Original Research Article

Dietary supplementation of Chromium Picolinate does not affect growth performance and feed conversion ratio of Rainbow Trout (*Oncorhynchus mykiss*)

ABSTRACT

Aims: A growth experiment was conducted to explore the impact of dietary chromium on the growth performance and feed conversion ratio of rainbow trout (*Oncorhynchus mykiss*).
Study design: Original Research Article
Place and Duration of Study: Fisheries Research Station (FRS), Trishuli Nepal, between November 1 to January 29, 2023.
Methodology: A total of 270 healthy and equal sized- *O. mykiss* (initial weight: 27g) were fed on a non-supplemented basal diet (Cr-Pic0), or a basal diet supplemented with 0.4g/kg Cr-Pic (Cr-Pic0.4), or a basal diet supplemented with 1.2g/kg Cr-Pic (Cr-Pic1.2) for 90 days. Each diet was randomly assigned to three replicate groups of 30 fish per flowthrough raceway tanks for 90 days and fed to apparent satiation twice daily.
Results: Upon conclusion of the feeding trial, the results showed no significant differences in final weight, weight gain, specific growth rate, or condition factor among treatments (P>0.05). There were also no significant differences in the survival rate and feed conversion ratio of the rainbow trout fed various experimental diets (P>0.05).
Conclusion: The results suggest that growth performance, food conversion, and survival of rainbow trout fingerlings are not affected by 0.4 or 1.2 mg/kg supplementation of Cr-Pic in diet.

Keywords: Rainbow trout, Chromium picolinate, Growth performance, Feed conversion ratio

1. INTRODUCTION

Chromium (Cr) is a naturally occurring ubiquitous element mostly found in rocks, plants, animals, and soil [1]. Chromium (Cr) is considered as an essential trace element for humans and animals because it plays a considerable role as vital micronutrients for better growth of humans and animals [2]. Chromium salt such as Chromium picolinate (Cr-Pic), in its oxidized form are found dissolved in aquatic medium [1]. Cr-Pic is also known for its high-quality benefits in aquaculture as feed additive due to its prevalence role in antioxidative activity [3], and carbohydrate, fats, and protein metabolism [1]. As carbohydrates are being considered as an alternative to fish meal in aqua feeds, there is significant investigation into the importance of chromium picolinate (Cr-Pic) due to its role in the glucose tolerance factor (GTF) and high nutritional value. Chromium, a pivotal element of GTF, is essential as a coenzyme for insulin in facilitating the transfer of glucose from the bloodstream to peripheral tissues [4]. The involvement of chromium in regulating carbohydrate metabolism has been documented in studies involving turkeys [5], humans [6], and fish [7–9].

Previous experiments have suggested that Cr supplementation in humans' diet can increase lean body mass while decreasing body fat [10,11]. Similarly, in pigs and poultry, inclusion of Cr-Pic has been demonstrated to increase the carcass protein and decrease the carcass fat [12,13]. Studies involving poultry have indicated that supplementing with chromium can help reduce the adverse effects of stressful conditions during cold winters and hot summers [14,15]. However, there are scanty studies on the effects of CR-Pic on the growth performance, body composition, and resistance against disease and stress conditions of aquatic animals. Research investigating the impacts of chromium (Cr) on the growth of aquaculture species like Tilapia (*Oreochromis niloticus*× *Oreochromis aureus*) [16] and rainbow trout (*Oncorhynchus mykiss*) [17] has yielded conflicting findings. So, the present study was carried out to investigate how chromium picolinate (Cr-Pic) affects the growth performance and feed conversion ratio of Rainbow trout (*Oncorhynchus mykiss*) with a aim of searching for feed ingredients that can be used to formulate significant trout feed and enhance rainbow trout production.

