

Effect of dietary supplementation of chromium on growth and biochemical parameters of *Labeo rohita* (Hamilton) fingerlings

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ABSTRACT

A 60-day feeding experiment was conducted to evaluate the effect of dietary chromium on growth, feed efficiency and biochemical parameters of *Labeo rohita* fingerlings. Four isonitrogenous (crude protein 35%) and isocaloric (415 kcal 100 g⁻¹) experimental feeds were prepared by supplementing different levels of dietary chromium picolinate viz., control (0.0 mg kg⁻¹), T1 (0.4 mg kg⁻¹), T2 (0.8 mg kg⁻¹) and T3 (1.2 mg kg⁻¹). Weight gain WG (%), specific growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio (PER) and apparent net protein utilisation (ANPU %) were significantly improved (p<0.05) when chromium was supplemented at 0.8 mg kg⁻¹ feed. The protein retention (PR %) value increased with the dose of chromium, showing the highest value in T2 group. Chromium supplementation significantly increased (p<0.05) liver glycogen in T1 and T2 groups but decrease was observed at high level of chromium supplementation in T3 group. Chromium supplementation significantly reduced (p<0.05) serum cholesterol and triglycerides in all the experimental groups compared to control showing the highest reduction in T2 group. The serum high density lipoproteins-cholesterol (HDL-C) was increased (p<0.05) in all experimental groups due to chromium supplementation and the highest blood HDL-C was observed in T2 group. However, no difference (p<0.05) in the serum low density lipoproteins-cholesterol (LDL-C) and phospholipid was observed in any of the experimental groups. Similarly, highest muscle protein as well as lowest liver AST and ALT were observed in T2 group. The results of the present study indicates that growth, feed utilisation and biochemical parameters in *Labeo rohita* can be significantly improved by feeding the fingerlings with chromium picolinate supplemented diet (0.8 mg kg⁻¹ feed).

Keywords: Cholesterol, Chromium, Glycogen, Growth, *Labeo rohita*

Introduction

Chromium (Cr) is an important trace element which plays a vital role in animal physiology (Mertz, 1993). It regulates carbohydrate metabolism as a structural component of glucose tolerance factor (GTF) by potentiating the action of insulin (Rosebrough and Steele, 1981; Mertz, 1993) which increases the absorption of glucose from circulation into peripheral tissues (Anderson, 1987). This essential trace element is also involved in the metabolism of lipid, protein, and nucleic acid (Rosebrough and Steele, 1981; Okada *et al.* 1983; Ohba *et al.* 1986; Press *et al.* 1990; McCarty, 1991). Cr supplementation increases animal growth performance by enhancing energy metabolism (Jacques and Stewart, 1993). Organic forms of chromium have a higher bioavailability than the

inorganic forms (NRC, 1997). Research on animal models confirmed that organic form of dietary chromium such as chromium picolinate (CrPic), chromium nicotinate (CrNic), and chromium-enriched yeast, is absorbed more efficiently, about 25-30 % more than inorganic forms like chromic chloride (CrCl₃) or chromic oxide (Cr₂O₃), which are poorly absorbed (1-3%) regardless of dose or dietary chromium status (Underwood, 1977; Mowat 1994; Olim *et al.*, 1994). Chromium picolinate, an organic and low-toxic form of trivalent chromium (Cr³⁺), is an essential element for optimum carbohydrate, lipid, protein and nucleic acid metabolisms (McCarty, 1991; Mertz, 1993), as well as for activating certain enzymes and stabilising proteins and nucleic acids (Anderson, 1987; Mertz, 1993).

