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Gelatinized Carbohydrates in the Diet of *Catla catla* Fingerlings: Effect of Levels and Sources on Nutrient Utilization, Body Composition and Tissue Enzyme Activities

Sona Yengkokpam, N. P. Sahu*, A. K. Pal, S. C. Mukherjee[1] and Dipesh Debnath
Department of Fish Nutrition and Biochemistry. Central Institute of Fisheries Education
Fisheries University Road. Mumbai-400 061, India

ABSTRACT : A Feeding trial was conducted to study the effects of three different sources and two levels of dietary gelatinized carbohydrate (GC) on nutrient utilization, growth, tissue composition and tissue enzyme activities of fingerlings of *Catla catla* (15.1-15.3 g). Six isocaloric (17.1-17.5 kJ/g) semi-purified diets were prepared either with rice, corn or tapioca at 40 or 50% GC each. The crude protein (CP) level used in the diet was 35% and 25% for 40% and 50% GC level, respectively to study the protein sparing effect of GC. The degree of gelatinization was higher for corn and tapioca than rice under similar cooking conditions. After a 60-d feeding trial, dry matter, carbohydrate, protein and lipid digestibility were higher in tapioca fed groups at both the levels of GC. However, the highest specific growth rate (SGR) and protein efficiency ratio (PER) were observed in the corn fed groups at 50% GC level indicating better utilization of nutrients from gelatinized corn. Feed conversion ratio (FCR) was almost similar in corn and tapioca fed groups between two levels of GC but in rice fed groups, FCR was lower in 40% GC than 50% GC level. The results indicated higher protein-sparing effect in corn and tapioca fed groups than rice fed groups. The order of gelatinized carbohydrate utilization in *Catla catla* fingerlings at 50% GC level was corn>tapioca>rice. At 40% GC level, corn and tapioca were comparable and more efficiently utilized than rice. In the corn fed groups, 50% GC was comparable with 40% GC level, whereas in rice and tapioca fed groups the 40% GC was better in terms of nutrient utilization. Liver glycogen content and hepatosomatic index were significantly ($p<0.05$) higher in those groups fed high GC (50%) irrespective of carbohydrate sources. Higher intestinal amylase and glucose-6-phosphate dehydrogenase activities were observed in higher GC fed groups than the lower GC groups. No mortality was found in any groups at any levels of GC. (**Key Words :** Corn, Rice, Tapioca, Gelatinization, *Catla catla*, Enzyme, Glycogen)

INTRODUCTION

The use of carbohydrate-rich ingredients in fish diet is gaining importance because of their ability to spare the more expensive protein component of the diet to be used for growth (Shiau, 1997). Although lipids constitute an important source of non-protein energy for fish (Kaushik et al., 1989) they are more expensive compared to carbohydrates. Carbohydrate-rich ingredients, apart from being the most economical source of energy in the diet, are also abundantly available throughout the world. In spite of its lower utilization, in omnivorous warm water fish such as carps, the carbohydrate utilization is more important (Shikata et al., 1993; Shimeno and Shikata, 1993; Wilson, 1994). There are reports of limited ability to utilize carbohydrate in carnivorous fish like cod (Hemre et al., 1993), Atlantic salmon (Hemre et al., 1995a) and white sturgeon (Deng et al., 2000). However, improved growth performance was observed in different species of fish upon feeding high carbohydrate diets viz., eels (Degani and Viola, 1987), rainbow trout (Kim and Kaushik, 1992; Grisdale-Helland and Helland, 1997), catfish (Hung et al., 2002) and carp (Mohapatra et al., 2003).

Utilization of carbohydrate by fish depends on three major factors-degree of gelatinization, type of starch and dietary inclusion level (Singh and Nose, 1967; Wilson, 1994; Shiau, 1997). Starch can be a valuable source of energy and its utilization is enhanced by gelatinization (Bergot and Breque, 1983) due to its increased digestibility

