Effects of Different Chromium Sources on Growth Performance, Serum Biochemical Indices, and Hepatic and Pancreatic Glucose Metabolism Enzyme Activities in Carp Fed High-Glucose Diets: Postprint

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Abstract

This study was conducted to investigate the effects of different dietary chromium sources on growth performance, serum biochemical indices, and hepatopancreatic glucose metabolic enzyme activities in common carp fed high-glucose diets. Four purified diets were formulated: a basal diet without chromium supplementation and three experimental diets supplemented with chromium(III) oxide (Cr2O3), chromium picolinate (CrPic), and chromium methionine (CrMet), respectively, with chromium supplementation levels in the experimental diets at approximately 2.60 mg/kg (calculated as trivalent chromium ions). A total of 720 common carp with an initial body weight of (40.95$±$4.80) g were randomly allocated into 4 groups, with 3 replicates per group and 60 fish per replicate. Each group was fed one diet, with the group receiving the basal diet serving as the control. The feeding trial lasted for 60 days. The results showed that, compared with the control group, supplementation with CrPic and CrMet significantly improved weight gain rate (WGR), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER) in common carp ($P<0.05$); Cr2O3 supplementation significantly improved WGR and SGR ($P<0.05$); supplementation with the three chromium sources had no significant effects on whole-body moisture, ash, or protein content ($P>0.05$), while CrPic and CrMet supplementation significantly increased whole-body lipid content ($P<0.05$); all three chromium sources significantly increased muscle glycogen content ($P<0.05$), whereas hepatic glycogen content was significantly increased only in the CrMet group ($P<0.05$); CrMet supplementation significantly decreased serum triglyceride (TG) and total cholesterol (TC) levels ($P<0.05$); CrMet supplementation significantly increased insulin (INS), insulin receptor (ISR), and growth hormone (GH) levels, as well as lactate dehydrogenase (LDH) and creatine kinase (CK) activities ($P<0.05$), and significantly decreased serum glucose and cortisol (COR) levels ($P<0.05$); all three chromium sources significantly increased the activities of pyruvate kinase (PK) and hexokinase (HK) in the hepatopancreatic glycolytic pathway ($P<0.05$), while CrPic and CrMet supplementation significantly decreased phosphoenolpyruvate carboxykinase (PEPCK) activity in the hepatopancreatic gluconeogenic pathway ($P<0.05$). In conclusion, for common carp fed high-glucose diets, CrMet exhibited the most pronounced effects in promoting growth, improving feed utilization, and enhancing glucose utilization capacity, followed by CrPic, while Cr2O3 showed the least pronounced effects.

Full Text

Effects of Different Chromium Sources on Growth Performance, Serum Biochemical Indices and Hepatopancreas Glucose Metabolism Enzyme Activities of Common Carp Fed High Glucose Diets

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Abstract

This study investigated the effects of different chromium sources in feed on growth performance, serum biochemical indices, and hepatopancreas glucose metabolism enzyme activities of common carp (*Cyprinus carpio*) fed high glucose diets. Four purified diets were formulated: a basal diet without chromium supplementation and three experimental diets in which the basal diet was supplemented with chromic oxide (Cr_2O_3), chromium picolinate (CrPic), or chromium methionine (CrMet). The chromium supplementation level in experimental diets was approximately 2.60 mg/kg (calculated as trivalent chromium ion). A total of 720 common carp with initial body weight of (40.95$±$4.80) g were randomly divided into four groups with three replicates per group and 60 fish per replicate. Each group was fed one of the four diets, with the basal diet serving as the control. The feeding trial lasted for 60 days.

The results showed that compared with the control group, supplementation with CrPic and CrMet significantly increased weight gain rate (WGR), specific growth rate (SGR), feed efficiency (FE), and protein efficiency ratio (PER) ($P < 0.05$). Cr_2O_3 supplementation significantly increased WGR and SGR ($P < 0.05$). The three chromium sources had no significant effects on whole-body moisture, protein, or ash content ($P > 0.05$), but CrPic and CrMet significantly increased whole-body lipid content ($P < 0.05$). All three chromium sources significantly increased muscle glycogen content ($P < 0.05$), while hepatic glycogen content was significantly increased only in the CrMet group ($P < 0.05$). CrMet supplementation significantly decreased serum triglyceride (TG) and total cholesterol (TC) contents ($P < 0.05$). CrMet significantly increased serum insulin (INS), insulin receptor (ISR), and growth hormone (GH) contents, as well as lactate dehydrogenase (LDH) and creatine kinase (CK) activities ($P < 0.05$), while significantly decreasing serum glucose and cortisol (COR) contents ($P < 0.05$). All three chromium sources significantly increased hepatopancreas hexokinase (HK) and pyruvate kinase (PK) activities in the glycolytic pathway ($P < 0.05$), while CrPic and CrMet significantly decreased hepatopancreas phosphoenolpyruvate carboxykinase (PEPCK) activity in the gluconeogenesis pathway ($P < 0.05$). In conclusion, for common carp fed high glucose diets, CrMet demonstrated the most pronounced effects in promoting growth and improving feed utilization and glucose utilization capacity, followed by CrPic, while Cr_2O_3 showed the least beneficial effects.