2. MATERIAL AND METHODS

2.1 Study location and experimental diets

This experiment was conducted at Fishery Research Station (FRS), Trishuli Nuwakot (27.93625°N, 85.15275°E)from November 1 to January 29, 2023. All the ingredients required to prepare the experimental diets were purchased from the local market. Chromium (Cr) in the form of chromium picolinate (Cr-Pic) was a commercial product obtained from Health Vit ™in the form of tablet. All the ingredients including Cr-Pic were grounded into fine powder and mixed to prepare three experimental diets at the feed house of FRS, Trishuli. The control diet had 0 mg/kg Cr-Pic while the other two diets had 0.4 and 1.2 mg/kg Cr-Pic. The dry matter, crude protein, crude fat, and ash content of the experimental diets were determined according to the guidance of the Association of Official Analytical Chemist [18]. The formulation and chemical composition of each experimental diet are given in Table 1.

Table 1 Ingredients and proximate composition of experimental diets.

Ingredients (g/kg)	**Experimental groups**		
	Control (T1)	T2 (0.4 mg/kg)	T3 (1.2 mg/kg)
Prawn meal	540	540	540
Soybean meal	200	200	200
Wheat flour	150	150	150
Rice barn	80	80	80
Vitamin premix[a]	10	10	10
Minerals premix[b]	20	20	20
Chromium picolinate	0	0.0004	0.0012
Proximate composition (%)			
Dry matter	91.28±0.01	90.21±0.62	91.88±0.12

Crude protein	45.07±0.01	44.18±0.14	45.85±0.42
Crude lipid	7.31±0.11	6.45±0.21	6.45±0.41
Total Ash	11.8±0.04	11.05±0.11	10.46±0.17

[a] *Vitamin mixture/kg premix containing the following: 33000IU vitamin A, 3300IU, vitamin D3, 410IU vitamin E, 2660mg Vitamin BI, 133mg vitamin B2, 580mg vitamin B6, 41mg vitamin B12, 50mg biotin, 9330mg choline chloride, 4000mg vitamin C, 2660mg Inositol, 330mg para-amino benzoic acid, 9330mg niacin, 26.60mg pantothenic acid.* [b] *Mineral mixture/kg premix containing the following: 325mg Manganese, 200mg Iron, 25mg Copper, 5mg Iodine, 5mg Cobalt.*

2.2Fish and experimental design

Rainbow trout (*Oncorhynchus mykiss*) were sourced from a Rainbow Trout Fishery Research Station (RTFRS), Dhunche Rasuwa and then acclimated in a large raceway tank at FRS with feeding farm made feed (crude protein 45%) for 1 week. At the outset of the experiment, a total of 500 apparently healthy fish (initial body weight: 27.00 ± 00 g) were fasted for 24 hours and then randomly stocked into nine flow-through cemented raceway tanks of 300 L at a stocking density of 30 fish per tank. Each dietary regime, labeled as Cr-Pic0 (Control: 0 mg/kg Cr-Pic), Cr-Pic0.4 (0.4 mg/kg Cr-Pic), and Cr-Pic1.2 (1.2 mg/kg Cr-Pic), was randomly assigned to triplicate tanks. Continuous water flow was provided to all culture tanks, and the fish were hand fed twice daily (at 0900 and 1500 hours) until apparent satiation. Tank maintenance involved the removal of feces and algal residues every two days in the evening, with thorough tank cleaning conducted every two weeks. The experiment was conducted under ambient temperature conditions with natural photoperiod, with average water temperature at 11.75 ± 0.42 ℃, pH at 7.71 ± 0.16, and dissolved oxygen levels above 7 mg/L throughout the study period.

2.3Sample collection, growth, and feed utilization analysis

At the end of the experiment, fish were fasted for 24 hour and thenall fish were sampled. Fish were sedated using clove powder (200 mg/L) as per the method described by Naderi et al.[19] and thenmeasured for individual weight and length to determine morphological parameters such as weight gain (WG), specific growth rate (SGR), condition factor (K), feed conversion ratio (FCR), and survival rate (SR). The growth, survival rate, and feed utilization parameters for each replicate were calculated using the following formula [20].

