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Apparent digestibility coefficients of carbohydrates and enzymes activity in major carps in response to Chromium Chloride Hexahydrate

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Abstract

This study investigated the effect of carbohydrate-based corn diets with chromium chloride hexahydrate supplementation on nutrient digestibility and enzyme activity in major carps *(Cirrhinus mrigala, Catla catla,* and *Labeo rohita)* for a period of 90 days. One control (T_1) and five experimental diets (T_2, T_3, T_4, T_5, T_6) were prepared by using gelatinized and non-gelatinized corn with the combination of various levels (0.0, 0.6, and 0.8 mg/kg) of $CrCl_3H_{12}O_6$. After acclimatization with a control diet (T_1) (32% CP), twenty fingerlings of these species were randomly stocked in eighteen digestibility tanks, each with one replicate. At the end of the trial, results showed that gelatinized corn with chromium supplementation significantly enhanced ($P \leq 0.05$) digestibility of nutrients and enzyme activities (amylase and protease) in liver and gut in all treated groups. Crude protein (76.05±0.54), crude lipid (84.53±0.46), carbohydrate (69.15±2.19), and gross energy (76.92±0.74) showed the highest apparent digestibility in *Labeo rohita* as compared to *Catla catla* and *Cirrhinus mrigala.* While highest amylase (liver:3.27±0.126 U/ml and gut: 1.29±0.045 U/ml) and protease activity (liver:1.22±0.116 U/ml and gut 3.42±0.087 U/ml) was recorded in *Catla catla* subsequently followed by *Labeo rohita* and *Cirrhinus mrigala*. Among the treatments, highest amylase activity was observed in the corn gelatinized group at 0.6 mg/kg chromium level, while maximum protease activity was observed at 0.8 mg/kg of chromium level (gelatinized group). Comparison among gelatinized and non-gelatinized corn diets suggested that gelatinization in fish feed performed best as a carbohydrate energy source with chromium supplementation at an appropriate level (0.6 mg/kg) for three major carps as compared to non-gelatinized corn.

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Introduction

Global aquaculture production has increased in current years and it is expected that aquaculture will provide the most dependable supply of food in the future. Aquaculture has been started to contribute appreciably to the intercontinental food supply and countryside livelihood about 30 years ago and in 2018 it reached 96.4 million tonnes (FAO, 2020). Global consumed fish, which is 50% produced through aquaculture, and it is prognosticated that by 2030 it will be the cardinal source of fish (Fazio, 2019). Fish, the main and important component of the aquatic environment and human community have had strong connections with aquatic animals for several years (Lynch et al., 2016).

Pakistan is an agricultural country, rich in aquatic and inland water resources. This type of resource has a tremendous capacity for the economic development of this region (Mohsin et al., 2017). When we flash overworld export, Pakistan contributed only 0.25% which is the rock bottom in the world export market. Many global coastal projects are introducing fisheries management but to establish a fishery economy the main role goes to fish feed and people who tangled themselves effusively and honestly (Nazir et al., 2015). Of all other species cultured in Pakistan, carps are a significant foodstuff that is commercially important fish in Pakistan. For poor consumers, the best and most reasonable protein source are carps, which can sustain both aquaculture and domestic capture (Laghari, 2018). To enhance food security and to reduce the economic threat, carps can be cultured with different integrated structures, and also because of their huge commercial importance, these species have to be cultured on a priority base (Sheikh et al., 2017).

Regarding the production of healthy and efficient fish culture, the fish feed has always been a priority base and shows a dynamic part in the basic economy of different fish culture practices and acts as a vital chunk of investment for cultural purposes (Craig et al., 2017). By using different animal and plant-based artificial feeds, the nutritional requirements of various fish species are fulfilled (Prabu et al., 2017). Carbohydrate is being assessed as a substitute for protein sources in fish feed. It is feasible to replace fishmeal with a plant-based carbohydrate source without any harmful effect on the growth performance of fish? It can be replaced up to 80% with different plant concentrates and mixed with the organic form of Cr (Ngugi et al., 2017). Normally carps can bear a high range of carbohydrates in their feed, show an ability for tissue replacement, and protein-sparing, and grow better than carnivorous fish (Zhao et al., 2020).

