Research Article

Effect of Inorganic Cr (Chromium Chloride on Carbohydrate Metabolism in *Cirrhinusmrigala* (mori)

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Abstract | A feeding trial was carried out to assess the effect of dietary chromium supplementation on apparent nutrient digestibility coefficient (%) of gelatinized and non-gelatinized corn in *Cirrhinusmrigala* fingerlings for 90 days. Using various levels of chromium chloride hexahydrate six test dietsdesignated as; T1(G/0.0, $CrCl_{3.}6H_2O$ mg Kg^{-1}), T2(NG/0.0, $CrCl_{3.}6H_2O$ mg Kg^{-1}), T3(G/0.2, $CrCl_{3.}6H_2O$ mg Kg^{-1}), T4(NG/0.2, $CrCl_{3.}6H_2O$ mgKg^{-1}), T5 (G/0.4, $CrCl_{3.}6H_2O$ mg Kg^{-1}) and T6(NG/0.4, $CrCl_{3.}6H_2O$ mg Kg^{-1}) were prepared. Results showed highest apparent digestibility coefficient (ADC) of nutrientsdry matter, crude lipid and gross energy in test diet T5 that was gelatinized and supplemented with chromium 0.4 mg/Kg while, for crude protein higher value of nutrient digestibility was recorded in test diet T3(G/0.2, $CrCl_{3.}6H_2O$ mg/Kg).It was concluded that chromium supplementation with gelatinized corn in fish (*Cirrhinusmrigala*) diet can improve the nutrients digestibility more efficiently as compared to non gelatinized and Cr-free diet.

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Introduction

In many parts of the world aquatic food products are essential component of human diet and especially are source of protein. Due to increasing population we are facing shortage of food especially in protein form, fish farming has expanded as substitute cheap source of protein (Zeitoun and Mehana, 2014). During the past four years global annual aquaculture production has been expanding at an average rate of 2.9% (FAO-FISHSTAT, 2012). Intensive and semi-intensive fish cultures are gaining more importance for production of low cost diet (Khan et al., 2015). For growth and survival of fish species nutrition is most important. Fish meal is a rich source of protein and also containing essential amino acids, low carbohydrates content and fatty acids (Altan and Korkut, 2011). But it is most expensive and not a reliable source. Fish meal can be replaced with other inexpensive sources that would be of different types and originated from plants (Slawski et al., 2013). *Cirrhinusmrigala*is commonly called mori, white carp and mrigal. It is one of Indian major carps developed mostly in Southeast Asian nations. It feeds generally on natural vegetation but in the absence of natural feed *Cirrhinusmrigala* feeds on artificial feed (Khan, 2015).

To reduce feed cost carbohydrates are utilized in fish feed formulation because carbohydrates are most economical, low cost than lipids and protein and a rich source of energy (Yengkokpam et al., 2007). Some factors such as dietary level, source or type and gelatinization treatment are responsible for better digestibility of carbohydrates in fishes (Krogdahl et al., 2005). As compared to other fishes major carps have enhanced digestibility for carbohydrates (Kumar et al., 2009). In this experiment corn was utilized as carbohydrate source because it is a rich source carbohydrates and its digestibility and utilization can be enhanced by gelatinization of corn. Gelatinization is a procedure in which corn is treated in the presence of water and heated as a result breaking down of intermolecular bonds occurs and swelling of starch granules improves digestibility and palatability (Yengkokpam et al., 2007).

Some other factors are also responsible for carbohydrate digestibility and utilization. In all physiological processes lipids are vital for normal growth and reproduction (Tocher, 2003). Chromium has various oxidation states from Cr^{+2} and Cr^{+6} but in nature mostly trivalent and hexavalent form of chromium are found (NRC, 2005). Chromium chloride hexahydrate reacts with protein and amino acids because it can easily cross all biological membranes that's why Cr^{+6} is a powerful oxidizing agent (Pechova and Pavlata, 2007).

The purpose of this experimental trial is to utilize low cost diet carbohydrates that improve palatability and digestibility of *C. mrigala*. Chromium choridehexahydrate works as a cofactor for insulin activity and in this experiment, it was utilized as food additives which improves metabolism of *C. mrigala*.

Materials and Methods

This nutritional trial was performed in research lab, Department of Zoology, Government College University, Faisalabad, Pakistan.