WG (g) = Final weight (g) - Initial weight (g)
SGR (%) = 100 × [In (final weight) – In (initial weight)]/days
K = 100 × [final weight/ (final length)^3]
FCR = Dry feed intake (g)/ weight gain (g)
SR (%) = 100 × (final number of fish/initial number of fish)

2.4STATISTICAL ANALYSIS

The data on growth performance and feed utilization are expressed as mean ± standard error of mean (SEM) from three replicate groups. Statistical analysis was performed using one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) tests, utilizing SPSS software (Version 25, IMB, Armonk). Significance was determined at $P < 0.05$.

3. RESULTS

The outcomes regarding growth parameters and feed utilization; final body weight, specific growth rate, condition factor, feed conversion ratio, and survival rate are outlined in Table 2. In the Cr-Pic0 (control) group, the average final body weight was 64.23 g, whereas in the Cr-Pic0.4 group, it was 60.76 g, and in the Cr-Pic1.2 group, it was 61.83 g. Specific growth rates were 0.96 and 1.08, 0.90 in the Cr-Pic0, Cr-Pic0.4, and Cr-Pic1.2 groups, respectively. Similarly, feed conversion ratios were 1.06, and 0.92 and 1.09 in the Cr-Pic0, Cr-Pic0.4, and Cr-Pic1.2 groups, respectively. Likewise, condition factors were 1.08, 1.06 and 1.09in the Cr-Pic0, Cr-Pic0.4, and Cr-Pic1.2 groups, respectively. There were no significant differences among the experimental groups in terms of growth and feed conversion ratio, and condition factors ($P>0.05$). Nonetheless, despite statistical similarity, the group receiving 0.4 mg/kg Cr-Pic exhibited the highest survival rate at 84.44%, followed by the Cr-Pic0 group at 72.22%, and the Cr-Pic1.2 group at 65.56%.

Table 2. Growth performance of Rainbow trout juveniles cultured under different treatments of chromium picolinate (Cr-Pic)

	Experimental diets		
	Cr-Pic0	Cr-Pic0.4	Cr-Pic1.2
Initial body weight (g)	27.16±0.01	26.98±0.21	26.96±0.12
Final body weight (g)	64.23±2.11	60.76±1.20	61.83±1.52
Weight gain (g)	37.07±2.16	33.78±1.31	34.88±1.45
Specific growth rate (%)	0.96±0.04	0.90±0.03	0.92±0.02
Condition factor	1.08±0.03	1.06±0.02	1.09±0.02
Feed conversion ratio	3.07±0.31	3.2±0.41	3.74±0.61
Survival rate (%)	72.22±6.67	84.44±13.92	65.56±13.65

Values are means from triplicate groups of fish (Mean ±SEM)

4. DISCUSSION

Chromium (Cr) is known as an important element involved in carbohydrate metabolism and growth promotion in different farmed animals [21]. Several studies have shown that Cr plays a significant role as a growth inducer and important element required in fish for facilitating carbohydrate, protein, and fat metabolism [2, 22–24]. A significant improvement in the final body weight, weight gain, specific growth rate, and feed utilization was reported by Li et al. [2] when chromium picolinate (Cr-Pic) was given to Nile tilapia (*Oreochromis niloticus*) at 1.2 or 1.8 mg/kg feed. Similarly, Liu et al. [24] reported that dietary Cr at 0.8 mg/kg improved the growth performance of grass carp (*Ctenophryngodon Idella*). Likewise, pronounced growth and feed efficiency ratio was reported by Asad et al. when Cr-Pic was fed to fingerlings of rohu (*Labeorohita*) at the rate of 0.3 mg/kg [1]. Likewise, the positive effect of dietary chromium at 0.5 mg/kg was also reported in common carp (*Cyprinus carpio*) growth performance [25]. Given that chromium (Cr) potentially plays a part in the glucose tolerance factors which enhance insulin function, as evidenced in mammals [26], its function in fish nutrition might be associated with the activities of insulin. Duguay and Mommsen [27] characterized insulin as the primary anabolic hormone in fish, highlighting its role in promoting glucose and amino acid uptake by skeletal muscle and liver, and augmenting protein synthesis rates in these tissues.

