Research Article

# Supplemental Organic and Inorganic Chromium Effects on Feed Digestibility and Muscle Composition in *Labeo rohita* (Rohu)

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**Abstract** | A fish nutritional trial was conducted for 90 days to find the effects of inorganic chromium and organic chromium on feed digestibility and muscle composition of (*Labeo rohita* fish). Six treatment groups were prepared with one control group (each with two replicates), three groups supplemented with inorganic chromium chloride hexa hydrate ($CrCl_3 6H_2O$ by 0.3, 0.5 and 0.6mg/kg) and three groups supplemented with organic chromium picolinate (Cr-Pic-0.3, 0.5 and 0.6mg/kg) were used. It was found that *Labeo rohita* fed on inorganic chromium ($CrCl_2$ Hexahydrate) showed maximum digestibility coefficient for ash and dry matter nutrients. In case of body muscle composition, fish fed on organic chromium (picolinate) showed highest deposition for dry matter, crude protein and gross energy. While comparing organic and inorganic Cr efficiency in fish feed, it was concluded that organic chromium (picolinate) inclusion increased the nutrients digestibility and enhanced the nutrients deposition in body muscles of fish.

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## Introduction

In socio economic area of South-Asian countries, fish occupies fundamental perspective (Khabade et al., 2015). It is the primary source of protein containing essential amino acids and minerals with essential nutrient providing source so, that masses being greatly relying on these cheap protein source. Growing population of the planet is still facing food deficiency now days (Khan et al., 2015). For fish culture practices intensive and semi-intensive cultures are generally used. Now days, nutrition is a serious problem in fish farming because it signifies almost 50% of total production cost (Craig et al., 2017). The ability to utilize the carbohydrates and plant proteins sources varies among different fish species. Some species are considered at their best to utilize dietary components of carbohydrates, *Labeo rohita* is among those species (Barbosa and Soto-Blanco, 2015). For fish feed formulation large amount of organic and inorganic source of feed is acquired in hand in Pakistan (Khan et al., 2015). Fish meal is enriched with protein source and around the world used as dietary protein source for fish feed formulation, but there are some limitations in feed using fishmeal such as higher price, minimum supply and uneconomical aspects (Krogdahl et al., 2005) To increase the nutrients bioavailability Cr metal play best role on its part. By adding organic chromium picolinate and inorganic chromium chloride hexa hydrate in fish diet enhanced the production rate of fish (Pechova and Pavlata, 2007). Different methods have been

used earlier to get maximum feed digestibility and better body composition such as inorganic chromium chloride hexa hydrate, organic chromium picolinate, chromium yeast, chromic oxide, probiotic inclusion and carbo-chelate (Ahmed et al., 2012). By the use of various compounds of chromium with different graded levels a little research work is performed on Indian major carps. In this experiment organic and inorganic forms of chromium was added in fish feed for better digestibility and body composition. Chromium is a vita1 micro minera1 it has been assumed as critical ingredient in the nutritiona1 and physiological responses in fishes (Ahmed et al., 2012). Generally, $Cr^{+3}$ and $Cr^{+6}$ form of chromium are found in environment. Hexavalent chromium is a strong oxidizing agent, simply cross biological membranes barrier and can react among protein and amino acids (Asad et al., 2017). Chromium is present in various forms $Cr^{+2}$ to $Cr^{+6}$ in nature. Mostly fish feed supplemented with $Cr^{+3}$ and $Cr^{+6}$ forms of chromium because of its role in fats, carbohydrates and protein metabolism (Dalsgaard et al., 2009). More work is required to expose the effects of organic chromium picolinate and inorganic chromium picolinate on fish body chemical composition and feed nutrients digestibility. Therefore, this research work was planned to determine the effects of organic and inorganic chromium (chromium picolinate and chromium hexa hydrate) on feed digestibility and body composition of *Labeo rohita*.

## Materials and Methods

### *Collection of fingerlings*

The fingerlings of *Labeo rohita* with initial size (2 to 3 cm were bought from fish seed hatchery, Satyana road Faisalabad, Pakistan. Fingerlings were acclimatized for ten days with feed (control diet 32%CP) before starting the experiment.

### *Experimental outline*

In post acclimatization work, *Labeo rohita* fingerlings of equal size were dispersed to seven aquaria (90cm L × 30cm W × 45cm H with 29 L water holding capacity) each with two replicate. The standardize parameters of water like DO (5-6 ppm), pH, temperature (32-35 $^0$C) were monitored and maintained on daily basis by YSI Proseries multi Parameter Professional plus meter. Fecal matter collected by siphoning method and manual water exchange were also performed on daily basis.