* Corresponding Author: N. P. Sahu. Tel: +91-22-26361446/7/8 (ext.475), Fax: +91-22-26361573, E-mail: npsahu1@rediffmail.com
[1] Department of Fish Pathology and Microbiology, Central Institute of Fisheries Education, Fisheries University Road, Mumbai-400 061, India.
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(Bergot, 1991; Podoskina et al., 1997; Mohapatra et al., 2003). Gelatinization results in hydratization and swelling of starch granules (Silano et al., 1975; Hofer and Sturmbauer, 1985) and thus improves digestibility by increasing the susceptibility of starch granules to enzyme. It has been reported that the digestibility of tuber starch (potato and tapioca) is lower than that of cereal starch in rainbow trout (Bergot, 1991) and carp (Schwarz and Kirchgessner, 1991). Corn fed pigs were found to have higher feed conversion ratio than wheat fed groups (Han et al., 2005). Rations based on corn are also commonly used in poultry for their high available energy (Wang et al., 2005). Low digestibility of starch at higher inclusion level has also been reported (Bergot, 1979; Bergot and Breque, 1983; Hemre et al., 1989). However, significant increase in digestibility was reported with increase in gelatinized carbohydrate level in the diet of a carp *Labeo rohita* fry (Mohapatra et al., 2003). An excess of digestible carbohydrate in the diet however, causes growth retardation in rainbow trout (Hilton et al., 1982; Hilton and Slinger, 1983). The dietary carbohydrates are known to cause prolonged hyperglycemia in a number of fish (Hilton and Slinger, 1983; Kaushik and De Oliva-teles, 1985; Walton, 1986; Brauge et al., 1994). As compared to carnivorous fish, the capacity of glucose regulation of carp (an omnivorous fish) is considerably higher (Furuichi and Yone, 1981). High levels of dietary carbohydrate in rainbow trout resulted in increased deposition of glycogen in liver (Hilton and Slinger, 1983; Walton, 1986; Pfeffer et al., 1991; Kim and Kaushik, 1992) and increased liver size (Kim and Kaushik, 1992). Increased hepatosomatic index was also observed in cod (Hemre et al., 1989) and Atlantic salmon (Hemre et al., 1995b) fed high carbohydrate diets.

Catla catla, one of the Indian major carps, is an economically important aquaculture species in India. It is an omnivorous freshwater species cultured traditionally in earthern ponds and fed with mixtures of rice bran and groundnut oil cake in equal proportions as dry powder or as moist paste. Very few nutritional studies have been carried out on this species. Fingerlings of *Catla catla* grew better with a 35% protein diet containing 4% and 35% fat and carbohydrate, respectively (Seenappa and Devaraj, 1995). In an earlier study, it was reported that 40% gelatinized starch was optimum for growth of *L. rohita* fry (Mohapatra et al., 2003).

In the present study, two levels of gelatinized carbohydrate i.e. 40% and 50%, from three different sources (rice, corn and tapioca) were used at two crude protein levels (35% and 25%). At higher level of GC, the CP level was reduced to study the protein sparing action of the carbohydrate. Rice, corn and tapioca were selected due to their abundant availability in the country. Aim of the present study was to evaluate the effect of different sources and levels of GC on growth, conversion efficiency, body composition, nutrient digestibility and enzyme response in *Catla catla* fingerlings.

MATERIALS AND METHODS

Experimental diets

Six isocaloric diets with three carbohydrate sources (rice, corn and tapioca flour) each at two GC levels (40% and 50%) were formulated (Table 1). The crude protein content of the feeds was kept around 35% or 25% at 40 and 50% GC, respectively for all the sources. The flour of rice, corn and tapioca were added with approximately 80% water (v/w) and autoclaved at 120°C for 1 h to achieve maximum gelatinization. These gelatinized carbohydrates were then spread over a tray and dried in oven at 60°C. The dried mass was then pulverized in a hammer mill (DP Pulveriser works, Mumbai, India) through a 0.5-mm screen and used in feed. Gelatinized rice, corn and tapioca flour were analyzed for their degree of gelatinization before the feed formulation. The amount of different carbohydrate sources in the feed was adjusted to achieve a GC level of either 40 or 50%. The GC level in the diet was calculated by multiplying % of carbohydrate inclusion with its degree of gelatinization. All the ingredients were mixed thoroughly and water was added to form dough. It was then steam conditioned for 5 min. Pellets were prepared by a hand pelletizer into 2-mm diameter, dried in an oven at 60°C for 24 h and stored in an airtight polyethylene bags at room temperature (28-30°C) until use.