The effect of chromium supplementation on growth performance of targeted animal is equivocal. Chromium has been reported to enhance carbohydrate metabolism in both turkeys (Rosebrough and Steele, 1981) and humans (Levine *et al.*, 1968). It has been reported that organic chromium supplementation to diets of rats (Gray and Bowman, 1992), mice (Morris *et al.*, 1995), chicken (Lien *et al.*, 1999) and feeder calves (Kegley *et al.*, 1997) had positive effects on glucose metabolism and insulin activity. It has been reported that dietary inorganic Cr supplements as Cr₂O₃ (Shiau and Liang, 1995; Shiau and Shy, 1998) and CrCl₃ (Shiau and Lin, 1993) can significantly improve growth and feed utilisation parameters in hybrid tilapia fed diets containing high levels of glucose. Similar results have also been obtained on the same species when organic Cr (*i.e.*, Cr-Nic or Cr-Pic) was supplemented to the diet rich in glucose content (Pan *et al.*, 2002a). The growth enhancement by chromium supplementation at certain dosages has also been reported by Tacon and Beveridge (1982) in trout and by Jain *et al.* (1994) in Indian major carp. In contrary, it has also been reported that Cr supplementation has no significant effect on weight gain in hybrid tilapia when fed as Cr-Pic (Pan *et al.*, 2003) or Cr-Nic (Pan *et al.*, 2002b). Similarly, no significant improvement was observed in growth performance of gilthead seabream (Gatta *et al.*, 2001) and rainbow trout (Bureau *et al.*, 1995; Selcuk *et al.*, 2010) when fed diet supplemented with Cr-yeast or Cr-Pic. Moreover, no significant effect on growth performance of Nile tilapia was observed by feeding Cr-Pic supplemented feed (El-Sayed *et al.*, 2010; Mehrim, 2012). The results of the previous experiments suggest chromium plays an important role in fatty acid metabolism and can alter the serum fatty acid profile (Evock-Clover *et al.*, 1993, Kitchalong *et al.*, 1995; Min *et al.*, 1997; Wang *et al.*, 2007, Zha *et al.*, 2007, Wang *et al.*, 2009). Krolczewska *et al.* (2004) as well as Patil *et al.*, (2008) reported decrease in serum total cholesterol, LDL-C, triglycerides and increased serum HDL-C when broiler chickens were fed with diet supplemented with chromium picolinate. A similar decrease in the serum lipid profile has been reported in grass carp when fed diet supplemented with chromium picolinate (Liu *et al.*, 2010). The present study was conducted to assess the effect of dietary chromium on growth performance and other biochemical parameters to elucidate its role in lipid and protein metabolism in the Indian major carp, rohu (*Labeo rohita*).

Materials and methods

Experimental animals

Two hundred and seventy *Labeo rohita* fingerlings (13.59 ± 0.02 g) were procured from Hans Aquaculture,

Raigad, Maharashtra. The animals were acclimatised for 45 days prior to the experiment in a 3000 l capacity rectangular tank and fed on an isocaloric basal diet containing 35% crude protein to satiation. Continuous aeration was provided along with 50% replacement of water with fresh borewell water.

Preparation of experimental diets

Chromium piconilate was procured from Oceanic Laboratories (P) Ltd., Tarapur, Mumbai, India, and four isonitogenous (crude protein 35%) and isocaloric (415 k cal 100 g⁻¹) experimental feeds were prepared by supplementing different levels of dietary chromium picolinate *viz.*, control (0.0 mg kg⁻¹), T1 (0.4 mg kg⁻¹), T2 (0.8 mg kg⁻¹) and T3 (1.2 mg kg⁻¹) (Table 1).

Table 1. Formulation and composition of the experimental diet

Ingredients (g 100 g ⁻¹)	Control	T2	T3	T4
Casein	31.0	31.0	31.0	31.0
Gelatin	12.0	12.0	12.0	12.0
Dextrin white	11.0	11.0	11.0	11.0
Starch soluble	27.0	27.0	27.0	27.0
Cellulose powder	8.50	8.50	8.50	8.50
Carboxy methyl cellulose	1.0	1.0	1.0	1.0
¹ Sunflower oil	4.0	4.0	4.0	4.0
² Cod liver oil	3.50	3.50	3.50	3.50
³ Vitamin-mineral mix	1.92	1.92	1.92	1.92
Vitamin C	0.03	0.03	0.03	0.03
Betaine hydrochloride	0.03	0.03	0.03	0.03
Butylatedhydroxy toluene	0.02	0.02	0.02	0.02
Chromium piconilate (supplemented)	0.00	0.00004	0.00008	0.00012
Proximate composition (g 100 g ⁻¹)				
Moisture	8.02	7.99	7.87	7.93
Carbohydrate (%)	52.64	51.81	52.53	52.61
Crude protein (%)	34.77	35.47	35.23	34.77
Crude fat (%)	7.22	7.07	7.22	7.00
Ash (%)	5.37	5.65	5.02	5.62