### *Feed ingredients and diets preparation*

**Experimental diet:** Two forms of Cr (Cr-Picolinate) organic and (CrCl2 Hexahydrate) in-organic were used to assess the apparent digestibility of prepared diet. Basic diet ingredients were fishmeal, soybean meal, yellow corn, wheat bran and maize gluten corn oil, vitamin and premixes (purchased from local market) along with Cr compounds were milled and homogenized with water to make dough and subsequently dried at 60 $^0$C in oven. Chromic oxide ($CrO_2$) was also used in experimental feed as inert marker at the rate of 1% of total weight feed ingredient. Seven experimental diets were prepared one control T1, three graded level of inorganic chromium chloride hexa hydrate T2(CCH1,0.3mg/kg), T3(CCH2,0.5mg/kg) T4(CCH3,0.6 mg/kg), organic Chromium picolinate T5(CP1,0.3mg/kg), T6(CP2,0.5mg/kg) T7(CP3,0.6mg/kg) and whereas the control diet was maintained without Cr supplementation and formulated by using the linear formulation method of Winfeed 2.6 (Winfeed U.K) Ltd., Cambridge, UK). After acclimatization experimental diets were given to *Labeo rohita* fingerlings at 4% live wet body weight for 90 days of trail.

### *Fecal matter collection*

Fecal collection was done on daily basis by siphoning method and allowed them to dry at 55 °C in oven. After accomplishment of trial duration, fecal matter was milled for beginning of analysis (AOAC, 1990).

### *Statistical analysis*

At the end of trial obtained data of feed digestibility and body composition was subjected to one-way analysis of variance, ANOVA. The difference among means evaluate by Tukey's honest significant difference test and considered significant difference test $P<0.05$.

## Results and Discussion

### *Apparent nutrient digestibility*

*Labeo rohita* (rahu) fed with carbohydrates and Cr containing diet showed significant results ($P < 0.05$). Apparent nutrient digestibility coefficient (ADC %) and body muscle composition of rahu exposed to experimental diets are summarized in Table 2 and 3. Results showed that feed digestibility can be increased with the use of extrusion technology. The present study showed that the ability to be digestible of feed varied among nutrients.

**Table 1:** *Nutrient analysis and Percentage composition of experimental diets.*

| Ingredients % | T1 Control diet | T2 CCH1 0.3mg/kg | T3 CCH2 0.5mg/kg | T4 CCH3 0.6mg/kg | T5 CP1 0.3mg/kg | T6 CP2 0.5mg/kg | T7 CP3 0.6mg/kg |
|---|---|---|---|---|---|---|---|
| Fish meal | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 |
| Soybean meal | 31.0 | 31.0 | 31.0 | 31.0 | 31.0 | 31.0 | 31.0 |
| Yellow corn | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Maize gluten | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Wheat bran | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| Corn oil | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Vitamins and minerals premix | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Chromic oxide | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Chromic picolinate (mg/kg) | 0.00 | 0.3 | 0.5 | 0.6 | 0.00 | 0.00 | 0.00 |
| Chromium chloride Hexa hydrate(mg/kg) | 0.00 | 0.00 | 0.00 | 0.00 | 0.3 | 0.5 | 0.6 |
| Proximate composition (%) | | | | | | | |
| Moisture | 07 | 07 | 7.40 | 08 | 06 | 12 | 03 |
| Dry matter | 93 | 93 | 96 | 92 | 94 | 88 | 97 |
| Ash | 98 | 91 | 97 | 94 | 99 | 96 | 98 |
| Crude Lipid | 20 | 32.5 | 10.75 | 11.25 | 22.75 | 18.57 | 30.75 |
| Crude protein | 5.78 | 3.13 | 4.69 | 5.47 | 5.47 | 6.72 | 6.25 |
| Chromic Oxide | 0.96 | 0.96 | 0.98 | 0.97 | 0.97 | 0.96 | 0.96 |
| Gross Energy (Kcal g-1) | 497.67 | 560.25 | 459.05 | 446.47 | 515.95 | 470.95 | 572.13 |