Contrary to expectations and positive effects of the Cr-Pic on many domesticated animals, there are varying results on the effects of Cr-Pic on growth performance and feed utilization of fish. Li et al. [2] in their experiment reported, Cr-Pic at 1.6 mg/kg decreased the growth performance of grass carp. Likewise, in an experiment with tilapia (*Oreochromis niloticus* × *Oreochromis aureus*), Cr-Pic at 2 mg/kg diet had no effect on growth and carbohydrate utilization. Ng and Wilson [28] and Selcuk et al. [17] found that adding organic chromium to the diet of channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) did not enhance growth performance, consistent with findings in this study. Similarly, previous research, such as that conducted byTacon and Beveridge [29], indicates that supplementing rainbow trout diets with trivalent chromium at 1, 3 or 6 mg/kg did not lead to improvements in growth performance or feed efficiency. Likewise, 1.6 mg/kg Cr-Pic did notimprovefinal body weight, weight gain or feed conversion ratio in rainbow trout [17]. The current study's findings align with those of prior research on rainbow trout [17,29], underscoring the lack of significant effects from additional dietary chromium on growth performance of rainbow trout.Differences in experimental approaches, including the use of static water systems, closed water recirculation rearing systems, or flow-through systems, might explain the discrepancies observed in these results. In addition, the influence of chromium in the diets of omnivorous fish may be notable, given its enhancement of carbohydrate metabolism. Nonetheless, carnivores might encounter multiple challenges in processing, absorbing, and utilizing nutrients from carbohydrate-rich diets, primarily because their ability to utilize carbohydrates as an energy source is restricted [32].Furthermore, in static water systems in culture of carps and tilapias, the recycling of chromium (Cr) may have taken place, facilitating its availability for uptake by fish from the water. Conversely, in flow-through systems, chromium dissolved in the aquatic medium is swiftly eliminated, rendering it inaccessible to fish [17]. The organic chromium (Cr-Pic) is absorbed more efficiently than inorganic forms [30] making it easily available for uptake from aquatic medium by fish. However, it's important to highlight that while organic chromium plays significant roles in nutrient metabolism in various farmed animals, the inclusion of inorganic chromium in diets may lead to genotoxic effects, impacting fish gill, liver, and blood [1,31]. Despite this fact, certain inorganic forms of chromium, such as Cr_2O_3, Na_2CrO_4, and $CrCl_3$, have been demonstrated to improve growth performance and feed efficiency in fish like tilapia, as indicated in the literature reviewed by [17], this could potentially be influence of the static or flowthrough rearing conditions as mentioned earlier.

Conclusion

In conclusion, the results of this study indicate that supplementing the diet with chromium picolinate does not affect the growth performance or feed conversion in rainbow trout cultured in flow-through raceways. Consequently, it is not yet advisable to use chromium picolinate for rainbow trout fingerlings at industrial scale.Hence, further research is necessary to assess the positive impact of chromium picolinate under varied rearing environments and life stagesof rainbow trout in order suggest the use of Cr-Pic on an industrial scale.

