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Chemical composition and growth performance of *Labeo rohita* under the influence of Chromium chloride hexahydrate marker

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Abstract

The aim of the experimental trial was to estimate the effects of various graded levels of chromium chloride hexahydrate on body composition and growth in *Labeo rohita* under laboratory study using two types of corn (Gelatinized &Non Gelatinized). For this trial, 480 juveniles were randomly distributed in six groups (0.0, 0.3 & 0.5 mg/Kg Chromium chloride hexahydrate each diet with G & NG corn) each group with two replicates. Each replicate group of 40 juveniles fed on experimental diet for eight fortnights. Gelatinized corn with Chromium chloride supplementation did not boost($p<0.05$) the growth performance of *Labeo rohita* whereas in case of body composition, chromium with G corn intensified the deposition of dry matter, crude fat, crude protein and gross energy. On the basis of these results, it is concluded that gelatinized corn and Chromium chloride hexahydrateare very effective supplements to increase the fat and lipid contents in the *Labeo rohita* body.

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Introduction

Fish is the most valuable source of protenaceous food for all animals. In Pakistan rohu is preferred on all other fishes due to its prestigious taste. *Labeo rohita* is a commonly cultured fish in sub-continent in polyculture system with other Indian major carps. Due to lack of research work in Pakistan on low cost feed formulation, fish culture is restricted to extensive and semi-intensive culture. Pakistan is an agricultural country produces a large amount of seed of animal and plant origin for feed formulation. Most expensive component of fish diet is fish meal, rich in proteneceous compounds, readily digestible but produces hazardous end products which create unhygienic condition for fish Krishnankutty (2005); Goda *et al.*, (2007); Umar *et al.*, (2011). Other major component of fish diet is carbohydrate which is abundant in environment, cheap in cost but its digestion depends on fish species and inclusion level. To increase its digestion the process of gelatinization is performed in which starch granules increase in size and are more susceptible to digestion. Mohapatra *et al.*, (2003); Yankokpam, (2007). Lipid is given to fish in diet as cod liver oil and sunflower oil. To increase the digestion of growth and to get better body composition of fish, various methods have been used in past: i.e. probiotic inclusion, phytasesupplementation, chromic oxide. Keong & Wilson (1999); Magzoub *et al.*, (2010). Chromium chloride hexahydrate Ahmad *et al.*, (2010 & 2012) chromium picolinate Liu *et al*, (2003); Seluck *et al.*, (2009 & 2010); & Mehrim, (2012) etc. Most of the work is performed on tilapia and rainbow trout, a limited work on carps and gilthead sea bream using different chromium compounds organic and inorganic, and different levels with various experimental durations and diet composition (Ahmad, 2012). In present study chromium chloride hexahydrate incorporated in diet to maximize growth and digestibility of corn. Chromium is an important trace element necessary for metabolism of carbohydrate by acting as co-factor of insulin. Its most important biological form is Cr^{+3}. Gelatinization of Carbohydrates also enhances its metabolism and improves growth of fish.

On the other hand, non-gelatinized carbohydrates support immune system. Gelatinization of carbohydrates has no effect on RBC's and has negative effect on survival Kumar *et al.* (2007). Gelatinization is a process of thermal modification of corn which results in hydration and swelling of starch granules to improve palatability, digestibility and susceptibility of starchgranules to enzymes Yankokpam, (2007). It is reported that requirement of carbohydrate for *L. rohita* is 26% but this requirement is increased up to 40% when gelatinized carbohydrate used without any detrimental effect on health Mohapatra *et al.*, (2003). Chromium is an important mineral necessary for the metabolism of carbohydrates, nucleic acid and lipid. It is a cofactor for insulin activity Pachova & Pavlata, (2007). It is found in different valence forms Cr^{+2} to Cr^{+6}Lushchak, (2011). Most abundant forms are +3 and +6 and are biologically more important NRC, (2005). Least toxic form is trivalent chromium. It enhances glucose tolerance, lipogenesis and effects glycogen accumulation in the presence of insulin. Chromium salts improve growth by efficient carbohydrate utilization Pan *et al.*, (2002); & Liu *et al.*, (2010). Many experiments were conducted to investigate the role of chromium on carbohydrate metabolism and growth in rainbow trout and tilapia.