Key words: chromium; growth performance; serum biochemical indices; glucose metabolism enzyme activity; common carp

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Carbohydrates are the most economical energy source in animal feeds. If inexpensive carbohydrates can be utilized as an energy source to replace part of the protein, achieving "protein-sparing effects," this could not only reduce dietary protein content and feed costs but also protect fishery resources and reduce nitrogen pollution in aquaculture systems [1]. However, compared with terrestrial animals, fish have poor carbohydrate utilization capacity [2-3]. Excessive dietary carbohydrate levels can lead to growth retardation, reduced feed utilization, weakened disease resistance, and even mortality in fish [4-5].

Trivalent chromium (Cr^{3+}) is an essential trace element that plays important roles in carbohydrate, protein, and lipid metabolism [6-7]. Studies have shown that Cr^{3+} enhances insulin action and improves glucose tolerance [8-9]. Previous research has confirmed that Cr^{3+} can effectively improve growth performance in grass carp (*Ctenopharyngodon idellus*) [10], rainbow trout (*Oncorhynchus mykiss*) [11], large yellow croaker (*Larmichthys crocea*) [12], and tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) [13], enhance immune function in Mozambique tilapia [14] and rainbow trout [15], and improve carbohydrate utilization in striped bass (*Morone saxatilis*) [16]. Additionally, Cr^{3+} can effectively reduce plasma cortisol content and improve stress status [17-18].

Some studies have examined the effects of Cr^{3+} on growth and feed utilization in common carp [19-20], but these investigations primarily used inorganic chromium sources. Research has demonstrated that organic chromium exhibits higher absorption rates, biological activity, and stability compared with inorganic chromium [21]. The most widely used chromium sources in livestock and poultry are chromium picolinate (CrPic) and amino acid-chelated chromium (CrMet). These two sources are chelates of Cr^{3+} with picolinic acid and methionine, respectively, which can effectively alleviate antagonistic competition among mineral elements and facilitate chromium absorption. However, few reports have addressed the safe dosage of organic chromium as a feed additive for common carp and its physiological effects.

This study utilized glucose—a poorly utilized carbohydrate source for common carp—to compare the effects of three different chromium forms (inorganic chromium, organic acid chromium, and amino acid chromium) on growth performance, serum biochemical indices, and hepatopancreas glucose metabolism enzyme activities in carp fed high glucose diets, providing a theoretical basis for chromium application in formulated feeds for common carp.

1.1 Experimental Diet Formulation

Four purified diets were formulated using casein as the protein source, soybean oil as the lipid source, and glucose as the carbohydrate source. The diets included a basal diet without chromium supplementation and three experimental diets supplemented with Cr_2O_3, CrPic, or CrMet, respectively. The chromium supplementation level in experimental diets was approximately 2.60 mg/kg (calculated as Cr^{3+}). CrPic was purchased from Sinopharm Chemical Reagent Co., Ltd. with an effective content of 98% and chromium content of 12.4%. CrMet was purchased from Hubei Tuochukangyuan Pharmaceutical Chemical Co., Ltd. with an effective content of 99% and chromium content of 80%. All solid feed ingredients were passed through an 80-mesh sieve, mixed by stepwise amplification, and processed into pelleted feeds using a twin-screw extruder (manufactured by the Mechanical Engineering Research Institute of South China University of Technology). Two pellet sizes were produced (diameters of 1.5 and 3.2 mm), and the feeds were dried in an oven (DK400, Yamato, Japan) at 40°C.