Experimental design and feeding trial

Fingerlings (15.1-15.3 g) of *Catla catla* were procured from Khopoli Govt. Fish Farm, Maharashtra, India. Ninety fingerlings were randomly distributed in six experimental groups with three replicates each. Fishes were stocked in plastic tubs (100 L capacity) with continuous aeration. It was a static system where at least 75% of water was exchanged every day with fresh water by siphoning out water with uneaten feeds and faecal matters. Before the experiment, the fishes were acclimatized to the experimental condition for 30 d and fed a 30% crude protein diet. The water quality parameters, i.e. temperature, pH, dissolved oxygen (DO), carbon dioxide (CO_2), ammonia-nitrogen and nitrite- nitrogen were recorded every week following standard methods (APHA 1985). Dissolved oxygen (DO) and pH ranged from 6.17 to 7.9 ppm and 7.5 to 8.49, respectively. The ammonia and nitrite levels varied between 0.27-0.67 ppm and 0.04-0.17 ppm, respectively. Water temperature varied from 25 to 27°C and CO_2 was not detected in any of the tubs. The fingerlings were fed to satiation twice daily (0800 and 2000 h) and daily feed

Table 1. Ingredient and proximate composition of experimental diets (%)

Ingredients	Rice		Corn		Tapioca	
	40	50	40	50	40	50
Casein[1]	32.7	17.5	35.2	20.6	38.6	24.9
Gelatin[2]	5.0	5.0	5.0	5.0	5.0	5.0
Carbohydrate sources[3]	46.9	58.7	42.4	53.0	41.4	51.7
Carboxymethylcellulose[4]	1.0	1.0	1.0	1.0	1.0	1.0
Cellulose[4]	5.3	8.8	7.4	11.4	5.0	8.4
Sunflower oil:cod liver oil[3] (2:1)	6.0	6.0	6.0	6.0	6.0	6.0
Vitamin-mineral mix[5]	2.6	2.6	2.6	2.6	2.6	2.6
Vitamin B complex[6]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin C[7]	0.1	0.1	0.1	0.1	0.1	0.1
Glycine[8]	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition % (Mean±SD; n = 3)						
Crude protein	34.6±0.0	25.5±0.2	33.3±0.2	25.0±0.2	33.3±0.2	25.0±1.0
Lipid	5.5±1.6	5.6±0.2	6.2±0.3	6.2±0.3	6.4±0.2	6.3±0.2
Ash	3.1±0.0	4.8±0.2	3.4±0.2	3.2±0.2	3.6±0.0	3.5±0.0
Total carbohydrate	56.3±0.7	64.1±0.3	57.1±0.3	65.7±0.2	56.7±0.3	65.2±1.4
Calculated digestible energy ($KJ\ g^{-1}$ diet)	17.3±6.4	17.1±2.3	17.4±2.6	17.5±1.9	17.5±1.7	17.5±6.4
Degree of gelatinization (%)	85.2±2.4	85.2±2.4	94.3±2.3	94.3±2.3	96.5±0.7	96.5±2.4
GC%*	40	50	40	50	40	50

The antioxidant Butylated Hydoxy Toluene (Himedia ltd, India) was added at 0.02% of the added oil.

[1] Casein fat free: 75%CP (Himedia ltd, India): [2] Gelatin: 96% CP (Himedia ltd. India).

[3] Procurred from local market, Mumbai, India.

[4] Sd Fine Chemicals Ltd., India.

[5] Composition of vitamin mineral mix (Agrimin, India) (quantity/kg): Vitamin A 625,000 IU; Vitamin D_3 62,500 IU; Vitamin E 250 mg; Nicotinamide 1 g; Cu 312 mg; Co 45 mg; Mg 6 g; Fe 1.5 g; Zn 2.13 g; Iodine 156 mg; Se 10 mg; Mn 1.2 g; Ca 247.34 g; P 114.68 g; S12.2 g; Na 5.8 mg; K 48.05 mg.

[6] Composition of vitamin B complex (Glaxo, India) (quantity/g): Thiamine mononitrate 20 mg; Riboflavin 20 mg; Pyridoxine hydrochloride 6 mg; Vitamin B_{12} 30 mcg; Niaciamide 200 mg; Ca pantothenate 100 mg; Folic acid 3 mg; Biotin 200 mcg.

[7] Roche, India.

[8] Himedia ltd., India.

* GC% = % of carbohydrate source×degree of gelatinization.

intake was monitored. The feeding trial lasted for 60 d.

Digestibility studies

The digestibility study was conducted by using chromium oxide (Cr_2O_3) as an external marker. Feed containing Cr_2O_3 (1%) was fed during the last 20 d of the experimental period. After 3 d of feeding Cr_2O_3-containing feeds, faecal matters were collected daily. The fingerlings were fed to satiation once daily at 0800 h and the tubs were cleaned to remove any uneaten feeds and faecal matters at 1700 h. Faeces were collected in the next day, in the morning by siphoning out and kept in an oven at 80°C for drying. Finally faecal matter for the whole 20 d were pooled for each group, finely ground, dried to constant weight at 100°C and stored at 4°C until further analysis.

