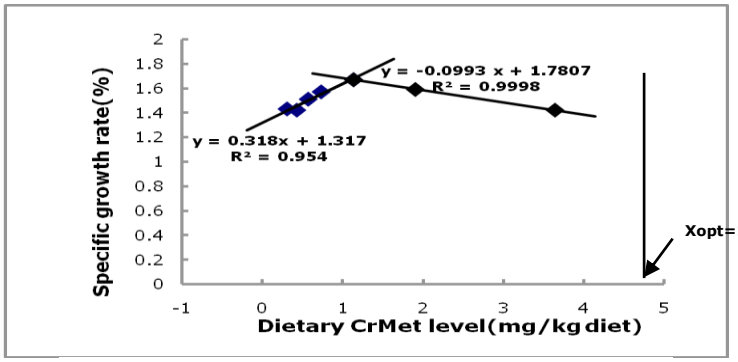

Fig 1. Relationship between SGR (%) and dietary Cr^{3+} levels based on a two-slope broken line regression analysis, where Xopt represents the optimal dietary Cr^{3+} level for achieving the maximum SGR (%) in common carp.

Whole body composition and tissue glycogen

Wholebody composition and glycogen in the hepatopancreas and muscle of juvenile common carp are shown in Table 3. Wholebody moisture, protein, lipid, and ash contents were not significantly affected by the dietary Cr^{3+} levels (P > 0.05), whereas the hepatic glycogen and muscle glycogen contents were significantly affected by the Cr^{3+} levels. Fish fed the 0.73 mg/kg diet had significantly higher hepatic glycogen than that fed the 0.31 mg/kg Cr^{3+} diet, but no significant differences were observed among the other treatments (P > 0.05). The muscle glycogen content significantly increased with dietary Cr^{3+} levels increasing up to 1.90 mg/kg and thereafter declined (P > 0.05).

Table 3. Proximate compositions of the whole body of *Cyprinus carpio* fed diets with different CrMet levels

Item	*Cr^{3+} level (mg/kg)*						
	0.31	*0.43*	*0.57*	*0.73*	*1.13*	*1.90*	*3.64*
Whole bady (%)							
Moisture	76.00±0.63	76.96±1.21	75.62±0.49	76.74±0.52	76.37±0.82	76.97±0.42	76.11±0.46
Protein	15.13±0.42	15.03±0.16	15.22±0.39	15.02±0.65	15.35±0.10	17.51±0.40	15.53±0.22
Lipid	4.51±0.33	6.09±0.25	5.99±1.06	5.73±0.13	6.70±0.67	6.66±0.10	5.77±0.27
Ash	4.01±0.24	4.03±0.11	4.05±0.13	4.04±0.12	4.04±0.15	4.17±0.18	4.11±0.25
Hepatic and muscle glycogen levels(mg/kg)							
Hepatic glycogen	26.45±3.27^{a}	29.67±2.91^{ab}	29.67±2.91^{ab}	42.90±7.36^{b}	42.90±7.36^{b}	37.90±5.83^{ab}	28.98±4.33^{ab}
Muscle glycogen	1.24±0.19^{a}	1.44±0.21^{ab}	1.48±0.20^{ab}	1.55±0.15^{ab}	1.68±0.23^{ab}	1.97±0.29^{b}	1.37±0.28^{ab}

Data represent mean ± S.D. of three replicates. Values in the same line with different superscripts are significantly different (P<0.05).

Hematological characteristics

The hematological characteristics of common carp fed different dietary Cr^{3+} levels are shown in Table 4. Fish fed the Cr-Met diets exhibited significantly lower glucose contents in the serum than those fed the basal diet, whereas the ISR level significantly increased with dietary Cr^{3+} increasing up to 1.90 mg/kg and then levelled off. Fish fed the Cr^{3+} diets from 0.43 to 1.90 mg/kg exhibited significantly higher ISR level in serum than those fed the basal diet and the excessive diets ($P < 0.05$). There were no differences in GH content among all treatments, except for the 3.64 mg/kg diet, which had a significantly higher GH content than that fed the 0.73 mg/kg diet ($P < 0.05$). Fish fed the basal diet had significantly higher cortisol concentrations than those fed the Cr-Met diets ($P < 0.05$), whereas the INS and GC concentrations were not significantly influenced by the dietary Cr^{3+} levels ($P > 0.05$). LDH activity increased significantly as dietary Cr^{3+} levels increased from 0.31 to 1.13 mg/kg; however, with a further increase of dietary Cr^{3+} levels from 1.90 to 3.64 mg/kg, LDH activity decreased significantly ($P < 0.05$).