¹Sunflower oil, Nature Fresh, Cargill India Pvt. Ltd.: Saturated fatty acids - 10.1; MUFA - 45.4; PUFA - 40.1; Trans fatty acids <-0.5)

²Cod liver oil (Type B) BP, Universal medicare Pvt. Ltd., Mumbai

³vitamin mineral mix (EMIX PLUS) (quantity/2.5kg): Vitamin A: 55,00,000 IU; Vitamin D3: 11,00,000 IU; Vitamin B2:2,000 mg; Vitamin E: 750 mg; Vitamin K: 1,000 mg; Vitamin B6: 1,000 mg; Vitamin B12 : 6 mcg; Calcium pantothenate: 2,500 mg; Nicotinamide: 10 g; Choline chloride: 150 g; Mn: 27,000 mg; I: 1,000 mg; Fe: 7,500mg; Zn: 5,000mg; Cu: 2,000mg; Co: 450mg; Ca: 500g; P: 300g; L- lysine: 10 g; DL.Methionine:10 g; Selenium: 50 ppm.

Experimental design

The experiment was conducted for a period of 60 days in the wet laboratory facility of Central Institute of Fishereis Education (CIFE) Mumbai. One hundred and eighty advanced fingerlings of *L. rohita* (13.59 ± 0.02 g) were randomly distributed in four distinct experimental groups with three replicates following a completely randomised design (CRD). Twelve rectangular plastic tubs of uniform size (300 l capacity) were used as experimental units for all the experimental trials where each tub contained fifteen fishes. Feeding was done to satiation twice a day and continuous aeration was provided along with 25% replacement of water at every 24 h. The water quality parameters in all the experimental tanks were within the normal range throughout the experimental period (temperature - 26 - 28 °C; dissolved oxygen - 6.5 to 7.0 mg l⁻¹ and pH - 7.0 - 7.5).

Growth and feed efficiency parameters

The growth parameters of the experimental fishes were assessed by taking their body weight at 15 days interval. The animals were kept starved overnight before body weight measurement. The growth performance was assessed using the following formulae:

Weight gain (WG %)	= [(Final weight gain-Initial weight gain)/ Initial weight gain] × 100
Specific growth rate (SGR %)	= [(ln final weight - ln initial weight)/ Experimental period in days] × 100
Feed efficiency ratio (FER)	= Net weight gain (wet weight)/ Feed given (dry weight)
Protein efficiency (PER)	= Net weight gain (Wet weight)/Crude protein fed
Apparent net protein utilization (ANPU)	= [(Total final carcass protein-Total initial carcass protein)/ Protein fed] × 100
Protein retention (PR %)	= (Gram protein gain/Gram protein fed) × 100

Biochemical analysis

After 60 days experimental period, fishes were collected from each tub and anaesthetised with clove oil (50 µl l⁻¹). Blood was withdrawn from the caudal vein using a syringe. For collection of serum, blood was withdrawn without the use of anticoagulant and allowed to clot for 2 h in slanting position till the serum separated out. This clotted blood sample was then centrifuged at 3500 rpm at 4 °C and the serum was collected as supernatant and stored at -18 °C until use. Serum protein was estimated by biuret method using commercial kit (Qualigen Diagnostics, India). Serum biochemical parameters such as cholesterol, triglycerides, high density lipoproteins-cholesterol (HDL-C), low density lipoproteins-cholesterol (LDL-C), and phospholipids, were quantified using

respective colorimetric assay kits procured from Merck, Germany and the analysis was done in the Auto blood analyser, Spectra Junior (Merck, Germany).

Statistical analysis

Data on growth, feed utilisation and biochemical parameters among treatment groups were tested by one way analysis of variance (ANOVA) and the comparison of mean values were made by Tukey's HSD test. At significance level $p < 0.05$. Statistical analysis was performed using the software program SAS version (2007).

Results

Growth and feed efficiency parameters

Significantly higher ($p < 0.05$) body weight gain (WG %) and specific growth rate (SGR) were observed in T2 group (0.8 mg kg⁻¹) (Table 2). Similarly, FER, PER and ANPU % significantly improved ($p < 0.05$) in T2 group, whereas no significant difference was observed among other treatment groups and control (Table 2). The PR % value increased with the dose of chromium, showing the highest value in T2 group which decreased as the chromium level increased above 0.8 mg kg⁻¹ feed (T3).