Sampling

Fish were weighed at the start and every 20 d interval thereafter till the termination of the experiment on the 60th d. At the end of experiment, 6 fish for each dietary treatment were anaesthetized with clove oil (50 µl/L) and dissected to collect liver and intestine for enzyme analysis. Immediately, a 5% homogenate in 250 mM sucrose was prepared for liver and intestine tissues. The homogenate was centrifuged at 10,000×g for 20 min and the supernatant was collected in a sample vial and stored at -20°C until use. Whole intestine was used for amylase and protease assays. Before homogenization the intestinal content was removed. All the enzyme assays were performed within 4 d. Another 6 fish per treatment were used to collect muscle and liver tissue to analyze the glycogen content. The liver and muscle tissues were digested in 30% KOH for glycogen estimation. The muscle samples were taken from the caudal peduncle region after scraping off the scales.

Chemical analysis

Starch gelatinization : The degree of gelatinization of different carbohydrate sources was estimated as follows (Guraya and Toledo, 1993). A known amount (0.2 g) of dried sample was mixed with 15 ml of 0.2 N potassium hydroxide followed by intermittent stirring for 30 min. The pH of the mixture was adjusted to 5.5 using 2 N phosphoric acid and the volume was made upto 100 ml with distilled water. Next, 100 µl of aliquot was transferred to a test tube and diluted to 5 ml with distilled water. Then 50 µl of standard iodine solution (4% KI, 1% I_2) was added and the

absorbance of the solution was measured at 600 nm (A_1) against the reagent blank. Another aliquot was made by the same procedure by mixing 0.2 g of dried sample in 15 ml of 0.6 N potassium hydroxide and the absorbance was measured at 600 nm (A_2) as above. The degree of gelatinization was calculated as follows:

Gelatinization % = (A_1/A_2) 100

Proximate analysis : Experimental diets were analyzed using standard methods (AOAC, 1995) for dry matter (DM), crude protein (CP), lipid and ash. Dry matter content was determined by drying the samples to constant weight at 100°C; crude protein by Kjeltec semi-automatic system (Tecator); lipid by Soxtec system (Model SD2, 1045, Tecator) and ash by muffle furnace incineration at 550°C for 6 h. Total carbohydrate (TC) was calculated by difference and digestible energy content of the feed was calculated (Halver, 1976). The initial and final tissue composition of the fish and the proximate composition of the faecal matters were analyzed for all the treatment groups as described above. All the analyses were done in triplicates.

Chromium assay : The chromium content in the feeds and faecal matter was estimated by using atomic absorption spectrophotometer. Wet digestion (AOAC, 1995) of the sample was carried out and the chromium content was estimated by flame ionization atomic absorption spectrophotometer (AAS 4129, Electronics Corporation of India Limited) using chromium cathode lamp (357.9 nm).

Analysis of glycogen and enzymes

Muscle and liver glycogen content was estimated colorimetrically by treating with anthrone reagent (Hassid and Abraham, 1957). In brief, tissue was put in 30% potassium hydroxide and boiled in water bath until digested. After cooling, 5 ml 95% ethanol was added and centrifuged at 6,000×g for 10 min. Supernatant was discarded and glycogen was again precipitated as above. Then the precipitate was dissolved in distilled water and an aliquot was reacted with anthrone and the optical density was read at 590 nm. A standard was run along with the sample to calculate the sample concentration.

Amylase activity of liver and intestine was measured by estimating the reducing sugars produced due to the action of glucoamylase and α-amylase on carbohydrates (Rick and Stegbauer, 1974). The reaction mixture consisted of 1% (w/v) starch solution, phosphate buffer and the tissue homogenate. The reaction mixture was incubated at 37°C for 30 min. Dinitro salicylic acid (DNS) was added to stop the reaction and kept in boiling water bath for 5 min. After cooling, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard and amylase activity was expressed as mmol of maltose released from starch per min at 37°C.

Total proteolytic activity of liver and intestine was measured using the casein hydrolysis method (Walter, 1984). Enzyme reaction mixture consisted of 1% (w/v) casein solution, phosphate buffer (pH 8.0) and the tissue homogenate, which was incubated for 1 h at 37°C. The reaction was stopped by adding 6% trichloroacetic acid (TCA). After holding for 1 h at 4°C, samples were centrifuged (Remi, India Ltd.) at 10,000xg for 10 min and the absorbance of the supernatant was recorded at 280 nm. The reagent blank was made by adding the supernatant just before stopping the reaction with TCA without incubation. Tyrosine was used as standard and one unit of enzyme activity is defined as the amount of enzyme needed to catalyze the formation of 1μg of tyrosine per min.