Test Specie

*C.mrigala*specie was selected as experimental fish. Its fingerlings (1-2 inches) were purchased from fish seed hatchery, Staina road Faisalabad, Pakistan.

Acclimatization of experimental fish

*C. mrigala*fingerlings were acclimatized for one weekand stocked in aquaria having 30 L water capacity. Throughout acclimatization period *C.mrigala*fingerlings were fed tosatiations on control diet (non gelatinized and Cr-free).By using following standard method (APHA, 1985) water quality parameters such as pH, dissolved oxygen and temperature were monitored on daily basis.

Stocking density

Six hundred fingerlings of equal sizewere dispersed arbitrarily in six experimental groups with two replicates of each treatment. Oxygen pumps with capillary system were used to supply oxygen (5-6 ppm) for proper aeration.

Experimental diets

By using corn (gelatinized and non-gelatinized) with various levels of chromium chloride (0, 0.2 and 0.4 $Cr_2Cl_3.6H_2O$ mg Kg^{-1}) six experimental diets were prepared (Table 1). Corn flour was added with almost 50% water (v/w) to form it dough. Then dough was placed in autoclave for one hour at 120 °C to attain maximum gelatinization. The dried and gelatinized corn flour with all test ingredients were then pulverized to pass through 5mm sieve size. All ingredients were mixed thoroughly for 8 min and fish oil was added gradually while mixing the diet. During mixing 10-12% water was added to form dough (Lovell, 1989). Dough was then steam conditioned for 10 min. Hand pelletizer was used to prepare pellets of 2mm diameter, oven dried for 24 h at 60 ^{0}C and stored in air tight jars at room temperature.

Feeding protocol

Cirrhinusmrigala fingerlings were fed twice a day on experimental diet at the rate of 4% live wet body weight. For proximate analysis this nutritional trial was continued for 90 days to collect appropriate amount of fecal matter. Chromic oxide was added at the rate of 1% as an inert marker. After the feeding period of 2 hours fingerlings were shifted to partitioned aquaria for the collection of fecal matter. By siphoning out fecal matter was collected from each aquarium, then dried at room temperature and stored for proximate analysis.

Digestibility studies

The ADC_s (%) of dry matter, ash, crude protein, crude lipid, crude protein, and gross energy was calculated by using following formulas (NRC, 2011):

$$Apparent\ nutrient\ digestibility = 100 \times [1 - (dietary\ CrO^3 / fecal\ Cr_2O_3) \times$$

$$(fecal\ nutrient\ or\ energy\ concentration / dietary\ nutrient\ or\ energy\ concentration)$$

Proximate analysis

The representative samples of dried feed ingredient (corn), test diets and feces were homogenized by using mortar and pestle separately and were analyzed using standard method (AOAC ,1995) for dry matter (DM), ash, crude lipid (CL) and crude protein (CP). Dry matter contents were measured by drying the

Table 1: *Percentage composition of test diets.*

Components (%)	T1	T2	T3	T4	T5	T6
	(G/0.0,$Cr_2Cl_3.6H_2O$ mg/kg)	(NG/0.0,$Cr_2Cl_3.6H_2O$ mg/kg)	(G/0.2,$Cr_2Cl_3.6H_2O$ mg/kg)	(NG/0.2,$Cr_2Cl_3.6H_2O$ mg/kg)	(G/0.4,$Cr_2Cl_3.6H_2O$mg/kg)	(NG/0.4,$Cr_2Cl_3.6H_2O$ 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
Carboxymethyl 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
B.H.T	0.02	0.02	0.02	0.02	0.02	0.02
Chromium Chloride Hexa hydrate(mg/kg)	0.0	0.0	0.2	0.2	0.4	0.4

[1] Casein fat free: 70%CP (Himedia ltd, India); [2] Gelatin: 90%CP (Himedia ltd, India); [3] Purchased from local market, Faisalabad, Pakistan. [4] Sigma Chemical, St. Louis, MO, USA.,[5] Composition of vitamins mineral mix (Quantity/Kg): Vitamin A, 15 M.I.U; Vitamin D_3, 3M.I.U; Vitamin B_1,5000mg; Vitamin E, 6000 IU; Vitamin B_2, 6000mg; Vitamin K_3, 4000mg, Vitamin B_6, 4000mg; Folic acid, 750 mg; Vitamin B_{12}, 9000 mg; Calcium pantothenate, 10000mg; VitaminC,15000mg; Nicotinic acid, 25000mg,[6] The antioxidant Butylated Hydoxy Toluene was added at 0.02% of the added oil.