Corn is a significant product of the starch industry available in substantial amounts in most countries and can be used in fish feed as a carbohydrate source (Fang et al., 2018). Because of its good protein digestibility, corn is a beneficial alternative to protein for fishmeal (Bu et al., 2018). Gelatinization of starch has efficiently enhanced the carbohydrate consumption in carp fingerlings because of an increase in the activity of endogenous a-amylase. Gelatinization improves the pellet quality and digestibility in some fish species (Romano & Kumar, 2019), although few studies reveal that it has harmful effects on body composition and growth in some animals (Zimonja & Svihus, 2009). To enhance the digestibility of carbohydrates in carps, some additives are needed to be added. Chromium is used as feed additives in different forms due to its dominant part in protein, fats, and carbohydrates metabolism (Khabade, 2015) as chromium salt, chromium chloride hexahydrate and chromium picolinate perform a vital role as a major micronutrient for enhancing the growth of humans and animals (Asad et al., 2017). This study aimed to investigate the effect of various levels of chromium with gelatinized and non-gelatinized corn on apparent digestibility coefficient, and gut and liver enzyme activity (amylase and protease) in major carps.

Materials and Methods

Our experimental trial aimed to investigate the effect of gelatinized and non-gelatinized corn with chromium chloride hexahydrate-based diets in major carps for a period of 90 days.

Experimental Fish

The fingerlings of thaila (*Catla catla),* mrigal *(Cirrhinus mrigala),* and rohu *(Labeo rohita)* were procured from a hatchery of fish seed at Jhaal road, Faisalabad, and were transported in air-filled bags to the fish lab of Zoology Department, Government College University, Faisalabad. Before the experimentation, it was acclimatized for fifteen days under experimental conditions and was fed on a reference diet (NG, 0.0 $CrCl_3H_{12}O_6$ mg/kg) at 4 percent of their wet body weight (Allan & Rowland, 1992) at their proximate station.

Experimental diets

Six experimental diets were supplemented with 0.0, 0.6 and 0.8 mg Cr kg^{-1} as chromium chloride hexahydrate ($CrCl_3H_{12}O_6$) containing G and NG carbohydrates was prepared as T_1(NG, Cr 0.0 mg/Kg); T_2 (G, Cr 0.0 mg/Kg); T_3(NG, Cr 0.6 mg/Kg); T_4 (G, Cr 0.6mg/Kg); T_5 (NG, Cr 0.8 mg/Kg); T_6 (G, Cr 0.8 mg/Kg). Corn is the main feed component. For making gelatinized and non-gelatinized feed, it was grounded to make a fine powder.

Gelatinization process

In the Gelatinization process, the first step is making the dough. For this purpose, an adequate volume of water was added to the grounded corn. For attaining high gelatinization, the dough was put in an autoclave for one hour at fifteen psi. After this process, the dough was spread in try and then placed in a drying oven for 1 hour. This dry mass was ground in a grinding machine and then preserved in an air-tight jar until use. The gelatinization degree was calculated as follows: Gelatinization % = A1/A2 · 100.

Feed ingredients and Experimental design

The experimental diet was assembled according to **Table 1**. Mixed all these ingredients gently, and an adequate amount of water was added to make the dough. The dough was pelletized in a laboratory pelletizer fixed with 2 mm diameter, and the pellets were collected in trays and dried in an oven at 60 ^{0}C. Chromic oxide was used at a one percent inclusion level in the diet.

After acclimatization, 720 fingerlings of three major carps were distributed in thirty-six V-shaped digestibility tanks designed especially for collecting fecal material following a completely randomized design (CRD). In each tank, twenty fingerlings of almost equal size were dispersed arbitrarily. It was a static framework where the water quality parameter was sustained by changing 75% of water and getting rid of water with fecal matter and uneaten feed.

Determination of digestibility and chemical analysis

Feces were accumulated from the fecal collection tube of each tank after two hours of the feeding period. To reduce nutrient discharge, proper attention should be adopted to avoid fragmentation of the fecal filaments. For chemical analysis, fecal material of each group was dried and placed in plastic containers and stored in a cold and dry place unless it was used. A representative sample of the dried feces of all replicates and the experimental diet was analyzed identically by Association of Official Analytical Chemists AOAC (1990) procedures. Before analyzing, these samples were homogenized in a mortar pestle. According to the AOAC, crude protein analysis was carried out in microkjeldhal apparatus; the dry matter was figured out after drying the samples to a constant weight at 105 °C in a drying oven, GE (gross energy) by oxygen bomb calorimeter, and ash was calculated by incineration the given samples for achieving constant weight in a muffle at 550 °C. Crude lipid was evaluated by extraction method using 10454 Soxtec system HTz. Chromic oxide was calculated in feces and feed by spectrophotometer using the way of (Divakaran, 2002) through this UV/VIS2001. Percentage of carbohydrate was measured by difference, i.e. percentage of carbohydrate **=** 100 – (moisture + ash + Protein + Fat)