Biochemical parameters

Chromium supplementation significantly increased ($p < 0.05$) liver glycogen in T1, and T2 groups but significant decrease ($p < 0.05$) was observed in T3 group when chromium supplementation increased above 0.8 mg kg⁻¹ feed (Table 3). In the present study, chromium supplementation significantly reduced ($p < 0.05$) serum cholesterol and triglycerides in all the experimental groups compared to control showing the lowest value in T2 group (Table 3). The serum HDL-C increased ($p < 0.05$) in all experimental groups due to chromium supplementation and the highest blood HDL-C was observed in T2 group. No significant difference in the serum LDL-C and phospholipid was observed in any of the experimental group. Similarly, highest muscle protein as well as lowest liver AST and ALT were observed in T2 group.

Discussion

In the present study, dietary supplementation of chromium piconilate (0.8 mg kg⁻¹ feed) significantly improved the WG, SGR, FER, PER, ANPU and PR of rohu fingerlings. The present results are in agreement with the previous observations obtained by supplementing diet with chromium picolinate (Liu *et al.*, 2010) in grass carp, chromic oxides (Shiau and Chen, 1993; Shiau and Liang, 1995; Shiau and Shy, 1998) and chromium chloride (Shiau and Lin 1993) in hybrid tilapia diets. However,

Table 2. Growth parameters (% weight gain, SGR, FCR, PER, PR and ANPU) of different experimental groups fed with different experimental diets (Mean \pm SE)

Parameters	Control	T1	T2	T3
WG (%)	114.76 ^a \pm 2.86	115.59 ^a \pm 12.49	175.15 ^b \pm 6.21	109.77 ^a \pm 5.61
SGR	1.27 ^a \pm 0.02	1.27 ^a \pm 0.10	1.69 ^b \pm 0.04	1.23 ^a \pm 0.05
FER	0.478 ^a \pm 0.01	0.482 ^a \pm 0.05	0.729 ^b \pm 0.03	0.457 ^a \pm 0.02
PER	1.36 ^a \pm 0.03	1.38 ^a \pm 0.15	2.08 ^b \pm 0.07	1.31 ^a \pm 0.06
PR (%)	27.24 ^a \pm 0.04	28.93 ^c \pm 0.07	32.03 ^d \pm 0.04	28.22 ^b \pm 0.16
ANPU (%)	18.68 ^a \pm 0.67	18.73 ^a \pm 0.50	21.76 ^b \pm 0.47	18.69 ^a \pm 0.49

Values in the same row having same superscript are not significantly different ($p > 0.05$)

WG : Weight gain, SGR : Specific growth rate, FER: Feed efficiency ratio, PER : Protein efficiency ratio, PR: Protein retention, ANPU : Apparent net protein utilisation

Table 3. Biochemical parameters (Cholesterol, Triglycerides, HDL-C, LDL-C, Phospholipids, Insulin, GOT and GPT) of different experimental groups fed with different experimental diets (Mean \pm SE)

Parameters	Control	T1	T2	T3
Liver glycogen (mg g ⁻¹)	32.17 ^b \pm 0.14	35.57 ^c \pm 0.08	35.81 ^c \pm 0.07	30.26 ^a \pm 0.17
Serum cholesterol (mg dl ⁻¹)	91.33 ^a \pm 0.25	88.51 ^b \pm 0.28	81.06 ^a \pm 0.60	88.57 ^b \pm 0.17
Serum triglycerides (mg dl ⁻¹)	76.21 ^b \pm 0.34	70.33 ^a \pm 0.72	70.91 ^a \pm 0.48	70.98 ^a \pm 0.45
Serum HDL-C (mg dl ⁻¹)	42.25 ^a \pm 0.21	43.89 ^a \pm 0.11	46.90 ^a \pm 0.08	43.99 ^a \pm 0.10
Serum LDL-C (mg dl ⁻¹)	37.25 ^a \pm 4.69	36.92 ^a \pm 2.19	36.40 ^a \pm 5.89	43.27 ^a \pm 6.37
Serum phospholipids (mg dl ⁻¹)	162.55 ^a \pm 2.56	164.73 ^a \pm 4.76	164.27 ^a \pm 1.28	162.89 ^a \pm 5.71
Muscle protein (mg g ⁻¹)	20.45 ^a \pm 0.45	21.39 ^a \pm 0.82	28.95 ^b \pm 0.10	21.43 ^a \pm 1.38
Liver AST (nM mg ⁻¹ protein min ⁻¹)	26.26 ^b \pm 1.11	25.98 ^b \pm 2.21	18.99 ^a \pm 2.77	26.95 ^c \pm 1.12
Liver ALT (nM mg ⁻¹ protein min ⁻¹)	18.60 ^b \pm 2.46	18.19 ^b \pm 0.61	10.90 ^a \pm 0.49	29.32 ^c \pm 1.97