The aims of this study are to use such carbohydrates which are inexpensive source of energy rather than expensive protein. Gelatinized Corn has better effect on growth and digestibility of fish as compared to non-gelatinized corn. Chromium chloride hexahydrate is used to enhance carbohydrates metabolism.

Materials and methods.

Experimental fish

Labeo rohita fingerlings were purchased from fish seed hatchery, Satiana road, Faisalabad. The fingerlings were acclimatized for one week rearing on reference diet. After acclimatization test diets were given to fish at 4% live body weight of fish.

Feed Ingredients and Diet Preparation Procedure

Six test diets were prepared by using corn as carbohydrate source, purchased from local market and was ground to make powder, add almost 80% of water (v/w) and autoclaved for one hour at 15psi to get maximum gelatinization. This material then spread on a tray and placed in oven at 60°C. The dried mass was passed in a hammer mill having 0.5 mm screen and then stored in air tight jars until use. Protein source was gelatin and fat free casein, while lipid sources are cod liver oil and sunflower oil and carbohydrate source is corn (G & NG). All dry ingredients were measured carefully, ground and mixed thoroughly for 30 minutes. Spread this mass in tray and place in oven to dry it (Kumar V*et al.* 2012). Then pellets were made with hand pelletizer, placed in drying oven for 48 hours and stored until use. Chromic oxide was used as indigestible marker in all diets. Six experimental diets were prepared containing G and NG carbohydrates, each with three graded levels of Chromium chloride hexahydrate, chromium as Chromium chloride hexahydrate T1 (G, $CrCl_3.6H_2O$. 0.0 mg/Kg), T2 (NG, $CrCl_3.6H_2O$. 0.0 mg/Kg), T3(G, $CrCl_3.6H_2O$. 0.3mg/Kg), T4 (NG, $CrCl_3.6H_2O$. 0.3 mg/Kg), T5 (G, $CrCl_3.6H_2O$. 0.5mg/Kg) and T6 (NG, $CrCl_3.6H_2O$. 0.5mg/Kg). All diets were formulated through Winfeed 2.6 (Winfeed U.K) Ltd., Cambridge, U.K) using linear formulation method.

Feeding protocol and fecal collection

After acclimatization of one weak, fish fingerlings were transferred to glass aquria. For six test diets two replicates were used and in each replica fifty fingerlings were stocked. Fish were given control and experimental diets at the rate of 4% live wet body weight twice a day (morning and afternoon) in the feeding aquarium Noreen *et al.*, 2008). After feeding period of three hours, fingerlings were transferred in partitioned aquaria for collection of fish feces on daily basis. Fecal matter was accumulated by siphoning with care to avoid breaking of fecal strings to reduce the leaching of all nutrients. The accumulated fecal matter from all replicates was dried in petridishes at room temperature (Temp 30^{O}C-32^{O}C) on daily basis. Feces accumulation was continued for 120 days till enough quantity of fecal matter has been accumulated for all chemical analysis. The water quality parameters i.e. temperature, pH, and dissolved oxygen were checked daily according to standard methods (APHA 1998). Throughout the feeding trial fish was re-weighted after every fortnight to calculate the live 4% body weight of fish.

Analytical method

A representative sample of feed, oven dried feces and body meat of every replica were homogenized separately using a mortar and pestle and analyzed chemically by AOAC (1990) procedure. Representative samples of six experimental diets and dried samples of feces of every replica were homogenized separately in a mortar pestle and chemically analyzed by AOAC protocols, oven drying at 105°C for dry matter (DM), micro kjeldahl analysis for crude protein,

Chloroform methanol extraction method for crude fat through 10454 soxtec system HTz, electric furnace for ash. Chromic oxide (Cr_2O_3) was estimated in dried feed and feces according to Divakaran through UV/VIS2001 spectrophotometer.