1.2 Experimental Fish and Management

Experimental fish were provided by Tianjin Chenhui Feed Co., Ltd. and transported to the company' s aquaculture laboratory. After a two-week acclimation period during which they were fed the basal diet (without Cr^{3+}), healthy fish of uniform size [initial body weight (40.95$±$4.80) g] were randomly distributed into 12 blue circular plastic tanks (800 L) with three replicates per group and 60 fish per replicate. The culture system was static. Experimental water was aerated tap water (aerated for over 24 h). Fish were fed twice daily (07:00 and 16:00) at a rate of approximately 4-6% of body weight, with feeding amount adjusted every two weeks. Water was exchanged once daily with simultaneous siphoning of feces, with a replacement volume of one-third of the total water. During the experimental period, water temperature was 15-25°C, pH was 7.6-7.8, ammonia nitrogen was $ $0.05 mg/L, and dissolved oxygen was $ $6.0 mg/L. The feeding trial lasted for 60 days.

1.3 Sample Collection

After 60 days of feeding, fish were fasted for 24 h, anesthetized with eugenol (1:10,000), weighed, and counted. Six fish from each tank were stored at -20°C for whole-body composition analysis. Another 10 fish per tank were sampled via caudal vein puncture, and blood was centrifuged for 5 min (4°C, 3,500 r/min). Serum was collected and stored for biochemical analysis. After blood collection, fish were dissected on ice to obtain hepatopancreas and muscle tissues for determination of glucose metabolism enzyme activities and glycogen content. All samples were stored at -80°C until analysis.

1.4 Analytical Methods

Proximate composition of experimental diets and fish were determined according to AOAC (1995) [22]: moisture content by constant weight drying method (GB/T 5009.3-2010), crude protein by Dumas combustion method (GB/T 24318-2009) using a Thermo Fisher Scientific FLASH2000 automatic protein analyzer (Thermo, USA), crude lipid by Soxhlet extraction method (GB/T 5009.6-2010) using a Gerhardt SOXTHERM Soxhlet extractor (Gerhardt, Germany), and crude ash by gravimetric method after incineration (GB/T 5009.4-2010).

Dietary chromium content was determined by atomic absorption spectrometry (graphite furnace atomicization). Hepatopancreas and muscle glycogen contents were determined using kits from Nanjing Jiancheng Bioengineering Institute according to the method of Hassid et al. [23]. Briefly, hepatopancreas or muscle tissue was mixed with strong alkali at a 1:3 (mass:volume) ratio, boiled in a water bath, treated with acetaldehyde, and finally reacted with anthrone reagent. Absorbance was measured at 620 nm using a spectrophotometer.

Serum glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatine kinase (CK), and lactate dehydrogenase (LDH) were analyzed by Tianjin Kingmed Diagnostics using a Roche C311 automatic biochemical analyzer (Roche, Germany). Serum insulin (INS), glucagon (GC), insulin receptor (ISR), growth hormone (GH), and cortisol (COR) were determined by enzyme-linked immunosorbent assay (ELISA) kits. INS and GC kits were purchased from Assay Designs (USA), while ISR and GH kits were from Shanghai Enzyme-linked Biotechnology Co., Ltd.

Hepatopancreas hexokinase (HK), pyruvate kinase (PK), and succinate dehydrogenase (SDH) activities were determined using kits from Nanjing Jiancheng Bioengineering Institute. Hepatopancreas phosphofructokinase (6-PFK1), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphate dehydrogenase (G6PDH), glycogen synthase (GS), and fatty acid synthetase (FAS) activities were determined by double-antibody sandwich ELISA using kits from Assay Designs (USA). All assays were performed strictly according to kit instructions. For ELISA kits, samples were added to microplates followed by corresponding antibodies, incubated at 37°C for 60 min, and then washed.

1.5 Calculation Formulas

Weight gain rate (WGR, %) = [(Wt - W0)/W0] × 100
Specific growth rate (SGR, %/d) = [(ln Wt - ln W0)/t] × 100
Feed efficiency (FE) = (Wf + Wd - W0)/C
Protein efficiency ratio (PER) = (Wt - W0)/Cp
Survival rate (SR, %) = (Number of surviving fish/Total number of fish) × 100

Where: W0 = initial body weight of experimental fish; Wt = final body weight of experimental fish; t = experimental days; C = feed intake; Wf = total final weight of experimental fish; Wd = total weight of dead fish; Cp = protein intake.

1.6 Statistical Analysis

Data were analyzed by one-way ANOVA using SPSS 19.0 software. If significant differences were detected ($P < 0.05$), Tukey’ s test was used for multiple comparisons. All data are expressed as mean ± standard deviation (mean±SD).