Liver hexokinase activity was measured (Crane and Sols, 1955). The glucose-6-phosphate formed was measured by estimating the stable phosphorus. One unit of enzyme is that amount which can catalyze the phosphorylation of 1 μmol of glucose in 15 min at 30°C. The glucose-6-phosphatase enzyme in the liver tissue was assayed (Marjorie, 1964). In this case inorganic phosphorus (Pi) released was measured. Enzyme activity was expressed as μg of Pi released per min per mg protein at 37°C. The glucose-6-phosphate dehydrogenase in the liver tissue was assayed (DeMoss, 1955) following the formation of NADPH. The enzyme activity was expressed as units per mg protein per min. The aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were also estimated (Wooten, 1964). Enzyme activity was expressed as nmol of product released per min per mg protein at 37°C. The product was oxaloacetate and sodium pyruvate for AST and ALT, respectively. Quantification of protein in the liver and intestine was carried out using bovine serum albumin as the protein standard (Lowry, 1951). Glycogen and enzyme analyses were all done separately for each replicate.

CALCULATIONS

Specific growth rate (SGR)
= 100{ln (final weight)-ln (initial weight)
/experimental period}

Feed conversion ratio (FCR)
= dry feed consumed (g)/gain in wet weight (g)

Protein efficiency ratio (PER)
= gain in wet weight (g)/protein fed (g)

Table 2. Growth, survival, HSI and GSI (Mean±SD) of *Catla catla* fingerlings fed different experimental diets for 60 d

Source	GC level	Initial B.wt.(g)	Final B.wt.(g)	SGR[1]	FCR[2]	PER[3]	ANPU[4]	HSI[5]	GSI[6]
Rice	40	15.1±0.4	24.7±0.8	0.81^{X}±0.03	2.81^{a}±0.22	1.03^{X}±0.08	17.68±2.83	1.50^{a}±0.14	1.16^{XY}±0.15
	50	15.2±0.3	23.7±0.3	0.73^{X}±0.10	4.01^{bY}±0.53	0.95^{X}±0.03	14.56^{X}±0.83	1.74^{b}±0.25	1.08^{X}± 0.18
Corn	40	15.3±0.4	26.5±0.9	0.98^{Y}±0.05	2.60±0.85	1.15^{aY}±0.03	19.15^{a}±3.11	1.60^{a}±0.13	1.37^{Y}±0.13
	50	15.3±0.2	29.3±0.7	1.08^{Y}±0.07	2.75^{X}±0.21	1.52^{bZ}±0.12	24.10^{bZ}±0.59	1.81^{b}±0.11	1.45^{Y}±0.20
Tapioca	40	15.2±0.1	27.8±0.9	0.95^{bY}±0.02	2.50^{a}±0.10	1.22^{Y}±0.04	20.42±2.36	1.51^{a}±0.12	1.11^{X}±0.20
	50	15.1±0.3	23.3±0.2	0.76^{aX}±0.06	3.22^{bX}±0.24	1.19^{Y}±0.09	19.86^{Y}±2.48	2.04^{b}±0.22	1.18^{X}±0.14
ANOVA		---------- p value ----------							
Among sources at 40 GC		-	-	0.004	0.114^{ns}	0.016	0.522^{ns}	0.389^{ns}	0.044
Among sources at 50 GC		-	-	0.003	0.014	0.001	0.002	0.056^{ns}	0.007

Figures in the same column having the same superscript do not vary significantly (p>0.05).
[a, b] Between two levels within the source: [X, Y, Z] Among different sources at 40 GC: [X, Y, Z] Among different sources at 50 GC.
[1] SGR: Specific growth rate; [2] FCR: Feed conversion ratio; [3] PER: Protein efficiency ratio; [4] ANPU: Apparent net protein utilization; [5] HIS: Hepatosomatic index; [6] GSI: Gastro somatic index; n = 3 (n = 6 for HSI and GSI); ns: Not significant.