**Table 2:** *Apparent nutrient digestibility coefficient (%) of control and experimental diets.*

| | Nutrients% | Dry Matter | Ash | Crude Fat | Crude pr0tein | Gr0ss energy (Kca1 $g^{-1}$) |
|---|---|---|---|---|---|---|
| T1 | Contro1 | 17.63±0.25BC | 18.75±0.32C | 16.12±0.49B | 74.83±4.56A | 10.59±0.50B |
| T2 | CCH1(0.3mg/kg) | 16.72±0.31C | 26.15±0.963BC | 22.03±0.50BC | 49.48±2.42BC | 18.62±0.52B |
| T3 | CCH2(0.5mg/kg) | 23.11±1.95B | 28.61±1.261A | 42.24±1.09B | 62.42±1.69B | 28.16±1.04AB |
| T4 | CCH3(0.6mg/kg) | 26.21±0.880A | 26.19±0.62BC | 35.39±0.62C | 61.65±0.85C | 28.10±1.02AB |
| T5 | CP1(0.3mg/kg) | 7.09±0.23BC | 18.89±0.45C | 42.66±1.02AB | 72.92±2.05BC | 11.27±0.09C |
| T6 | CP2(0.5mg/kg) | 11.46±0.06D | 16.04±0.14D | 20.39±0.70BC | 55.81±0.49C | 10.13±0.15C |
| T7 | CP3(0.6mg/kg) | 22.36±0.15B | 26.40±0.920B | 47.65±2.61A | 70.34±3.01AB | 28.94±1.05A |

**Table 3:** *Approximate nutrient ana1ysis of fish body muscle reared under control and experimental diets.*

| Nutrients | T1 | T2 | T3 | T4 | T5 | T6 | T7 |
|---|---|---|---|---|---|---|---|
| | Contro1 | CCH1 (0.3mg/kg) | CCH2 (0.5mg/kg) | CCH3 (0.6mg/kg) | CP1 (0.3mg/kg) | CP2 (0.5mg/kg) | CP3 (0.6mg/kg) |
| Dry Matter | 93±1.74A | 94±1.54A | 96±1.60A | 95±2.26A | 96±1.75A | 97±2.23A | 96±1.31A |
| Ash | 91±1.44C | 92±2C | 93±1.33BC | 94±1.74ABC | 97±0.69AB | 98±0.51A | 98±1.28A |
| Crude Fats | 91.2±1.24A | 88±2.66AB | 86±2.52B | 79±0.42C | 86±2.10B | 90±0.91A | 89±1.71A |
| Crude protein | 14.69±0.08B | 11.56±0.09E | 3.13±0.10D | 13.90±0.11C | 13.13±0.11D | 15.47±0.16A | 14.69±0.20B |
| Gross Energy (Kcal g-1) | 874.43±15.91A | 857.76±10.28A | 861.8±11.86A | 803.48±8.11B | 857.73±19.73A | 899.75±10.06A | 876.12±19.06A |

The ADC of dry matter was highest in T4:CCH3 (26.21%) while lowest in T5:CP1 (7.09%). The maximum value of ADC of ash was observed for inorganic experimental diet T3:CCH2 by amount of 0.5mg/kg (28.61%). Experimental diet T7:CP3 exhibited highest ADC % of crude fat (47.65%) while lowest was recorded in T1: control (16.12%) when treated with organic (Cr- Picolinate) at level

of 0.6mg/kg. Control diet T1:74.83% having highest ADC % for crude protein. Organic experimental diet T7:CP3 having highest percentage (28.94%) of ADC for gross energy.

Body composition of experimental fish *Labeo rohita* was evidently influenced by Organic Chromium Picolinate and inorganic Chromium chloride hexa hydrate ($P<0.05$). Maximum deposition of nutrients in body muscle of fish for dry matter (97%), ash (98%), crude protein (15.47%) and gross energy (899.75 kcal/g) values were recorded in organic (Chromium Picolinate) experimental group T6:CP3; 0.5mg/kg. The content of body muscle crude fat did not significantly influence by dietary organic and inorganic chromium levels ($p> 0.05$), however high value was observed in fish fed with control diet. For crude fat highest deposition (91.2%) in body muscle was recorded in control group. Findings of this experiment indicated that feed utilization, body composition and biochemical parameters of *Labeo rohita* can efficiently be improved by utilizing organic chromium picolinate. The primary aim of this investigation was to understand the effect of dietetically used chromium on body composition. Briefly, Chromium stimulated the activity of insulin hormone which ultimately increases the protein utilization in tissue contents and ultimately at whole body composition. Basic ingredients used in feed formulation were enriched with plant protein sources that increased the nutrients availability and let them to digestible in more efficient way in the presence of organic Cr compound.