2 Results

The effects of different chromium sources on growth performance of common carp fed high glucose diets are shown in Table 2 . Survival rates ranged from 91.67% to 95.00% with no significant differences among groups ($P > 0.05$). WGR and SGR in chromium-supplemented groups were significantly higher than in the control group ($P < 0.05$), with the highest values observed in the CrMet group, though not significantly different from the CrPic group ($P > 0.05$). Compared with the control group, FE and PER were significantly increased in CrPic and CrMet groups ($P < 0.05$), but showed no significant change in the Cr_2O_3 group ($P > 0.05$).

The effects of different chromium sources on whole-body proximate composition of common carp fed high glucose diets are presented in Table 3 . Whole-body lipid content in CrPic and CrMet groups was significantly higher than in the control and Cr_2O_3 groups ($P < 0.05$). Hepatic glycogen content in the CrMet group was significantly higher than in the control and Cr_2O_3 groups ($P < 0.05$). Muscle glycogen content was significantly increased in all chromium-supplemented groups ($P < 0.05$). No significant differences were observed among groups in whole-body moisture, protein, or ash content ($P > 0.05$).

The effects of different chromium sources on serum biochemical indices of common carp fed high glucose diets are shown in Table 4 . Serum TG and TC contents were significantly decreased in the CrMet group compared with the control group ($P < 0.05$), while no significant changes were observed in Cr_2O_3 and CrPic groups ($P > 0.05$). No significant differences were detected among groups in serum HDL and LDL contents ($P > 0.05$). Serum INS and ISR contents were significantly increased in the CrMet group compared with the control group ($P < 0.05$), but showed no significant changes in Cr_2O_3 and CrPic groups ($P > 0.05$). Serum GH content and LDH activity were significantly increased in all chromium-supplemented groups ($P < 0.05$), while serum glucose and COR contents were significantly decreased ($P < 0.05$), with no significant differences among chromium sources ($P > 0.05$). Serum CK activity was significantly increased in Cr_2O_3 and CrMet groups ($P < 0.05$) but showed no significant change in the CrPic group ($P > 0.05$). No significant differences were observed among groups in serum GC content ($P > 0.05$).

The effects of different chromium sources on hepatopancreas glucose metabolism enzyme activities of common carp fed high glucose diets are presented in Table 5 . Hepatopancreas HK and PK activities were significantly increased in all chromium-supplemented groups compared with the control group ($P < 0.05$). SDH activity was highest in the CrPic group, significantly higher than in all other groups ($P < 0.05$). PEPCK activity was significantly decreased in CrPic and CrMet groups compared with the control group ($P < 0.05$), while FAS activity was significantly increased in these groups ($P < 0.05$). GS activity was significantly increased in all chromium-supplemented groups ($P < 0.05$). No significant differences were observed among groups in hepatopancreas 6-PFK1 and G6PDH activities ($P > 0.05$).

3 Discussion

The present results demonstrated that supplementation with all three chromium sources improved growth performance in common carp, consistent with findings in carp fed diets supplemented with chromium chloride ($CrCl_3$) [19], tilapia fed diets with CrPic and chromium nicotinate (CrNic) [24], large yellow croaker fed diets with CrNic [12], and tilapia fed glucose-based diets supplemented with Cr_2O_3 and $CrCl_3$ [25-28]. However, some studies reported that dietary CrPic supplementation had no significant effects on growth performance in rainbow trout and tilapia [11, 28-29], and similar results were observed in golden porgy (*Sparus aurata*) [30] and rainbow trout [31] fed yeast chromium. These discrepancies may be related to fish species, chromium form and level, culture conditions, and experimental design. The current study also showed that organic chromium was more effective than inorganic chromium in promoting carp growth. Generally, organic chromium exhibits higher biological activity and stability than inorganic chromium under physiological conditions, and its absorption rate is substantially higher. Studies have shown that inorganic chromium absorption is only 1-3% [32], whereas organic chromium absorption reaches 10-25% [33]. Therefore, the superior results observed with organic chromium in this study may be attributed to higher chromium absorption and more effective growth promotion, similar to findings in grass carp [34].

The results showed that different chromium sources had no significant effects on whole-body moisture, protein, or ash content, while organic chromium significantly increased whole-body lipid content, consistent with previous studies on tilapia [28,35]. Research has demonstrated that Cr^{3+} participates in nutrient metabolism by synergizing with insulin to enhance activities of relevant metabolic enzymes, thereby altering nutrient deposition patterns [36-37]. The increased whole-body lipid content in organic chromium groups in this study may be due to enhanced hepatopancreas FAS activity, affecting whole-body lipid content, similar to observations in pigs supplemented with chromium [38-39].