REFERENCES

1. Asad F, Mubarik MS, Ali T, Zahoor MK, Ashrad R, Qamer S. Effect of organic and in-organic chromium supplementation on growth performance and genotoxicity of *Labeorohita*. Saudi J Biol Sci. 2019;26(6):1140-1145. doi:10.1016/j.sjbs.2018.12.015
2. Li H, Meng X, Wan W, et al. Effects of chromium picolinate supplementation on growth, body composition, and biochemical parameters in Nile tilapia *Oreochromis niloticus*. Fish PhysiolBiochem. 2018;44(5):1265-1274. doi:10.1007/s10695-018-0514-0
3. Risha E, Ahmed F, Khaled AA, Hossain FMA, Akhtar N, Zahran E. Interactive effects of dietary betaine and chromium picolinate on the immunomodulation, antioxidative response and disease resistance of Nile tilapia (O*reochromis niloticus*). Aquac Res. 2022;53(9):3464-3477. doi:10.1111/are.15853
4. Anderson RA, Mertz W. Glucose tolerance factor: an essential dietary agent. Trends Biochem Sci. 1977;2(12):277-279. doi:10.1016/0968-0004(77)90280-8
5. Rosebrough RW, Steele NC. Effect of supplemental dietary chromium or nicotinic acid on carbohydrate metabolism during basal, starvation, and refeeding periods in poults. Poult Sci. 1981;60(2):407-417. doi:10.3382/ps.0600407
6. Levine RA, StreetenDHP, Doisy RJ. Effects of oral chromium supplementation on the glucose tolerance of elderly human subjects. Metabolism. 1968;17(2):114-125. doi:10.1016/0026-0495(68)90137-6
7. Pan Q, Liu S, Tan YG, Bi YZ. The effect of chromium picolinate on growth and carbohydrate utilization in tilapia, *Oreochromisniloticus*× *Oreochromis aureus*. Aquaculture. 2003;225(1-4):421-429. doi:10.1016/S0044-8486(03)00306-5
8. Gatta PP, Piva A, Paolini M, et al. Effects of dietary organic chromium on gilthead seabream (*Sparus aurata* L.) performances and liver microsomal metabolism. Aquac Res. 2001;32(S1):60-69. doi:10.1046/j.1355-557x.2001.00005.x
9. Paripatananont T, Lovell RT. Comparative Net Absorption of Chelated and Inorganic Trace Minerals in Channel Catfish (*Ictalurus punctatus*) Diets. J World Aquac Soc. 1997;28(1):62-67. doi:10.1111/j.1749-7345.1997.tb00962.x
10. Evans GW. The effect of chromium picolinate on insulin controlled parameters in humans. International Journal of Biosocial Medical Research. 1989:163-180.
11. Kaats GR, Blum K, Fisher JA, Adelman JA. Effects of chromium picolinate supplementation on body composition: a randomized, double-masked, placebo-controlled study. Current Therapeutic Research. 1996;57(10):747-756. doi:10.1016/S0011-393X(96)80080-4
12. Ward TL, Southern LL, Boleman SL. Effect of dietary chromium picolinate on growth, nitrogen balance and body composition of growing broiler chicks. Poult Sci. 1993;72(1):37.
13. Lindemann MD, Wood CM, Harper AF, Kornegay ET, Anderson RA. Dietary chromium picolinate additions improve gain:feed and carcass characteristics in growing-finishing pigs and increase litter size in reproducing sows. J Anim Sci. 1995;73(2):457-465. doi:10.2527/1995.732457x
14. Şahin K, Küçük O, Şahin N. Effects of dietary chromium picolinate supplementation on performance and plasma concentrations of insulin and corticosterone in laying hens under low ambient temperature. J Anim Physiol Anim Nutr (Berl). 2001;85(5-6):142-147. doi:10.1046/j.1439-0396.2001.00314.x

15. Sahin N, Sahin K, Onderci M, et al. Chromium picolinate, rather than biotin, alleviates performance and metabolic parameters in heat-stressed quail. Br Poult Sci. 2005;46(4):457-463. doi:10.1080/00071660500190918

16. Pan Q, Liu S, Tan YG, Bi YZ. The effect of chromium picolinate on growth and carbohydrate utilization in tilapia, *Oreochromis niloticus*×*Oreochromis aureus*. Aquaculture. 2003;225(1-4):421-429. doi:10.1016/S0044-8486(03)00306-5

17. Selcuk Z, Tiril SU, Alagil F, et al. Effects of dietary l-carnitine and chromium picolinate supplementations on performance and some serum parameters in rainbow trout (*Oncorhynchus mykiss*). Aquaculture International. 2010;18(2):213-221. doi:10.1007/s10499-008-9237-z