Growth studies

The morphometric characteristics which include net body weight (g) and total body length (cm) of fingerlings were measured from all test diets on the basis of fortnight. Total length was measured using a 30cm ruler as the distance from the tip of mouth to the most anterior tip of tail fin. Analytical was used to measure the body weight. After recording all the data, the fingerlings were transferred into their respective aquaria again. The growth of fish was determined by following calculations:

i. Increase in wet body weight (g) by following formula

Average body weight of 1st observed fortnight- Average body weight of next observed fortnight

ii. Increase in net total body length (cm)

Average body length of 1st observed fortnight- Average body length of next observed fortnight

iii. Condition factor by using following formula

$K= W \times 10^5 \div L^3$

Where,

W= Average wet body weight (g)

L=Average body length (cm)

iv. Specific growth rate (SGR) evaluated by using the following formula

v.

$$SGR = \frac{\ln(\text{Final wet body weight}) - \ln(\text{Initial wet body weight})}{\text{Time duration (Days)}} \times 100$$

Statistical analysis

After finding the possible results, data of digestibility, growth and body composition was subjected to statistical analysis ANOVA and ± SE values was calculated by using SPSS (Noreen, S. *et al.*, (2007) and the differences between the means ($p<0.05$) were evaluated by the Tukey's HSD Test (Snedecor & Ochran, 1991).

Results

This experiment showed significantly variant results for growth and body composition which are discussed below.

Table 1. Ingredient composition of experimental diet, (G & NG, $CrCl_36H_2O$ mg/Kg).

Components (%)	T1	T2	T3	T4	T5	T6
	(G,0.0,$CrCl_3.6H_2O$ mg/kg)	(NG,0.0,$CrCl_3.6H_2O$ mg/kg)	(G,0.3,$CrCl_3.6H_2O$ mg/kg)	(NG,0.3,$CrCl_3.6H_2O$ mg/kg)	(G,0.5, $CrCl_36H_2O$mg/kg)	(NG,0.5,$CrCl_3.6H_2O$ mg/kg)
Casein	30.57	30.57	30.57	30.57	30.57	30.57
Gelatin	8	8	8	8	8	8
Corn flour	42.43	42.43	42.43	42.43	42.43	42.43
Cellulose	7	7	7	7	7	7
Sunflower oil	8	8	8	8	8	8
Carboxy methyl cellulose	1	1	1	1	1	1
Vitamin premix	2.60	2.60	2.60	2.60	2.60	2.60
Chromic oxide	1	1	1	1	1	1
Butylene Hydroxy Toulene	0.02	0.02	0.02	0.02	0.02	0.02
Chromium Chloride Hexahydrate	0.0mg/kg	0.0mg/kg	0.3mg/kg	0.3mg/kg	0.5mg/kg	0.5mg/kg

(*the antioxidant Butylated Hydroxy Toluene was added at 0.02% of the added oil.).

Growth

The maximum weight gain and increase in total body length is shown in Table 2. These results clearly exhibited that chromium had no effect on growth. Non-gelatinized diet without chromium chloride showed maximum weight gain, increased ($p < 0.05$) in body length and SGR in *Labeo rohita*. These results revealed that a growth value varies significantly in all groups.

Table 2. Growth performance of *Labeo rohita* in response to chromium chloride hexahydrate and corn (G & NG) diet.

Parameters	T1	T2	T3	T4	T5	T6
Initial weight	1.78	1.75	2.3	2.15	2.9	1.99
Final weight	4.08	5.55	6.08	4.6	4.44	4.34
Weight gain	2.3	3.8	3.78	2.48	1.54	1.35
Initial length	5.2	5.06	5.6	5.38	5.3	5.42
final length	7.39	8.14	8.18	7.5	7.65	7.5
Increase in length	2.19	3.08	2.58	2.12	2.35	2.08
SGR	0.69	0.96	0.81	0.63	0.35	0.65

Body Composition

Effects of dietary addition of G&NG corn, chromium chloride hexahydrate and their interaction are expressed in Table 6. Improved ($p<0.05$) deposition of dry matter, crude fat, crude protein and gross energy in response to dietary chromium and gelatinized diet as compared to non-gelatinized corn without chromium.

Table 3. Comparison of means of fortnights for body weight and total body length of *L. rohita*.