Calculation of Apparent digestibility coefficient

ADC % nutrients for the respective experimental diet were estimated by using this formula (NRC, 1993)

ADC of dry matter diet (%) = 100 × [1- (dietary chromium/ fecal chromium) × (fecal nutrient / dietary nutrient)

Enzymes Activity Analysis

After the experiment, the liver and gut were taken three fish from each tank. The fish was anesthetized with clove oil and dissected to collect the liver and intestine for estimation of digestive enzyme (amylase and protease). After extraction, the liver and gut were homogenized in 250 mg/ml of distilled water at 4ºC. The Homogenate mixture was centrifuged at 5000 rpm for 20 min at 4ºC. The subsequent supernatant was removed and stored at -80°C until the enzymatic analysis.

Amylase activity

The activity of amylase was measured by starch used as substrate (Bernfeld, 1955). At 37 ºC, one percent starch substrate and a well-diluted sample (1 ml) were incubated for 3 minutes. The reaction was stopped by adding 3.5 DNS reagent (2 ml). The solution was heated for 5 minutes, cooled, and then added the distilled water (20 ml) in a boiling water bath. Checked the absorbance against blank at 540 nm wavelength by using this formula.

$$Enzyme\ activity\ \left(\frac{U}{ml}\right) = \frac{\text{absorabance of enzyme - absorbanceof blank}}{incubation\ time\ \times dilution\ factor} \times S.F$$

Where'
S. F= standard factor which is 6.5
Incubation time = 3 minutes
Dilution factor = 1

Protease Activity

A protease assay was used to determine the activity of the protease enzyme. The amount of enzyme that catalyzed the hydrolysis of 1 μmol of substrate per minute at the assay temperature is the one unit of enzyme activity. Total proteolytic activity was measured using the casein hydrolysis method of Kunitz (1947) as modified by Walter (1984).

The reaction was conducted by using a wide range of PH. The enzyme reaction mixture was incubated at 37°C, comprised of 1 percent casein solution in 0.05 M Tris PO_4 buffer (pH 7.8) for almost 5 min. After ten minutes, the reaction was stopped by 10 percent TCA solution, then filtered the entire content. Before stopping the reaction, the reagent blank was prepared by adding tissue homogenate with no incubation. The tyrosine standard curve expressed as mole tyrosine, released /mg/min protein at 37°C, was used to determine proteolytic enzyme activity.

To calculate the enzyme activity following formula was used.

$$\text{Units/ml Enzyme} = \frac{(\mu\text{mol tyrosine equivalents released}) \times (11)}{(1) \text{ x } (10) \text{ x } (2)}$$

11= Total volume of assay in milliliters
10= total time (in minutes) as per the unit definition
1= volume of enzyme (in milliliters) of enzyme used
2= volume (in milliliters) used in the colorimetric determination

Statistical Analysis

Following the completion of the experimental trial, the obtained data from apparent digestibility coefficient and enzyme activity of liver and gut were subjected to statistical analysis (ANOVA), and mean values were evaluated by using SPSS (Steel et al., 1996) and the difference between means ($P \leq 0.05$) were evaluated by the Tukey's HSD Test (Snedecor & Cochran, 1991).