samples in oven at 105 ˚C for 12 hours; ash analysis was performed in electric muffle furnace at 650 ^{0}C for 6 hours (Eyela-TMF 3100); crude lipid by petroleum ether extraction method through Soxtec system (Model HT2, 1045); Crude protein (N x 6.25) was determined by micro kjeldahlapparatus. Percent N-free extract was calculated by subtracting the sum of % crude proteins, crude lipids and moisture from 100 i.e. NFE % = 100 – (crude protein% + crude fat% + moisture %). Gross energy was calculated by using the following formula:

$$GE\ (Kcalg^{-1}) = (5.64 \times proteins\ \%) + (9.44 \times lipids\ \%) + (4.11 \times NFE\ \%)$$

Chromium assay: The chromium oxide content in the feeds and fecal matter was estimated by using UV-VIS 2001 Spectrophotometer at 370 nm absorbance (Divakaran et al., 2002).

Statistical analysis

Highly significant difference among gelatinized and non-gelatinized carbohydrate diets with various levels of chromium chloride hexahydrate was tested by one way analysis of variance (ANOVA) and comparison of means was done by Tukey's Honestly significant difference test and considered significant at $p>0.05$ (Snedecor and Cochran, 1991).

Results and Discussion

The results for proximate analysis of fish feed, and fecalmatter are given in Table 2 and 3 respectively. While, apparent nutrient digestibility coefficient (%) of nutrientsdry matter, ash, crude lipid, crude protein and gross energy for test diets is given in Table 4.

Table 2: *Proximate nutrient analysis of fish test diets and estimation of chromic oxide (Cr_2O_3) %.*

Test Diets						
Nutrients	T1	T2	T3	T4	T5	T6
Dry matter (%)	98	96	97	98	99	98
Ash (%)	20.10	21.61	22.79	35.13	25.60	28.00
Crude lipid (%)	20	19	24	26	32	22
Crude protein (%)	39.06	35.93	31.25	37.5	35.93	32.81
Chromic oxide (%)	0.97	0.97	0.98	0.96	0.97	0.96
Gross energy(K-cal/g)	569.04	550.79	574.4	598.73	632.41	570.27

Apparent nutrient digestibility coefficient (%)

The digestibility of nutrients is not defined accurately in numerous commercial feedstuffs and it is revealed that feed performance and digestibility can be improved with the use of extrusion technology. In this research work we compared the carbohydrate digestibility of two types of corn (G and NG) at different levels of chromium chloride in fish feed. Obtained results showed maximum apparent nutrient digestibility coefficient (%) of nutrients dry matter, ash, crude lipid, crude protein and gross energy in experimental group that was fed on diet supplemented with gelatinized corn and chromium chloride.

Table 3: *Proximate nutrient analysis (%) of fish fecal matter and estimation of chromic oxide (Cr_2O_3).*

Fecal matter						
Nutrients	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Dry matter (%)	88.02	92.05	88	92	91	91
Ash (%)	17.19	19.31	21.72	31.62	24.50	24.12
Crude lipid (%)	10.15	11.05	9.5	9	11.5	10.5
Crude protein (%)	34.90	32.40	25.78	29.68	34.37	31.22
Chromic oxide	1.29	1.20	1.42	1.31	1.57	1.48
Gross energy(K-cal/g)	469.17	486.79	451.75	471.5	487.88	477.70

The apparent nutrient digestibility coefficient (ADC) of nutrient dry matter was found highest for the experimental group T5 (45.00 ± 2.00^{A}) and lowest value was recorded for T2 (24.00 ± 0.00^{D}). While, apparent digestibility coefficient for ash was found maximum in experimental group T6 (45.00) which was treated with higher inorganic Cr concentration. In case of nutrient crude lipid ADC was found highest in experimental group T5 (79.00 ± 2.00^{A}) and lowest values was observed for T2 (54.00 ± 2.00^{C}). For crude protein apparent nutrient digestibility was recorded highest in experimental group T3 (44.00 ± 4.00^{A}) that was treated with gelatinized corn and lowest for group T2 (28.00 ± 1.00^{B}) that was treated with non-gelatinized and non- supplemented diet. The highest value of apparent digestibility coefficient for gross energy was found maximum for group-T5 (54.00 ± 3.00^{A}) and minimum for group-T2 (30.00 ± 1.00^{C}).

