## *Original Research Article*

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



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**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:**Fish, with an average initial body weight of 27 g, were fed three experimental diets containing 45% crude protein, with chromium picolinate (Cr-Pic) added at levels of 0, 0.4, and 1.2 mg/kg organic chromium, over a period of 90 days in a flow-through raceway system. Each diet was randomly allocated to three replicate groups of 30 fish per raceway and fed to apparent satiation twice daily.**Results:**Upon conclusion of the feeding trial, the results indicated no significant differences in final weight, final weight gain, specific growth rate, or condition factor (P>0.05). Additionally, there were no notable variations in survival rate and feed conversion ratio among the rainbow troutin .
**Conclusion:**In summary, the findings suggest that dietary supplementation of chromium, in the form of Cr-Pic at levels of 0.4 or 1.2 mg/kg diet, does not confer any beneficial effects on enhancing the growth of rainbow trout.

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

**Comment [NA1]:** Incomplete sentence

**Comment [NA2]:** Study design, place and duration of study
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## 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 (*Oreochromisniloticus*×*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. MATERIALS AND METHODS

### 2.1 Study location and experimental diets

This experiment was conducted at Fishery Research Station (FRS), TrishuliNuwakot(Figure 1) from November 1 to January 29. 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 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.

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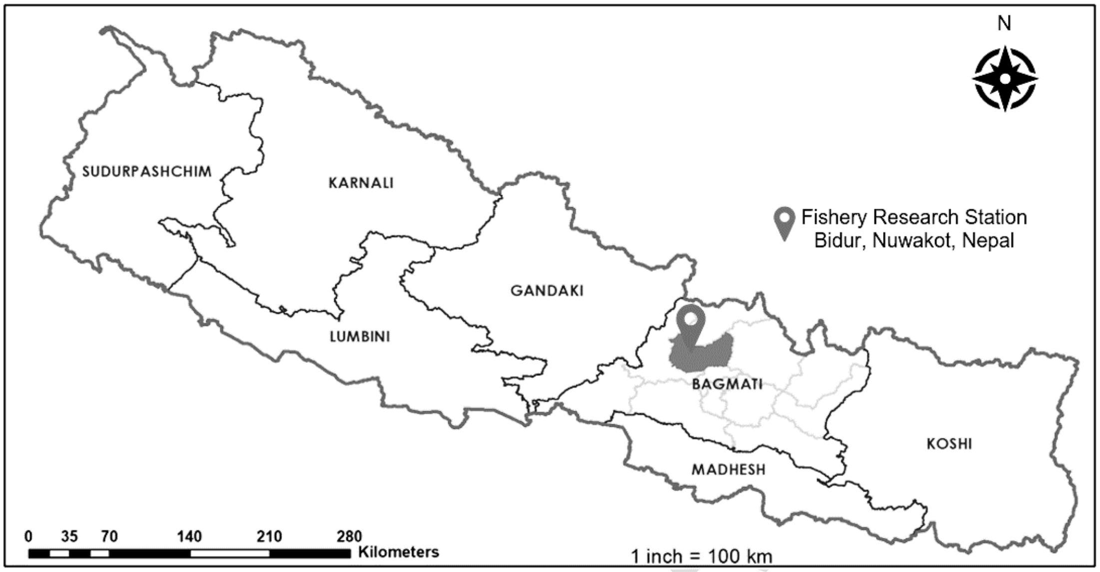


**Fig. 1. Location of Fishery Research Station in Bidur municipality of Nuwakot district, Nepal. Source: Nepal in Maps**
*(https://nepalinmaps.com/)*

**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), DhuncheRasuwa 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 900 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
$K = 100 \times [\text{final weight}/ (\text{final length})^3]$
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 weights 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 | | |
|---|---|---|---|
| | Control (T1) | T2 (0.4 mg/kg) | T3 (1.2 mg/kg) |
| 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 (*Oreochromisniloticus*) 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 (*CtenophryngodonIdella*). 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 (*Cyprinuscarpio*) 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.

**Comment [NA4]:** Is there any differences in the feeding habits of these fish species and O mykiss, if yes, could this be a reason for the differences observed in growth performance?

**Comment [NA5]:** The study's findings are compared with prior research on rainbow trout. What are the key similarities and differences between the current study and previous studies investigating the effects of chromium supplementation on rainbow trout growth performance? How do these comparisons contribute to our understanding of the potential effects of chromium supplementation in fish diets?

Contrary to expectations and positive effects of the Cr-Pic on many farmed and 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 (*Oreochromisniloticus* × *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 by [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 not lead to improvements in final 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 static water systems, the recycling of chromium (Cr) may take 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.

**Comment [NA6]:** Please comment if the studies showing the growth performance improvments have usd different experimental conditions or rearing systems than this study.

## Conclusion

The findings of this study suggest that adding chromium picolinate to the diet does not impact the growth performance or feed conversion ratio of rainbow trout under experimental feeding conditions. Future investigations could explore potential benefits of chromium picolinate (Cr-Pic) in rainbow trout across various life stages and under different experimental setups and rearing environments, such as closed tanks or flow-through systems like raceways.

**Comment [NA7]:** Based on the findings of this study and previous research, what are the practical implications for aquaculture practices regarding the use of chromium supplementation in fish diets? Are there specific recommendations for fish farmers or aquaculture industry stakeholders based on the current understanding of chromium's effects on fish growth performance?

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