Results

Apparent digestibility Coefficient

The apparent nutrient digestibility coefficient of major carps fed on control and experimental diets is presented in **Table 2**. When dry matter digestibility was statistically compared (ANOVA) of three major carp species fingerlings a significant ($P \leq 0.05$) difference between the three species was noted while a non-significant ($P \geq 0.05$) between the treatments was observed in each species. Results showed the highest dry matter digestibility (78.00±2.10 %) in *Labeo rohita* fingerlings for treatment T_4 and minimum dry matter digestibility (72.40±1.40) was also observed in fingerlings of *Labeo rohita* which fed on 0.8 mg/kg of chromium with non-gelatinized corn (T_5). For lipid and protein digestibility, a highly significant difference ($P \leq 0.01$) was observed between the treatments in each species. Results showed the highest ADC % of protein and lipid nutrient was noted in *Labeo rohita* fingerlings in treatment T_4 whereas, a minimum ADC % value was observed in *Catla ctala* and *Cirrhinus mrigala* fingerlings of treatment T_1 (control). In the case of carbohydrates, the highest nutrient digestibility (73.90±1.00) was observed in *Labeo rohita* in treatment T_2 followed by *Catla catla* (73.90±1.00) in T_4 and then in *Cirrhimus mrigala* which was also noted in treatment T_4. While the lowest carbohydrate digestibility (53.40±0.70) was seen in *Labeo rohita* subsequently followed by *Catla catla* and then in *Cirrhinus mrigala* in treatment T_6.

For ADC % of gross energy, results showed that the highest digestibility (79.90±1.10 %) was noted in *Labeo rohita* fingerlings for treatment T_4 followed by *Catla catla* and *Cirrhinus mrigala* while the lowest values (72.70±0.80) of ADC % was observed for treatments T_6. However, a significant difference ($P \leq 0.05$) for ADC% of gross energy was observed between all the treatments.

Table 2. Apparent digestibility coefficient % of major carps fed upon control and experimental diets

Species	Parameters	T_1 NG= $CrCl_3H_{12}O_6$ (0.0mg/kg)	T_2 G= $CrCl_3H_{12}O_6$ (0.0mg/kg)	T_3 NG= $CrCl_3H_{12}O_6$ (0.6mg/kg)	T_4 G= $CrCl_3H_{12}O_6$ (0.6mg/kg)	T_5 NG= $CrCl_3H_{12}O_6$ (0.8mg/kg)	T_6 G= $CrCl_3H_{12}O_6$ (0.8mg/kg)
Labeo rohita	Dry Matter	75.00±1.10a	77.00±1.30a	76.00±1.10a	78.00±2.10a	72.40±1.40a	73.10±1.40a
Catla catla		77.30±1.20a	75.00±1.90a	75.70±1.10a	77.30±1.30a	72.60±1.90a	73.60±1.10a
Cirrhinus mrigala		73.00±0.90a	73.20±0.80a	74.10±0.90a	74.70±0.90a	72.70±1.10a	72.90±1.40a
Labeo rohita		84.00±1.10ab	84.80±0.90ab	85.00±1.10ab	86.10±1.20a	83.00±1.30ab	84.30±1.40ab
Catla catla	Crude lipid	80.10±1.20ab	84.00±0.10ab	84.60±0.40ab	85.50±0.70a	81.20±0.70ab	84.20±0.80ab
Cirrhinus mrigala		80.10±0.90a	83.40±0.90	83.80±1.20	85.10±0.40a	80.60±1.40a	83.40±1.50a
Labeo rohita		74.30±0.90ab	76.20±0.80ab	75.90±0.90a	79.30±1.20ab	75.00±0.60ab	75.60±0.40ab
Catla catla	Crude Protein	73.50±0.90ab	76.50±0.60ab	76.70±0.80ab	77.70±0.90ab	74.20±0.80ab	74.80±0.20ab
Cirrhinus mrigala		73.30±0.90ab	73.60±0.90ab	76.80±1.00ab	77.70±1.30ab	76.30±1.00	76.70±0.70
Labeo rohita		70.00±1.20ad	74.00±0.70ae	73.00±1.10ab	72.60±1.60e	71.90±1.80b	53.40±0.70b
Catla catla	Carbohydrate	68.20±1.80ad	67.10±0.90ae	68.60±1.70ad	73.90±1.00cf	65.40±0.70ab	59.40±0.90b
Cirrhinus mrigala		66.70±0.90ad	69.40±1.50ad	66.60±1.30def	70.90±1.40ad	66.50±0.80ab	61.20±1.70b
Labeo rohita		75.90±0.70aj	78.30±0.70ab	78.00±1.10fg	79.90±1.10ef	76.70±1.30ab	72.70±0.80bc
Catla catla	Gross Energy	74.00±1.00bcd	76.40±0.60a-d	77.00±0.60a-d	79.00±1.10ab	74.10±0.20bcd	73.90±1.60bcd
Cirrhinus mrigala		73.70±1.30cd	75.60±0.40a-d	76.30±0.70a-d	78.40±0.20abc	74.20±1.40bcd	74.90±0.10a-d

Means within a column or in rows having similar letters are statistically non-significant ($P \geq 0.05$). Small letters symbolize comparison among interactions within species.