Table 4. Hematological characteristics of the juvenile *Cyprinus carpio* fed with different dietary CrMet levels

Item	*Cr^{3+} level (mg/kg)*						
	0.31	*0.43*	*0.57*	*0.73*	*1.13*	*1.90*	*3.64*
Glucose (mmol/l)	7.74±0.88^{b}	3.71±0.51^{a}	5.40±1.21^{a}	3.58±0.91^{a}	3.65±0.33^{a}	5.39±1.14^{a}	3.94±0.07^{a}
INS(mIU/L) [1]	4.84±0.00	4.67±0.01	4.59±0.65	4.73±0.92	5.02±0.48	5.39±0.13	5.35±0.37
ISR(nmol/L)[2]	54.54±6.96^{a}	79.87±9.85^{b}	77.45±1.80^{b}	77.62±0.89^{b}	82.65±0.77^{b}	77.58±0.01^{b}	50.04±1.31^{a}
GC(pg/ml) [3]	550.72±52.94	571.64±48.32	569.80±61.07	599.37±17.74	645.14±36.24	664.58±54.57	635.88±47.78
GH(μg/L)[4]	1.50±0.10^{ab}	1.29±0.19^{ab}	1.73±0.26^{ab}	1.24±0.13^{a}	1.76±0.22^{ab}	1.73±0.32^{ab}	1.86±0.14^{b}
Cortisol (nmol/l)	420.60±31.80^{c}	313.50±37.64^{b}	254.57±27.41^{ab}	224.93±22.41^{a}	205.00±18.08^{a}	270.40±22.06^{ab}	276.66±28.79^{ab}
LDH(U/mL)[5]	164.00±21.21^{a}	227.00±14.14^{ab}	235.50±27.58^{ab}	297.50±33.23^{b}	521.00±42.43^{c}	472.50±38.89^{c}	236.50±2.12^{ab}

Date represent mean ± S.D. of three replicates. Values in the same line with different superscripts are significantly different.
[1] INS:Insulin; [2]ISR: Insulin Receptor;[3]GC: Glycogen; [4]GH:Growth hormone; [5]LDH: Lactic dehydrogenase (P<0.05).

Glucose metabolism in hepatopancreas

Enzyme activities in hepatopancreas of juvenile common carp are presented in Table 5. HK and 6-PFK1 activities increased significantly with increasing dietary Cr^{3+} levels from 0.31 to 1.90 mg/kg ($P < 0.05$) and then decreased with a further increase of dietary Cr^{3+} levels. The maximum value of PEPCK activity was observed in fish fed the basal diet, which was significantly higher than those fed the other diets, except for the 0.43 mg/kg diet. PK and SDH activities increased significantly as dietary Cr^{3+} levels increased from 0.31 to 0.73 mg/kg ($P < 0.05$); however, PK and SDH activities decreased with a further increase of the dietary Cr^{3+} levels from 0.73 to 3.64 mg/kg. Fish fed Cr^{3+} diets ranging from 1.09 to 3.64 mg/kg had significantly higher G6PDH activity compared to the basal diet ($P < 0.05$). GS activity increased significantly as dietary Cr^{3+} levels increased from 0.31 to 0.73 mg/kg ($P < 0.05$) and then reached a plateau with further increases of the dietary Cr-Met.

Table 5. Hepatopancreas enzyme activities of the juvenile *Cyprinus carpio* fed with different dietary CrMet levels

Item	*Cr^{3+} level (mg/kg)*						
	0.31	*0.43*	*0.57*	*0.73*	*1.13*	*1.9*	*3.64*
HK[1] (U/mgprot)	6.20±0.38^{a}	9.76±1.07^{ab}	10.60±1.25^{ab}	11.29±0.15^{ab}	14.27±2.12^{b}	32.55±2.91^{d}	25.01±1.71^{c}
PK[2] (U/gprot)	10.61±1.09^{a}	16.34±2.06^{abc}	20.50±3.03^{bc}	22.44±0.53^{b}	17.58±0.73^{abc}	16.84±2.99^{abc}	15.11±1.60^{ab}
6-PFK1[3] (U/L)	78.21±7.33^{a}	81.58±11.88^{ab}	82.98±4.07^{ab}	93.65±3.77^{ab}	108.12±0.53^{bc}	126.56±15.80^{c}	105.84±4.04^{abc}
SDH[4](U/mgprot)	2.75±0.03^{a}	3.34±0.49^{ab}	4.32±0.00^{b}	5.68±0.52^{c}	4.04±0.09^{ab}	3.97±0.36^{ab}	4.38±0.38^{bc}
PEPCK[5] (ng/ml)	13.56±1.04^{c}	12.05±0.30^{c}	5.72±0.08^{ab}	6.54±0.97^{ab}	4.30±0.23^{a}	6.03±0.96^{ab}	8.05±0.57^{b}
G6PDH[6] (ng/ml)	1.09±0.12^{a}	1.17±0.19^{ab}	1.40±0.11^{abc}	1.42±0.15^{abc}	1.90±0.20^{b}	1.79±0.04^{bc}	1.76±0.33^{bc}
GS[7] (ng/ml)	3.40±0.41^{a}	5.28±0.04^{ab}	6.41±0.07^{b}	11.32±1.04^{c}	9.84±0.89^{c}	9.03±0.48^{c}	9.40±0.82^{c}