Apparent protein utilization (APU)
= increase in whole body protein (g)/protein fed (g)
Hepatosomatic index (HSI)
= 100{weight of liver (g)/weight of fish (g)}
Gastrosomatic index (GSI)
= 100{weight of gastrointestinal tract (g)
/weight of fish (g)}

Digestible energy (DE), kcal/100 g
= (protein%×4)+(carbohydrate%×4)
+(lipid%×9) (Halver, 1976)

Total carbohydrate (TC)%
= 100-(protein%+lipid%+ash%)

Dry matter digestibility
= 100-100(%marker in feed/% marker in faeces)

Apparent nutrient digestibility
= 100-100{(% marker in feed/% marker in faeces)
×(% nutrient in faeces/% nutrient in feed)}

Statistical analysis

Significant difference among the carbohydrate sources within a level of GC was tested by one-way analysis of variance (ANOVA) and the comparison of any two mean values was done by Duncan's multiple range test (DMRT). Effect of GC levels within a carbohydrate source was tested by students 't' test. All the statistical analysis was performed by using the software programme SPSS (version 11).

RESULTS

Growth, feed conversion efficiency, HSI and GSI data are given in Table 2. The specific growth rate (SGR) was significantly different ($p<0.05$) with respect to the different carbohydrate sources at both levels of GC. Corn fed groups showed highest SGR at 50% tapioca fed groups and lowest in rice fed groups. Within the source at different levels, only the tapioca source had significantly ($p<0.05$) higher SGR at 40% than at 50% level. At 50% GC, the SGR was higher in corn fed group and at 40% GC, corn and tapioca had higher SGR than the rice fed group. The feed conversion ratio (FCR) was significantly different ($p<0.05$) between different GC levels within the source. At 50% GC level, highest FCR was found in rice fed groups, whereas it was not significant ($p>0.05$) at 40%. A general trend of higher FCR was observed at 50% GC level than its lower counterpart (40%), though it was not significantly different ($p>0.05$) in corn fed groups. In the rice and tapioca fed groups, protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were not significantly different ($p>0.05$) between two GC levels, but in the corn fed group, 50% level showed higher value than the 40% level. At 50%, both PER and ANPU were highest in corn fed group followed by tapioca and lowest in rice fed group.

The HSI was not significantly different ($p>0.05$) between different sources of carbohydrate at the same level of incorporation. However, there was significant difference ($p<0.05$) between the higher and lower GC groups. A trend of higher HSI was found in higher GC fed groups, irrespective of the source of carbohydrates. The trend was different for the GSI. Different levels of GC from the same source did not influence the GSI. But GSI between different sources at same GC level was significantly different ($p< 0.05$). Corn fed groups had higher GSI than the rice and tapioca fed groups at both GC levels.

The initial and final body composition of the tissues of *Catla catla* fingerlings are given in Table 3. The moisture, total carbohydrate and ash content decreased at the end of the experiment compared to the initial values, while the crude protein (CP) and lipid content increased. The moisture, CP and ash contents were not significantly different ($p>0.05$) among the different sources of carbohydrate. At 50% GC level, the lipid content was higher in corn fed group. Between two GC levels, not much

Table 3. Body composition of *Catla catla* fingerlings (%wet wt.) fed different experimental diets for 60 d (Mean±SD)

Source	GC level	Moisture	CP[1]	Lipid	TC[2]	Ash
Initial		78.0±0.0	13.27±0.1	2.32±0.1	2.50±0.2	3.92±0.1
Rice	40	74.2±1.0	14.95±1.3	5.56±0.4	1.77±0.5	3.86±0.7
	50	73.6±0.8	14.41±0.8	6.82^{X}±0.2	1.57^{XY}±0.2	3.58±0.04
Corn	40	74.3^{b}±1.0	15.10±0.4	5.24^{a}±0.3	2.21±0.5	3.55±0.1
	50	71.7^{a}±0.9	14.49±0.3	8.85^{by}±1.1	1.74^{Y}±0.1	3.48±0.3
Tapioca	40	74.7±0.7	14.32±0.1	6.03±1.0	1.65±0.05	3.34±0.2
	50	74.6±1.4	14.74±0.8	6.30^{X}±1.2	1.35^{X}±0.08	3.33±0.2
ANOVA		---------- p value ----------				
Among sources at 40 GC		0.825^{ns}	0.481^{ns}	0.374^{ns}	0.242^{ns}	0.359^{ns}
Among sources at 50 GC		0.051^{ns}	0.819^{ns}	0.033	0.019	0.419^{ns}

Figures in the same column having the same superscript do not vary significantly ($p>0.05$).
[a,b] Between two levels within the source: [X,Y,Z] Among different sources at 40 GC: [X,Y,Z] Among different sources at 50 GC.
[1] CP: Crude protein; [2] TC: Total carbohydrate; n = 3.
ns: Not significant.