Fortnights	Weight (g)	Length (cm)
16 Jul	2.15±0.158 E	5.33±0.074 G
26 Jul	2.35±0.158 E	5.63±0.071 F
7 Aug	2.46±0.100 E	6.00±0.058 E
22 Aug	3.52±0.178 D	6.82±0.099 D
5 Sep	3.65±0.176 CD	6.98±0.097 D
19 Sep	4.06±0.168 BC	7.29±0.100 C
3 Oct	4.33±0.162 B	7.43±0.098 BC
17 Oct	4.43±0.209 AB	7.54±0.101 AB
31 Oct	4.85±0.262 A	7.73±0.122 A

Means sharing similar letters are statistically non-significant ($P>0.05$).

Discussion

Growth

Not any case of mortality or abnormal growth was found during this feeding trial, same results of survival of fish were found by Ahmad (2012). Highest value of weight gain was found in group-T2 (NG, Cr 0.0 mg/Kg) while this group also showed maximum value of increase in length. Group-T2 includes non-gelatinized corn with 0.0 mg/Kg chromium chloride hexahydrate.

It showed that non-gelatinized carbohydrates with no chromium chloride hexahydrate had beneficial effects on growth rate these results are contrary to Ahmad (2012) who found that at 0.5mg/Kg common carp showed maximum growth. Pores and Oliva-Teles (2002) stated that non-gelatinized corn have positive effects on growth as compared to gelatinized corn. Saha and Ray (2001) reported same results for corn at 40% non-gelatinization.

Table 4. Comparison of means of body weight and length for treatments with various levels of chromium and G & NG corn for *Labeo rohita*.

Treatments	Weight (g)	Length (cm)
T1 G, 0.0mg/Kg $CrCl_3.H_2O$	3.10±0.217 C	6.53±0.191 C
T2 NG, 0.0mg/Kg $CrCl_3.H_2O$	3.57±0.319 B	6.79±0.260 B
T3 G, 0.3mg/Kg $CrCl_3.H_2O$	4.25±0.342 A	7.16±0.235 A
T4 NG, 0.3mg/Kg $CrCl_3.H_2O$	3.34±0.204 BC	6.67±0.180 BC
T5 G, 0.5mg/Kg $CrCl_3.H_2O$	3.61±0.175 B	6.65±0.192 BC
T6 NG, 0.5mg/Kg $CrCl_3.H_2O$	3.33±0.226 BC	6.72±0.187 B

Means sharing similar letters are statistically non-significant ($P>0.05$).

Yengkokpam (2007) compared the three diets corn, rice and tapioca for growth, corn diet showed maximum growth and specific growth rate at 40% gelatinized corn among all diets.

There are many reasons for different results obtained by different researchers with same chemical compound and with same experimental design.

Harpaz (2005) stated that initial body weight of fish is important in final body weight of fish at the end of the experiment. Other factors which effect the results are; experimental design, type, use of static water system, close water system, recirculation rearing system or flow through system are responsible for variety of results.

Dry matter

Highest dry matter deposition was reported in group-T3 while lowest value was evaluated in group-T4, same results by Mehrim (2012) at 400ppb level of chromium increased dry matter in fish body. Mehrim (2013) reported that up to 400μg/Kg increased dry matter content in dorsal muscle of nile tilapia.

Table 5. Analysis of variance table for total body weight and length of *Labeo rohita* fed on different experimental diets.

Parameters	Weight (g)	Length (cm)
Fortnights	44.93**	119.88**
Treatments	10.76**	10.85**
F × T	0.88 NS	0.55NS

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01).

Crude protein

In case of crude protein significant variation observed in different groups with maximum value recorded for group-T5 which contain gelatinized corn with 0.5mg/Kg chromium chloride hexahydrate, same results obtained by Yengkokpam (2007) with 40% gelatinized corn observed maximum protein in body meat. Contrary results were practiced by Liu *et al* (2010) who stated that chromium had no effects on protein retention in fish body meat. Mehrim (2012) reported that 400ppb level of chromium increased protein in fish body meat in dorsal muscle of nile tilapia while El-Sayyed *et al* (2010) described that 1000 and 1200 μg/Kg chromium increased protein retention in body meat of tilapia. Magzoub (2010) observed highly significant results for protein retention fish body meat with chromium in diet while contrary results by Dias *et al* (2001) who reported that L-cartinine did not affect the composition of body meat in tilapia. Pan *et al* (2002b) reported that chromium improves protein retention in fish body meat same results by Mehrim (2012) at 400ppb level of chromium.