Values in the same row having same superscript are not significantly different ($p > 0.05$)

HDL-C : High density lipoprotein-cholesterol, LDL-C: Low density lipoprotein-cholesterol, AST : Glutamate oxaloacetate transaminase

ALT : Alanine amino transferase

the present results are not found to be in congruence with the earlier studies conducted with hybrid tilapia (Pan *et al.*, 2003, Pan *et al.*, 2002b), gilthead seabream (Gatta *et al.*, 2001), rainbow trout (Bureau *et al.*, 1995; Selcuk *et al.*, 2010), Nile tilapia (El-Sayed *et al.*, 2010; Mehrim, 2012), where no significant improvement in growth performance was noticed by Cr supplementation. The variation among the results obtained from different studies could be attributed to several factors such as the form of chromium used, carbohydrate source and level of diet, dose and duration of treatment as well as feeding behaviour of the target species used for the experiment. It has been proven that adequate levels of non-protein energy sources (carbohydrate and lipid) in the diet can minimise the catabolism of protein by their protein sparing property (Cho and Kaushik, 1990). Wilson (1994) reported that an adequate level of carbohydrates in fish diet reduces catabolism of protein and lipid for energy purposes and provides metabolic intermediates for the synthesis of other biologically important compounds. In the present study, the increase in growth performance

may be due to the protein sparing action of carbohydrate resulted by the increased carbohydrate utilisation due to chromium piconilate supplementation. However, all the growth and feed efficiency parameters declined when the chromium piconilate supplementation were higher than 0.8 mg kg⁻¹ feed, which indicated that high-chromium supplementation was intolerable to *L. rohita* leading to reduced growth rate.

Glycogen level in liver was found to be significantly higher in rohu fed with the diet containing low level of chromium (0.8 mg kg⁻¹). This is in agreement with the finding of Liu *et al.* (2010) where higher liver glycogen level was reported in grass carp fed with the diet containing low level of chromium. It has been reported that chromium supplementation increases liver glycogen levels as a result of increasing activity of the enzyme glycogen synthetase (Rosebrough and Steele, 1981). Chromium piconilate supplementation also increases the carbohydrate utilisation and the excess glucose is stored in the form of glycogen in liver and muscle. However, liver glycogen was significantly reduced when chromium level in the diet

exceeded 0.8 mg kg⁻¹. This concurs with the observation in the freshwater field crab, *Barytelphusa guerini* (Sridevi *et al.*, 2000) and in *Anabas scandens* (Venugopal and Reddy, 1992) where liver and kidney glycogen contents were depleted by the higher level of chromium. Chromium at higher level induces release of adrenal catecholamines causing glycogenolysis (Sridevi *et al.*, 2000).