Glucose is synthesized into glycogen via gluconeogenesis catalyzed by glycogen synthase (GS) and stored in various tissues, with liver and muscle being the primary storage sites [40]. Steele et al. [41] reported that $CrCl_3$ effectively increased glycogen content in turkey poults. Similarly, the present study found that all three chromium sources significantly increased muscle glycogen content, with hepatic glycogen content also increased and significantly higher in the CrMet group compared with the control. Detection of hepatopancreas GS activity revealed that all three chromium sources significantly increased this enzyme' s activity, indicating significant effects in promoting glycogen synthesis in hepatopancreas and muscle. Comparable results were reported in mice by Campbell et al. [42].

The primary role of chromium in lipid metabolism is maintaining normal blood cholesterol levels. Studies have shown that dietary CrPic supplementation effectively reduced serum TC content in tilapia [43]. The present results are similar, with CrMet significantly decreasing serum TG and TC contents and improving lipid profiles. However, Liu [34] found no effect of different chromium forms on serum TC content in grass carp, possibly due to differences in fish species, dietary nutrient composition, and culture conditions.

Research has confirmed that Cr^{3+} can accelerate blood glucose disappearance rate and shorten glucose half-life, thereby reducing serum glucose content [44]. Studies on tilapia demonstrated that different chromium sources (Cr_2O_3, $CrCl_3$, CrNic) improved dietary carbohydrate utilization [24,25,28,43]. Liu [34] also confirmed that CrPic and CrNic improved carbohydrate utilization in grass carp. The present results showed that CrMet significantly reduced serum glucose content, consistent with the above findings, whereas Cr_2O_3 and CrPic had no effect. This suggests that chromium in the CrMet form may enhance chromium absorption, increase insulin sensitivity, and strengthen glucose metabolism, thereby reducing serum glucose content. The significant increase in serum LDH activity after CrMet supplementation also indicated enhanced glucose metabolism.

Liu et al. [10] found that appropriate CrPic supplementation significantly increased serum INS content in grass carp. The present study showed similar results, with CrMet significantly increasing serum INS content. Research has demonstrated that Cr^{3+} increases cell glucose sensitivity by increasing ISR content or promoting INS binding to cell membrane ISR [9,33]. The significant increase in serum ISR content in the CrMet group confirmed this mechanism. Meanwhile, serum COR content was significantly decreased in all chromium-supplemented groups, reflecting the typical metabolic relationship between INS (anabolic) and COR (catabolic), where INS and COR contents are inversely correlated. Chromium supplementation did not alter this relationship. The increased serum INS content demonstrated chromium' s physiological role as a cofactor for insulin, consistent with findings in laying hens where CrPic increased plasma INS and decreased COR under low temperature conditions [45].

Since Cr^{3+} directly stimulates tissue glycolysis and accelerates glucose oxidation, glucose content decreases while ATP generation increases. However, Ahmed et al. [19] reported that $CrCl_3$ supplementation had no significant effect on HK activity in carp. This discrepancy may be due to different chromium forms and supplementation levels. Besides HK, 6-PFK1 and PK are also key enzymes controlling glycolytic rate. PK catalyzes the final step of glucose conversion to pyruvate, converting phosphoenolpyruvate to pyruvate. The present results showed that PK activity in hepatopancreas was significantly increased in carp fed diets supplemented with all three chromium sources, indicating that these chromium sources can promote the glycolytic pathway.

PEPCK catalyzes the first step of gluconeogenesis, converting oxaloacetate to phosphoenolpyruvate [46]. Studies have found that Cr^{3+} can directly inhibit PEPCK activity by forming nucleic acid derivatives in animals [47]. Gardner et al. [48] confirmed that INS itself can inhibit PEPCK activity by suppressing PEPCK gene expression. Since chromium synergizes with INS, chromium supplementation can indirectly inhibit PEPCK activity by enhancing INS action. The present results are consistent with these studies, with CrPic and CrMet significantly inhibiting PEPCK activity in carp hepatopancreas. Reduced PEPCK activity may slow gluconeogenesis and decrease endogenous glucose production.

G6PDH is involved in NADPH synthesis and maintains cellular redox status [49]. In this study, supplementation with all three chromium sources had no significant effect on G6PDH activity, consistent with results in tilapia [29].

4 Conclusion

Supplementation of 2.92 mg/kg Cr_2O_3, 16.46 mg/kg CrPic, or 2.52 mg/kg CrMet in diets containing 35% glucose all promoted growth, feed utilization, and glucose utilization capacity in common carp, with CrMet showing the best effects, followed by CrPic, and Cr_2O_3 showing the poorest effects.

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