Table 4. Nutrient digestibility (%) for different experimental diets fed to *Catla catla* fingerlings for 60 d (Mean±SD)

Source	GC level	DM[1]	TC[2]	Protein	Lipid
Rice	40	78.8^{bY}±1.9	79.7^{bY}±1.1	87.4^{bX}±2.6	93.8^{XY}±2.2
	50	73.8^{a}±1.2	75.1^{a}±1.0	81.5^{a}±2.0	91.1^{X}±0.8
Corn	40	71.3^{X}±0.6	65.5^{aX}±0.5	86.8^{X}±1.2	89.5^{X}±3.0
	50	73.3±3.5	70.1^{b}±3.1	84.5±2.9	93.0^{X}±3.6
Tapioca	40	85.6^{bZ}±0.5	85.6^{bZ}±0.7	92.4^{bY}±0.5	96.9^{Y}±0.5
	50	77.5^{ac}±3.5	76.9^{a}±4.3	87.3^{a}±2.3	97.6^{Y}±0.6
ANOVA		---------- p values ----------			
Among sources at 40 GC		0.000	0.000	0.012	0.017
Among sources at 50 GC		0.288^{ns}	0.179^{ns}	0.069^{ns}	0.024

Figures in the same column having the same superscript do not vary significantly ($p>0.05$).
[a,b] Between two levels within the source: [X,Y,Z] Among different sources at 40 GC: [X,Y,Z] Among different sources at 50 GC.
[1] DM: Dry matter; [2] TC: Total carbohydrate; n = 3.
ns: Not significant.

Table 5. Effects of different experimental diets on liver and muscle glycogen of *Catla catla* fingerlings (means±SD)

Source	GC level	Muscle glycogen (mg/100 ml)	Liver glycogen (mg/g tissue)
Rice	40	1.7^{X}±0.4	62.83^{aX}±10.0
	50	2.4^{X}±0.5	99.61^{b}±12.6
Corn	40	3.4^{Y}±0.5	79.46^{aY}±9.3
	50	3.3^{Y}±0.6	96.98^{b}±11.3
Tapioca	40	3.5^{Y}±0.3	84.70^{aY}±10.4
	50	3.7^{Y}±0.6	95.32^{b}±10.0
ANOVA		---------- p values ----------	
Among sources at 40 GC		0.000	0.004
Among sources at 50 GC		0.026	0.805^{ns}

Figures in the same column having the same superscript do not vary significantly ($p>0.05$).
[a,b] between two levels within the source; [x,y,z] Among different sources at 40 GC: [X,Y,Z] Among different sources at 50 GC.
ns: Not significant.

difference in body composition was observed except for moisture and lipid contents of corn fed group where 50% GC had significantly higher ($p<0.05$) lipid content and lower moisture content.

The dry matter and apparent nutrient digestibility coefficients of protein, carbohydrate and lipids are given in Table 4. Increased GC either from rice or tapioca decreased the dry matter digestibility significantly ($p<0.05$). Similar trend was also found for protein digestibility. Carbohydrate digestibility increased at increased level of GC in corn fed group only, with a reverse trend in rice and tapioca fed groups. Lipid digestibility was affected ($p<0.05$) by the different sources, but not by different levels of GC in the diet. Lipid digestibility in tapioca fed group was higher than the rice and corn fed groups at 50% GC level. At 40% GC, there was significant difference ($p<0.05$) in digestibility among different carbohydrate sources, with tapioca showing higher digestibility than rice and corn fed groups.

Glycogen content of muscle was much lower in rice fed groups, which was significantly different ($p<0.05$) than the corn and tapioca fed groups at both GC levels (Table 5). However, no difference was found in tapioca and corn fed groups irrespective of their levels in the diets. Among the carbohydrate sources, muscle glycogen level was much lower in rice fed group than corn and tapioca fed groups at both GC levels. Glycogen content of liver was significantly ($p<0.05$) different among different sources at 40% GC level

Table 6. Effects of experimental diets on some enzymes activities of carbohydrate and protein metabolism in *Catla catla* fingerlings (means±SD)