Data represent mean ± S.D. of three replicates. Values in the same line with different superscripts are significantly different (P<0.05).
[1]HK：Hexokinase; [2]PK：pyruvate kinase; [3]6-PFK1：6-phosphofructo-1-kinase; [4]SDH: succinic acid dehydrogenas; [5]PEPCK：Phosphoenolpyruvate; carboxykinase; [6]G6PDH：Glucose-6-phosphate dehydrogenase; [7]GS: Glycogen synthase.

Discussion

In the present study, in juvenile common carp fed Cr-Met supplemented diets, WG, SGR, FE, and PER improved, glycogen levels in hepatopancreas and muscle increased, and ISR and LDH activities in the serum were enhanced compared to the basal diet. Optimum dietary Cr^{3+} levels also enhanced HK, PK, 6-PFK1, SDH, G6PDH, and GS activities in the liver, reduced glucose and cortisol contents in the serum, and weakened PEPCK activity in the hepatopancreas. These results clearly demonstrate that Cr-Met supplementation improved growth performance and feed utilization. Moreover, dietary Cr^{3+} supplementation showed a positive impact on the carbohydrate utilization of juvenile common carp fed a high dextrin diet.

Fish fed the diet containing 1.13 mg/kg Cr^{3+} demonstrated improved growth performance mainly due to the significantly higher PER and improved FE. These results are in accordance with previously reported results, which were obtained using chromium chloride (Shiau and Lin, 1993), and chromic oxide (Shiau and Shy 1998) supplementation in tilapia, chromium picolinate supplementation in grass carp (Liu *et al*., 2010) and chromium polynicotinate supplementation in juvenile large yellow croaker *Larmichthys crocea* (Wang *et al*., 2014). Research in humans and rats has shown that Cr^{3+} enhanced glucose tolerance, facilitated the glycolysis pathway, and eventually improved carbohydrate utilization (Sahin *et al*., 2001). Therefore, in the present study, improved PER in fish fed Cr-Met supplementation diets may be due to Cr^{3+} improving carbohydrate utilization and inhibiting gluconeogenesis from amino acids. It is noteworthy that high levels of Cr^{3+} led to depressed growth performance in this experiment. Decreases in growth and feed efficiency in fish fed high-chromium supplementation diets have also been observed in large yellow croaker (Wang *et al*., 2014), grass carp (Liu *et al*., 2010), tilapia (Shiau and Liang, 1995) and rainbow trout (Tacon and Beveridge, 1982). These results may be related to the toxic effects of high levels of Cr supplementation. Cr exposure was found to have a toxic effect of *Colisa fasciatus*, as evidenced by damaged gills, enhanced mucus secretion and increased blood lactaten (Nath and Kumar, 1987). These findings provided an explanation for the depressed growth performance of common carp supplemented with the 3.64 mg/kg Cr^{3+} diet in the present study.

Dietary Cr^{3+} supplementation had no significant effect on carcass composition in this study, and similar results were also observed in tilapia (Pan *et al*., 2003), gilthead seabream *S. aurata L.* (Gatta *et al*., 2001), and common carp with chromium chloride (Ahmed *et al*., 2013). Glucose is stored as glycogen in the liver and muscle via glycogenesis, catalyzed by glycogen synthase. Glycogen synthesis is an important component of glucose metabolism. In the current study, the highest hepatopancreas and muscle glycogen levels were observed in 0.73 and 1.90 mg/kg of the supplementation diets, respectively, which was attributed to the increased activity of glycogen synthase (GS). These results indicated that Cr-Met supplementation promoted carbohydrate anabolism.

Trivalent Cr is generally believed to be associated with the function of insulin. The present assay showed no effect of Cr-Met supplementation on the insulin concentration, while significantly higher ISR levels and lower glucose concentrations were observed in fish fed the diets containing not more than 1.90 mg/kg Cr^{3+}. These findings are consistent with previous findings in which Cr stimulated the biological activity of insulin by increasing the insulin receptor number or binding activity, thus regulating the uptake of blood glucose and utilization by cells (Anderson et al., 1991). In addition, the decreased glucose concentration and increased ISR level probably indicated that increased glucose utilization led to an improvement in WG and FE, which explain the growth-promoting effect observed in the current study.