Liver and Gut enzyme activity of major carps

Amylase activity

From the present study, it was revealed that amylase activity in liver and gut of three major carps was significantly affected by the chromium level in fish feed. The gut amylase activity clearly depicted the highest value (1.44±0.05 U/ml) in treatment T_4 (0.6 mg/kg $CrCl_3H_{12}O_6$) which was given gelatinized corn while control (T_1) showed the minimum (0.99±0.04 U/ml) enzyme activity with respect to all other treatments. However, treatment T_4 showed highly significant ($P \leq 0.01$) difference from control (T_1) and all other treatments (T_2, T_3, T_5, T_6) when all species were compared. Whereas species comparison of means values showed the highest gut amylase activity (1.29±0.045 U/ml) in *Catla catla* and lowest amylase activity (1.14±0.052 U/ml) was noted in *Cirrhinus mrigala* **(Table 3).** The result showed the general enhancement of liver amylase activity as compared to the gut enzyme activity of three major carps. **Table 4** shows the mean values of liver amylase activity, treatment T_4 showed the peak value (3.68±0.06 U/ml) in liver of three major carps while minimum activity (2.56±0.05 U/ml) was seen in T_1 (control). Regarding experimental fish species, comparison of mean showed the highest elevation of liver amylase activity (3.27±0.126 U/ml) in *Catla catla*, moderate activity (3.13±0.115 U/ml) in *Labeo rohita* and lowest (2.94±0.110 U/ml) activity was noted in *Cirrhinus mrigala.*

Table 3. Gut amylase activity (U/ml) of major carps fingerlings fed on control and treatment diets.

Treatments	Species			Mean
	Labeo rohita	*Catla catla*	*Cirrhinus mrigala*	
T_1	0.98±0.005ef	1.10±0.010def	0.90±0.030f	0.99±0.04C
T_2	1.28±0.060bcd	1.34±0.020abc	1.16±0.050cde	1.26±0.04B
T_3	0.99±0.005ef	1.15±0.050cde	0.91±0.040f	1.02±0.05C
T_4	1.48±0.055ab	1.54±0.050a	1.32±0.005bc	1.44±0.05A
T_5	1.32±0.000bc	1.30±0.025bcd	1.27±0.025cd	1.29±0.01B
T_6	1.33±0.040bc	1.33±0.050bc	1.27±0.040bcd	1.31±0.02B
Mean	1.23±0.057B	1.29±0.045A	1.14±0.052C	

Table 4. Liver amylase activity (U/ml) of three major carps fingerlings fed on control and treatment diets.

Treatments	Species			Mean
	Labeo rohita	*Catla catla*	*Cirrhinus mrigala*	
T_1	2.62±0.055hi	2.65±0.055hi	2.42±0.010i	2.56±0.05E
T_2	2.98±0.020def	3.29±0.065c	2.88±0.020efg	3.05±0.08C
T_3	2.83±0.045fgh	2.93±0.045efg	2.73±0.055gh	2.83±0.04D
T_4	3.67±0.035ab	3.84±0.055a	3.53±0.010b	3.68±0.06A
T_5	3.09±0.010cde	3.21±0.020cd	2.81±0.040fgh	3.04±0.08C
T_6	3.58±0.035b	3.73±0.055ab	3.26±0.040c	3.52±0.09B
Mean	3.13±0.115B	3.27±0.126A	2.94±0.110C	

Means within column or in rows having similar letters are statistically non-significant ($P \geq 0.05$). Capital letters are used for overall mean while small letters symbolize comparison among interactions within species.

Protease activity

In addition, protease activity also supported the same trend of amylase activity which was highly affected by the inclusion of chromium. This finding clearly illustrated the highest value (3.58±0.11 U/ml) of gut protease activity in treatment T_4 (0.6 mg/kg $CrCl_3H_{12}O_6$) which was given gelatinized corn while treatment T_5 showed the minimum (3.05±0.07 U/ml) enzyme activity with respect to all other treatments. However, treatment T_5 showed highly significant ($P \leq 0.01$) difference from control (T_1) and also with remaining treatments (T_2, T_3, T_5, T_6). Whereas comparison of means values regarding species showed the highest gut protease activity (3.42±0.087 U/ml) in *Catla catla* while minimum enzyme activity (3.36±0.092 U/ml) was noted in *Cirrhinus mrigala* **(Table 5).** However, for liver protease activity the highest value (1.34±0.18 U/ml) was showed in treatment T_4 (0.6 mg/kg $CrCl_3H_{12}O_6$) which was given gelatinized corn while treatment T_5 showed the minimum (0.62±0.13 U/ml) enzyme activity with respect to all other treatments. Whereas in case of species, comparison of means values showed the highest liver protease activity (1.22±0.116 U/ml) in *Catla catla* and lowest protease activity (0.92±0.097 U/ml) in *Cirrhinus mrigala* **(Table 6).**