18. AOAC. Official Methods of Analysis. 22nd Edition. (AOAC, ed.). Association of Official Analytical Chemist; 2023.

19. Naderi M, Keyvanshokooh S, Salati AP, Ghaedi A. Effects of chronic high stocking density on liver proteome of rainbow trout (*Oncorhynchus mykiss*). Fish PhysiolBiochem. 2017;43(5):1373-1385. doi:10.1007/s10695-017-0378-8

20. Aqmasjed BS, Sajjadi MM, Falahatkar B, Safari R. Effects of dietary ginger (*Zingiber officinale*) extract and curcumin on growth, hematology, immunity, and antioxidant status in rainbow trout (*Oncorhynchus mykiss*). Aquac Rep. 2023;32:101714. doi:10.1016/j.aqrep.2023.101714

21. Ahmed AR, Jha AN, Davies SJ. The Effect of Dietary Organic Chromium on Specific Growth Rate, Tissue Chromium Concentrations, Enzyme Activities and Histology in Common Carp, *Cyprinus carpio* L. Biol Trace Elem Res. 2012;149(3):362-370. doi:10.1007/s12011-012-9436-3

22. Qing P, Ying-zuo B, Ying-yuan P, Cheng Z. Effect of organic chromium on growth and carbohydrate utilization in hybrid tilapia *Oreochromis niloticus x O.aureus*. Acta HydrobiologicaSinica. 2002;26(4):393-399.

23. Shiau SY, Lin SF. Effect of supplemental dietary chromium and vanadium on the utilization of different carbohydrates in tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture. 1993;110(3-4):321-330. doi:10.1016/0044-8486(93)90379-D

24. Liu T, Wen H, Jiang M, et al. Effect of dietary chromium picolinate on growth performance and blood parameters in grass carp fingerling, *Ctenopharyngodonidellus*. Fish PhysiolBiochem. 2010;36(3):565-572. doi:10.1007/s10695-009-9327-5

25. Lin Y hua, Lu J min, Fu H guang, Liang Z long, Zhao S min, Ma J jie. Effect of chromium on growth and plasma biochemical indexes of *Cyprinus carpio* juveniles. Journal of Dalian Ocean University. 2003;18(1):48-51.

26. Mordenti A, Piva A, Piva G. Chromium in animal nutrition and possible effects in human health. In: Biotechnology in the Feed Industry. Proceedings of Alltech's Thirteenth Annual Symposium. Nottingham University Press; 1997.

27. Duguay SJ, Mommsen TP. Molecular aspects of pancreatic peptides. In: Sherwood NM, Hew CL, eds. Fish Physiology. Vol 13. Academic Press; 1994:225-271. doi:10.1016/S1546-5098(08)60069-2

28. Ng WK, Wilson RP. Chromic oxide inclusion in the diet does not affect glucose utilization or chromium retention by channel catfish, *Ictalurus punctatus*. J Nutr. 1997;127(12):2357-2362. doi:10.1093/jn/127.12.2357

29. Tacon AGJ, Beveridge MM. Effects of dietary trivalent chromium on rainbow trout. Nutr Rep Int. 1982;25(1):49-56.

30. de Oliveira LM, Ma LQ, Santos JAG, Guilherme LRG, Lessl JT. Effects of arsenate, chromate, and sulfate on arsenic and chromium uptake and translocation by arsenic hyperaccumulator Pteris vittata L. Environmental Pollution. 2014;184:187-192. doi:10.1016/j.envpol.2013.08.025

31. Mallesh B, Pandey PK, Kumar K, Vennila A, Shukla SP, Raman, RP et al. Bioconcentration of hexavalent chromium in different organs and induction of micronuclei in peripheral blood cells of *Cirrhinusmrigala* (Ham, 1822). Indian J Anim Sci. 2015;85(5):528-532. doi:10.56093/ijans.v85i5.48590

32. Colombo SM. Physiological considerations in shifting carnivorous fishes to plant-based diets. Aquaculture. 2020;38:5382. doi:10.1016/bs.fp.2020.09.002