Cortisol is generally considered to be a stress indicator in teleost fish (Barton and Iwama, 1991), as it prevents glucose entry into peripheral tissues (e.g., muscle and fat) so that it can be available for use by tissues that have a greater need for glucose, for example the brain and liver (Borgs, 1998). In this trial, cortisol concentration in fish fed the basal diet was significant higher than in those fed diets supplemented with Cr-Met. The results indicated that Cr-Met also has an "anti-stress" effect by lowering plasma cortisol. These

results are in accordance with previous findings by Marcello *et al*. (2014) who observed that a chromium carbochelate supplementation diet in Nile tilapia could diminish cortisol.

Trivalent Cr affects glucose metabolism of the body by regulating the glucose metabolism enzymes. Hexokinase acts as the initial and key enzyme in the glycolytic pathway and plays a vital role in glucose homeostasis by catalyzing the conversion of the γ-phosphate group from ATP to the 6th-hydroxyl group of D-glucose, thereby forming D-glucose-6-phosphate and ADP. In the present study, fish fed diets containing Cr^{3+} ranging from 1.13 to 3.64 mg/kg had significantly higher HK activities than those fed the basal diet. This may be due to the direct stimulation of glycolysis in tissues with increased glucose oxidation, resulting in a decreased glucose concentration and increased ATP production. However there was a report that the HK activity of common carp was not affected by dietary $CrCl_3$ supplementation (Ahmed *et al*. 2013). These differences may be due to the different forms of chromium employed (organic Cr-Met versus inorganic $CrCl_3$). Organic forms of Cr have higher bioavailability and stability in the physiological milieu than inorganic forms (NRC, 1997). Organic Cr (yeast-incorporated and Cr Cr-amino acids complex) was found to be more effective than inorganic Cr due to the much higher rate of absorption from the gastrointestinal tract (Kornegay, 1996). In addition to HK, 6-phosphofructo-1-kinase (PFK-1) and pyruvate kinase (PK) were also key controlling enzymes in the regulation of the glycolytic pathway. In our study, 6PFK-1 activity in the hepatopancreas of fish fed the 1.13 and 1.90 mg/kg Cr^{3+} diets showed a remarkable increase compared to fish fed the basal diet in our study, whereas fish fed a diet containing 0.73 mg/kg Cr^{3+} showed an significant increase in PK activity in the hepatopancreas, which may be responsible for the enhanced glycolysis and diminished gluconeogenesis.

Glucose-6-phosphate dehydrogenase (G6PDH) is the key enzyme in the pentose phosphate pathway of glucose metabolism, as it provides pentose phosphates for nucleic acid synthesis, nicotinamide adenine dinucleotide (NADPH) for synthetic reactions and maintenance of the cellular redox status (Cappai *et al*., 2011). In the current trial, G6PDH activity in the hepatopancreas significantly increased with the increase of dietary Cr^{3+} levels from 1.13 to 3.64 mg/kg, which suggested an enhancement in glucose metabolism through the phosphogluconate oxidation pathway. This result can be explained by improved insulin secretion and action. Cr^{3+} supplementation stimulated the influx of glucose into the pentose mono-phosphate shunt, contributing to the increase in glucose production of the reducing agent NADPH with a concomitant decrease in oxidative stress. Inconsistent results were observed by Pan *et al*. (2013), who reported that tilapia fed a Cr-Pic supplementation diet exhibited no change in G6PDH activity. Different aquaculture models (static water system versus flow-through system), species-specific differences in carbohydrate utilization, and dietary formulations may provide explanations for the difference between the present experiment and previous result.

Phosphoenolpyruvate carboxykinase (PEPCK) serves as a key rate-limiting enzyme of gluconeogenesis and catalyzes the transfer of oxaloacetate to phosphoenolpyruvate (NRC, 2011). It is reported that Cr^{3+} directly inhibits PEPCK activity by forming a nucleic acid derivative in the body. Cr^{3+} complexes were observed to be linear competitive inhibitors of avian PEPCK (Kramer and Nowak, 1988). Similarly, the present study demonstrated that fish fed diets with 0.57 to 3.64 mg/kg Cr^{3+} had significantly lower PEPCK activities than that fed the basal diet. The reduction in the activity of this enzyme could lead to decreased gluconeogenesis, thereby reducing endogenous glucose production.

In summary, the present study indicates that dietary addition of Cr-Met can improve growth performance and feed utilization efficiency as well as have a significant impact on the carbohydrate utilization of juvenile common carp fed a high dextrin diet. A two-slope broken-line model based on SGR against dietary Cr^{3+} levels demonstrated that the optimal dietary Cr^{3+} level was estimated to be 1.09 mg /kg.

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