Table 5. Gut protease activity (U/ml) of three major carps fingerlings fed on control and treatment diets.

Treatments	Species			Mean
	Labeo rohita	*Catla catla*	*Cirrhinus mrigala*	
T_1	3.66±0.050bc	3.47±0.045cde	3.26±0.030ef	3.46±0.08B
T_2	3.60±0.005bcd	3.73±0.055ab	3.40±0.010de	3.57±0.06A
T_3	3.42±0.005de	3.17±0.045fg	3.55±0.035bcd	3.38±0.07BC
T_4	3.32±0.010ef	3.54±0.060bcd	3.89±0.010a	3.58±0.11A
T_5	3.26±0.060ef	2.94±0.040h	2.96±0.045gh	3.05±0.07D
T_6	3.14±0.015fgh	3.71±0.055ab	3.12±0.005fgh	3.32±0.12C
Mean	3.40±0.056AB	3.42±0.087A	3.36±0.092B	

Table 6. Liver protease activity (U/ml) of three major carps fingerlings fed on control and treatment diets.

Treatments	Species			Mean
	Labeo rohita	*Catla catla*	*Cirrhinus mrigala*	
T_1	0.97±0.005def	0.99±0.000de	0.95±0.035def	0.97±0.01D
T_2	0.96±0.005def	1.36±0.015bc	1.17±0.045bcd	1.16±0.07B
T_3	1.06±0.065de	0.99±0.005de	0.97±0.005def	1.00±0.02CD
T_4	0.99±0.005de	1.89±0.095a	1.15±0.050cd	1.34±0.18A
T_5	0.91±0.020def	0.72±0.065f	0.23±0.020g	0.62±0.13E
T_6	0.89±0.030ef	1.41±0.090b	1.05±0.060de	1.12±0.10BC
Mean	0.96±0.019B	1.22±0.116A	0.92±0.097B	

Means within columns or in rows having similar letters are statistically non-significant ($P \geq 0.05$). Capital letters are used for overall mean while small letters symbolize comparison among interactions within species.

Discussion

In the current study, processing corn with an appropriate level of chromium ameliorated the apparent digestibility coefficient (ADC%) of nutrients in three major carps. The highest ADC % of CP was observed in treatments that fed on gelatinized corn with chromium supplementation. Whereas, minimum digestibility values were observed in fish species that were fed on a control diet prepared by using only non-gelatinized corn. However, the digestibility of CP in *Labeo rohita* and *Catla catla* could not be significantly improved at higher supplementation of chromium s but was better than control. This ameliorated the effect of gelatinization was also revealed by Asad et al. (2019) who investigated the effect of chromium with gelatinized corn on the digestibility of nutrients in *Crrhinus mrigala,* which demonstrated that the highest digestibility coefficient of CP was observed in treatment when fish fed with gelatinized corn at 0.2 mg/kg of chromium level. They also found that ADC % CP could not be further improved on a higher level of chromium inclusion in the fish diet.

In current findings, the higher ADC % of crude protein was observed in *Labeo rohita* followed by *cirrhinus mrigala* and *Catla catla*. This difference may be due to their feeding behavior. Abbas et al. (2019) observed similar findings of higher ADC % of the protein in *Labeo rohita* rather than *Catla catla* and *Cirrhinus mrigala*. The ADC % of the protein in this study was also in good agreement with Azaza et al. (2020) who observed the highest digestibility of crude protein in the *Oreochromis niloticus* fed with a corn starch diet as compared to glucose, maltose, and dextrin. However, this study was in disagreement with the work done by Ren et al. (2015) who observed the lowest crude protein digestibility in *Megalobrama amblycephala* fed with corn starch.