In the present study, chromium supplementation significantly reduced serum total cholesterol, triglycerides and increased serum HDL-C. This is in congruence with the findings of Kroliczewska *et al.* (2004) and Patil *et al.* (2008), who reported decrease in serum total cholesterol, LDL-C, triglycerides and increased serum HDL-C when broiler chickens were fed diet supplemented with chromium. The results of the previous experiments suggest that chromium plays an important role in fatty acid metabolism and can alter the serum fatty acid profile (Evoek-Clover *et al.*, 1993; Kitchalong *et al.*, 1995; Min *et al.*, 1997; Wang *et al.*, 2007; Zha *et al.*, 2007; Wang *et al.*, 2009). Cholesterol is an important biomolecule which is essential for the synthesis of cell membrane, bile salts and steroid hormones. It is synthesised predominantly in liver and transported by blood. However, excess cholesterol in blood has a negative impact as it can lead to arterial congestion and heart disease (Cabin *et al.*, 1982; Castelli *et al.*, 1988). The positive effect of chromium supplementation on lowering the serum cholesterol has been well documented (Page *et al.*, 1993; Kucukbay *et al.*, 2006; Jain *et al.*, 2007; Wang *et al.*, 2007; Liu *et al.*, 2010). Similarly, triglycerides are important form of storage fat which are stored mainly in the adipocytes and used during starvation. Like cholesterol, excess triglycerides in serum increase the chance of arterial congestion and heart disease (Menotti *et al.*, 1994, Miller *et al.*, 1999, Onat *et al.*, 2006). Chromium supplementation increases the biological activity of Insulin which decreases adipocyte lipolysis by reducing the activities of adenylate cyclase and hormone-sensitive lipase (Lambert and Jacquemin, 1979). Insulin can also decrease triglycerides rich lipoprotein by increasing the lipoprotein lipase activity (Garfinkel *et al.*, 1976; Howard *et al.*, 1993) which in turn increases serum triglyceride clearance (Lien *et al.*, 1999). The beneficial effect of chromium on lowering the serum triglycerides has been supported by previous studies (Jain *et al.*, 2007; Wang *et al.*, 2007). The HDL-C which is also known as good cholesterol plays a beneficial role in clearing and transporting the excess cholesterol back to the liver for its disposal and thus prevents arterial congestions and heart disease (Gordon *et al.*, 1977; Goldbourt *et al.*, 1979; Jacobs *et al.*, 1990). Insulin decreases the liver LDL receptor and thus decreases the serum LDL-C content with a concurrent increase in HDL-C (Brindley and Salter,

1991). Similar increase in serum HDL-C due to chromium supplementation has also been reported in previous studies (McCarty, 1991; Lien *et al.*, 1999; Zha *et al.*, 2007; Liu *et al.*, 2010)

Increase in muscle protein can be used as an indicator of enhanced protein synthesis. Chromium supplementation stimulates insulin activity, increases glucose utilisation and thus may indirectly plays a vital role in the protein-sparing mechanism. Insulin plays an important role in protein metabolism rather than carbohydrate metabolism in fish (Jobling, 1994). Insulin increases protein synthesis in muscle tissues (Jefferson *et al.*, 1980, Duguay and Mommsen, 1994; Davis *et al.*, 2002; Craig *et al.*, 2003). Insulin facilitates amino acid transport into the muscle cell (Tovar *et al.*, 1991, Bonadonna *et al.*, 1993), increases the ribosomal content of cell as well as their translation efficiency (Proud and Denton, 1997; Proud, 2006) and thus enhances protein anabolism in muscle cells. Moreover, insulin reduces proteolysis by downregulating cellular lysozyme activity (Jefferson *et al.*, 1974, Fulks *et al.*, 1975). This can be further correlated with reduced ALT and AST level in the liver tissue. ALT and AST are important transaminase in fish which plays important role in amino acid catabolism (Asadi *et al.*, 2006; Melo *et al.*, 2006). Fish fed with high protein diet usually show higher aminotransferase activity in liver which catabolise excess amino acid for energy purpose (Sa *et al.*, 2006) More over ALT and AST level also increases during stress to supply amino acid for gluconeogenesis (Chatterjee *et al.*, 2006; Tejpal *et al.*, 2008; Hoseini *et al.*, 2011). In the present study, lowest ALT and AST levels observed in T2 group is an indicator of reduced amino acid catabolism for energy purpose resulting in better somatic growth.

The results of the present study have clearly shown that the growth and feed utilisation parameters of *L. rohita* improved significantly ($p < 0.05$) when the animals were fed with experimental diet supplemented with Cr-Pic at a level of 0.8 mg kg⁻¹ feed. Chromium supplementation increased liver glycogen, muscle protein and reduced serum cholesterol, HDL-C, triglycerides as well as liver AST and ALT which shows its regulatory effect on biochemical parameters of fish. However, the present findings are based on the study at laboratory scale and further studies should be conducted at field level to test practical applicability at culture scale.

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