The results obtained in apparent nutrients digestibility coefficient for Chromium Picolinate compound in all experimental diets were found to be improved. Chromium Picolinate increased the digestibility, protein in body muscles and utilization of diet. Strongly convincing results acquired that digestibility of ingredients and chemical compositions of feed ingredients were directly related mechanisms.

The development of fish farming on commercial scale has become constricted due to lack of knowledge about fish supplemental diet ingredients and their biological importance. A combination of feed ingredients containing carbohydrates and specific additives may enhance growth and survival rates in fish. The current study results are consistent with the results of (Magzoub et al., 2010) reporting that highest deposition of dry matter in body of fish recorded at 400ppb level of chromium, surely demonstrate highest deposition of dry matter in body of fish in body muscles.

In case of ash, highest value was recorded in organic experimental diet-T6: CP2 by 0.5 mg/kg and T7:CP3 by 0.6mg/kg. Conversely, contradictory findings were recorded by Liu et al. (2010) stated that organic form of chromium had no positive effect on ash retention in fish. Maximum value of crude fat deposition in fish body meat was noted in experimental diet-T1 (control). Similar findings were recorded by AOAC (1990) concluded that crude fat deposition in fish body meat was lowest at level of chromium 0.5 mg/kg and control experimental diet. These findings are in contrary to Asad et al. (2017) who stated that highest level of chromium displayed lowest deposition of fats in fish body meat. Asad et al. (2017) reported that chromium had no beneficial effects on deposition of fat in fish body meat. In case of crude protein significant difference were observed among different treatments and in organic experimental diet-T6:CP2; 0.5 mg/kg these similar findings with (Ahmed et al., 2012) who reported that 0.5 mg/kg organic Chromium Picolinate displayed highest value for crude protein in fish body meat. Asad et al. (2017) reported that chromium showed maximum retention of protein in fish body. Results of other researchers **(**Ahmed et al., 2012; Magzoub et al., 2010; Barbosa and Soto-Blanco, 2015**)** favored the current study who stated that chromium had beneficial effect on nutrient crude protein retention in fish body meat, Contradictory findings were obtained by Dias et al. (2001) who stated that L-cartinine displayed no beneficial effect on fish body meat.

In this study gross energy values significantly different among various treatments and maximum percentage value of gross energy in fish body meat was recorded for organic experimental diet-T6:CP2;0.5mg/kg (899.75%) similar findings were obtained by Dalsgaard et al. (2009) who stated that at level of 0.5 mg $Kg^{-1}$with chromium showed maximum gross energy values. These results are in accordance with Mehrim (2012) who stated that improving level of chromium in fish body meat of carps showed beneficial results. The ADCs of dry matter was highest in experimental diet T4. These results are in accordance with Barbosa and Soto-Blanco (2015) who found that the inclusion of Cr in fish feed can lead to improvement of chemical

composition in tilapia. In fish body the ash contents displayed significant differences among all inorganic and organic experimental diets. Gatta et al. (2001) reported maximum ADC of ash contents in treatment fed test diet at 30% protein level which navigate from present investigation. The value of ash content ADC (%) was recorded highest in inorganic experimental diet T3. Similar results were reported by Khabade (2015) and Ahmed et al (2012) which concluded that at level of 0.5mg/kg chromium chloride hexa hydrate maximum crude fat deposition occur in *Labeo rohita*. In case of crude protein significant variation were observed among different treatments. The ADC protein was recorded maximum in control diet-T1. These results are in contrary to AOAC (1990) who reported that the supplement of chromium can improve utilization of protein in fish feed and also increase digestibility. While similar findings were reported by Barbosa and Soto-Blanco (2015) who concluded that L-cartinine had no positive effect on body meat of tilapia. In the study of gross energy values were significantly different among various treatments and organic experimental diet-T7 showed maximum gross energy percentage in agreement with the result reported by Hoseini et al. (2011) who concluded that chromium chloride at level 0.5mg/kg had beneficial effect on gross energy values for *Labeo rohita*.

## Conclusions and Recommendations

The obtained results of present research revealed that organic chromium picolinate at all the levels of 0.3, 0.5 and 0.6 (mg $kg^{-1}$) showed maximum apparent nutrient digestibility coefficient % and body composition of *Labeo rohita*. Organic and in-organic Cr compounds and carbohydrates combination may become interesting for foster fish culture system.

## Author's Contribution

F.Asad conceived the idea of the study. S.Qamer and A.Faiz provided the material and technical input. A.Faiz performed statistical analysis and data arrangement. R. Arshad and T. Yasmin wrote the manuscript and Z.Shaheen collected data from field.

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