The present study indicated that the highest apparent digestibility coefficient of crude lipid was observed in *Labeo rohita* fed on gelatinized corn with 0.6 mg /kg of chromium supplementation followed by *Catla catla* and then *Cirrhinus mrigala.* . However, in *Catla catla* and *Cirrhinus mrigala* the minimum apparent digestibility of crude lipid was observed in control. Current results showed that a further increase from 0.6 mg /kg of chromium substitution cannot improve the digestibility of lipid in all three major carps. The higher lipid digestibility recorded in T_4, T_3, T_2 may attribute to the complementary effect of chromium with gelatinized corn which helps in the digestion of nutrients and brings about a balanced carbohydrate that acts as binders and also ameliorates the pellet stability in water and also helps during mechanical stress due to handling, storage and transport (Romano et al.,2018). Similar to the present findings, Asad et al. (2019) found maximum lipid digestibility in *Cirrhimus mrigala* fingerlings when fed with gelatinized corn at 0.5 chromium chloride supplementation. In contrast to our results, Asad et al. (2014) found the maximum apparent digestibility coefficient of crude lipid in non-gelatinized corn, and the lowest was observed in the gelatinized fed group. This discrepancy in results for lipid digestibility maybe because of sources of carbohydrates, pH of the stomach, different fish species, feed ingredients, or feed drying method (Luo et al., 2013).

In the current study, the digestibility of carbohydrates varies significantly among the control and treated groups. The overall highest ADC% of carbohydrate was observed in Treatment T_4 similar to lipid and protein digestibility while the lowest was observed in treatment T_6 which was also gelatinized corn but the inclusion of chromium was 0.8 mg/kg. however, among other treatments the maximum digestibility of carbohydrates was observed in non-gelatinized fed groups but less than lipid and protein apparent digestibility. This finding is congruent to the observation obtained by Sandre et al. (2017) on *Colossoma macropomum*. They observed low carbohydrate digestibility with high carbohydrate inclusion.

The dietary level, quantity and chemical composition of carbohydrates are essential factors for digestibility and should be within the bearable limit of the fish (Talukdar et al., 2018).

In this study, there is a significant difference in liver and gut enzyme activity (amylase and protease) of three major carps. These findings depicted that with chromium supplementation, the activity of these enzymes increased more than the control. The activity of amylase in the liver and gut was higher at 0.6 mg/kg with gelatinized corn while the dietary chromium above the optimum level reduces the activity of amylase in the liver and gut. However, amylase activity in the liver was comparatively higher than in the gut of three major carps. Similar results of amylase activity in the liver and gut were observed by Abdel-Warith et al. (2020) in African catfish. Higher amylase activity was noted in the liver than in the stomach. Our study was also supported by Talukdar et al. (2018) who reported the maximum amylase activity in a group of *Clarias batrachus* which fed a higher percentage of gelatinized carbohydrates. The inclusion level of carbohydrates also correlates with the intestinal and gut amylase activity which is more eminent in the herbivorous and omnivorous fish. In the present study, higher activity of amylase with increasing chromium and gelatinization process suggest that three experimental carps were able to digest carbohydrates more efficiently. In the comparison of three experimental fish species, the current result demonstrated the higher activity of amylase in the liver and gut was observed in *Catla catla* followed *Labeo rohita* and then in *Cirrhinus mrigala*. This was also observed by Murataza et al. (2016) who noted the higher amylase activity in *Catla catla* than *Cyprinus carpio* and *Hypophthalmichthys molitrix* administrated with a corn-based diet. This might be because of the feeding habit and the ecological niche of these three major carps (Li et al. 2012).

The protease activity in the liver and gut of three major carps was significantly varied from each other. In *Labeo rohita* fingerlings the protease activity was not affected by the gelatinization of corn. The overall protease activity in the liver and gut was maximum in *Catla*

catla then in *Labeo rohita* and *Cirrhinus mrigala*. . The higher activity of protease in current results with chromium supplementation is also in line with the Kole et al. (2017) who demonstrated that exposure to chromium caused a significant increase in activity of the digestive enzyme (amylase, Protease) in *Anabas testudineus*. However, contradicts the finding of Sunita et al. (2015), who observed a decrease in the protease activity of three major carps (*Catla catla*, *Labeo rohita*, *Cirrhinus mrigala*) when supplementation with cadmium and zinc. A decreasing trend in protease activity might be of changing feeding behavior, i.e., under toxic stress, the fishes did not feed effectively.

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