Table 6. Proximate body composition of *Labeo rohita* in response to corn (G&NG), chromium and their interaction.

Components	T1 (G,0.0,$CrCl_3.6H_2O$ mg/kg)	T2 (NG,0.0,$CrCl_3.6H_2O$ mg/kg)	T3 (G,0.3,$CrCl_3.6H_2O$ mg/kg)	T4 (NG,0.3,$CrCl_3.6H_2O$ mg/kg)	T5 (G,0.5,$CrCl_3.6H_2O$mg/kg)	T6 (NG,0.05$CrCl_3.6H_2O$ mg/kg)
Dry matter(%)	89.19±1.140a	87.89±1.250a	90.33±0.560a	86.22±3.950a	89.96±12.95a	89.37±0.520a
Ash(%)	14.09±0.905ab	14.34±0.640ab	11.94±0.240c	14.19±0.055ab	13.24±0.395bc	16.04±0.765a
Crude fat(%)	35.48±1.655bc	40.08±2.300ab	34.29±3.100bc	26.43±3.840c	45.35±3.370a	30.30±1.545c
Crude protein (%)	10.72±0.095b	9.94±0.560b	9.82±0.825b	9.00±0.800b	13.78±0.280a	10.60±0.655b
Gross energy (Kcal/g)	572.31±4.260bc	595.22±15.660bc	569.20±17.060bc	522.98±19.410c	632.90±18.460a	539.22±22.230bc

Crude fats

In this trial, maximum value of crude fat in body meat was recorded for group-T5 which contains gelatinized corn with 0.5mg/Kg chromium chloride hexahydrate and value for crude fat varies in different groups significantly. Same results by Yengkokpam (2007); in case of corn feed, reported that 40% gelatinized corn improves crude fat retention in fish body meat. Ahmad (2012) stated that at 0.5mg/Kg chromium, lipid deposition was maximum. Dissimilar results by Liu *et al* (2010) who observed lowest lipid deposition with maximum chromium supplementation.

Opposing results by Gatta *et al* (2001), who stated that chromium had no effect on fat deposition in fish body meat and Pan *et al* (2003). Highest crude fat deposition may be due the available carbohydrates for utilization and retention of crude fat in body muscles. Pan *et al* (2003) reported that more complex dietary carbohydrates could enhance lipogenesis in tilapia. More lipid deposition is due to the activation of lipogenesis pathway which synthesizes fatty acids and glycerol from glucose substrate available from carbohydrate rich diet.

Gross energy

The maximum value of gross energy was recorded forgroup-T5 which contains gelatinized corn with 0.5mg/Kg chromium chloride hexahydrate and this value varies significantly in different groups, same results by Magzoub (2010) which stated that chromium had significant effects on gross energy value in different groups.

In case of ash group-T6 showed superior value of ash retention in body and its value varies significantly in different groups contrary to results of Liu *et al* (2010) and El-Sayyed (2010) who stated that chromium picolinate had non-significant effect on ash contents in fish body meat.

The mineral retention may be affected by the general health and chromium level in diet. Ahmad (2012) reported that chromium caused not any change in moisture contents of body meat when compared with control group. Magzoub *et al* (2010) who evaluated that non-significant difference was found in response to dietary chromium in moisture.

Conclusion

From this experiment it was concluded that chromium chloride hexahydrate with gelatinized corn had beneficial effects on protein profile of *Labeo rohita* but in case of growth maximum values were recorded for non-gelatinized corn without chromium supplementation. So in future, high protein fish can be produced by using chromium chloride hexahydrate with gelatinized corn. More research needed in this field.

The variations results of this study may be due the rearing conditions and different environmental conditions. This was a lab study more accurate results can be obtained by performing this experiment in field.

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