This feeding trial revealed that apparent nutrient digestibility coefficient values for all nutrients (dry matter, ash, crude lipid, crude protein and gross energy) were maximum in experimental groups that were treated with diets supplemented with gelatinized corn and chromium chloride. Inclusion of gelatinized corn improved the digestibility and utilization of fish feed. Some factors are responsible for better digestibility of carbohydrates i.e. type of carbohydrate, level of gelatinization and dietary addition level (Mohapatra et al. 2003). The apparent digestibility coefficient (ADC) of dry matter was found maximum in experimental group-T5 (G/0.4$CrCl_3.6H_2O$mg/kg). It may be due to the reason that gelatinized corn increased utilization of feed and as a result better growth attained that improved dry matter in fish body. These results are in favor of Yengkokpam et al. (2007). Who stated that apparent digestibility coefficient of dry matter improved by using gelatinized carbohydrates as compared to non-gelatinized carbohydrates. While Mohapatra et al. (2003) reported that apparent dry matter digestibility was greater in those experimental groups that were fed on non-gelatinized corn.

Highest value of ADC for ash was recorded in experimental group-T6. Liu et al., (2010) reported that Cr-pic had no beneficial effects on ash deposition in fish body meat. However, contrary results of our finding were observed by Asad et al. (2014) who concluded maximum ash value of ADC for non-gelatinized corn. Apparent nutrient coefficient for crude lipid was found highest in group-T5 (G/0.4$CrCl_3.6H_2O$ mg/kg). These results are in accordance with Shahzad et al. (2006) who reported that lipid digestibility was highest for corn as compared to feather and wheat meal. However, dissimilar conclusions were observed by Asad et al. (2014) who stated highest ADC of lipid in non-gelatinized corn as compared to gelatinized corn. Diet containing rich source of carbohydrates maximize lipogenic enzyme activity in omnivorous fish and as a result maximum lipid deposit in various tissues (Yengkokpam et al., 2007). Apparent protein digestibility was positively correlated with gelatinized feedbecause maximum value was recorded for experimental group T3 (G/ 0.2 mg kg^{-1}). Similar results were reported by Shahzad et al. (2006) who found highest apparent digestibility coefficient of crude protein in corn meal. Gelatinization of corn enhanced digestibility of feed which improved protein profile of fish. In case of gross energy maximum value of apparent digestibility coefficient was recorded for group-T5 (G/0.4$CrCl_3.6H_2O$mg kg^{-1}). However, contradictory results were observed by Asad et al. (2014) who reported maximum value of ADC for gross energy in non-gelatinized corn. It might be due to variation in feed ingredients or addition of chromium chloride.

Conclusions and Recommendations

From trial it was concluded that inclusion of dietary chromium (chromium chloride) with gelatinized corn in fish feed of *Cirrhinusmrigala* improved digestibility coefficient of dry matter, ash and crude lipid and

Table 4: *Apparent nutrient digestibility coefficient (%) of test diets used in trial.*

Sr.No	Dry matter	Ash	Crude lipid	Crude protein	Gross energy
T1	34.00±1.00^{C}	37.00	63.00±3.00^{BC}	34.0±1.00 ^{AB}	39.00±2.00^{BC}
T2	24.00 ± 0.00^{D}	36.00	54.00±2.00^{C}	28.00±1.00^{B}	30.00±1.00^{C}
T3	38.00±1.00^{BC}	35.00	74.00±1.00^{AB}	44.00±4.00^{A}	47.00±2.00 ^{AB}
T4	33.00 ± 0.00^{C}	39.00	76.00±3.00^{AB}	43.00±1.00^{A}	44.00±1.00 ^{AB}
T5	45.00 ± 2.00^{A}	43.00	79.00±2.00^{A}	43.00±3.00^{A}	54.00±3.00^{A}
T6	42.00±1.00^{AB}	45.00	70.00±4.00^{AB}	40.00±1.00^{AB}	47.00± 2.00^{AB}

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

gross energy at level of 0.4 mg kg^{-1}chromium chloride and improved digestibility coefficient of crude protein at 0.2 mg kg^{-1}level. However, additional work is needed to investigate the most appropriate amount of chromium chloride with varying gelatinized levels of other carbohydrate sources for more sustainable aquaculture production.

Authors Contribution

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

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