Source	GC level	Hexokinase[1]	G-6-Ptase[2]	G6PDH[3]	AST[4]	ALT[5]	Amylase (liver[1])	Amylase (intestine[1])	Protease (liver[1])	Protease (intestine[1])
Rice	40	0.15±0.03	0.21±0.04	37.61^{aX}±6.4	17.05^{bY}±1.1	10.20±1.9	2.05^{a}±0.34	1.10^{a}±0.04	0.83^{bZ}±0.06	6.43^{a}±1.03
	50	0.13±.03	0.23±0.05	62.81^{bX}±0.6	11.94^{a}±1.2	11.67^{Y}±2.9	2.95^{b}±0.66	1.89^{b}±0.44	0.13^{aX}±0.04	10.99^{bY}±0.82
Corn	40	0.13±0.04	0.20±.05	89.22[aZ]±2.2	13.69±2.3	8.59±0.3	2.54±0.02	1.20^{a}±0.12	0.34^{bX}±0.03	7.40±1.78
	50	0.14±0.01	0.19±0.03	115.4^{bZ}±4.9	11.92±0.8	7.50^{X}±0.3	2.45±0.12	1.66^{b}±0.24	0.15^{aX}±0.01	6.50^{X}±0.86
Tapioca	40	0.10±0.04	0.22±0.04	114.69^{aZ}±5.3	14.10^{b}±3.3	9.68±1.8	1.84^{a}±0.51	1.17^{a}±0.17	0.34^{X}±0.03	7.66±1.19
	50	0.13±0.03	0.21±0.07	99.51^{bY}±9.5	9.03^{a}±3.4	8.05^{X}±0.5	2.23^{b}±0.32	1.60^{b}±0.24	0.28^{Y}±0.04	7.24^{X}±0.76
ANOVA		P value								
Among sources at 40 GC		0.170[ns]	0.942[ns]	0.000	0.158[ns]	0.360[ns]	0.052[ns]	0.635[ns]	0.000	0.548[ns]
Among sources at 50 GC		0.764[ns]	0.687[ns]	0.000	0.144[ns]	0.018	0.108[ns]	0.541[ns]	0.001	0.001

Figures in the same column having the same superscript do not vary significantly ($p>0.05$).

[a, b] Between two levels within the source: [X, Y, Z] Among different sources at 40 GC; [X, Y, Z] Among different sources at 50 GC.

[1] U/mg protein.

[2] Glucose-6-phosphatase - µg phosphorus released/min/mg protein.

[3] Glucose-6-phosphate dehydrogenase - Δ 0.01 OD/min/mg protein.

[4] Aspartate amino transferase - n moles of oxaloacetate released/min/mg protein.

[5] Alanine amino transferase - n moles of sodium pyruvate released/min/mg protein.

ns: Not significant.

and between two levels of GC within the same source. Higher inclusion level of GC in the diet induced more glycogen deposition in liver in all the sources. At 40% GC level, liver glycogen level of rice fed group was lower than in corn and tapioca fed groups.

Different enzyme activities are presented in Table 6. A trend of higher intestinal specific amylase activity was observed in the 50% GC fed groups in all the carbohydrate sources. Different sources of carbohydrate did not have any influence ($p>0.05$) on intestinal and hepatic amylase activity at both GC levels. In rice and tapioca fed groups, the hepatic amylase activity was higher at 50% GC level than 40% GC level, but not significant ($p>0.05$) in corn fed group. The amylase activity was higher in the liver than the intestine. This was reverse in the case of protease activity, where much higher activity was observed in the intestine. The intestinal protease activity was not significantly different ($p>0.05$) among the different sources at 40% GC level. Liver protease activity showed decreasing activity with increased GC, though it was not significantly different in tapioca fed group. At 50% GC level, hepatic protease activity was significantly higher ($p<0.05$) in tapioca fed groups, whereas intestinal protease activity was higher ($p<0.05$) in rice fed groups. No significant difference was observed in the hexokinase and glucose-6-phosphatase activity among the experimental groups. Increased glucose-6-phosphate dehydrogenase (G6PDH) activity was observed in those groups fed with higher GC except in tapioca fed groups that showed a reverse trend. Among the different sources of carbohydrate, the rice fed groups showed significantly lower ($p<0.05$) G6PDH activity than the other groups at both the GC levels. Corn and tapioca fed groups showed higher G6PDH activity at 50 and 40% GC, respectively. The AST activity was higher in low GC fed groups though it was not different in corn fed groups. Rice fed groups showed higher ALT activity at 50% GC level and was significantly different ($p<0.05$) from corn and tapioca fed groups. The ALT activity within the carbohydrate group at both GC levels did not differ significantly ($p>0.05$).

DISCUSSION

Growth was not increased significantly in rice fed groups with increase in GC level, but FCR significantly increased. Same trend was also observed with tapioca fed groups. However, in corn fed groups higher SGR at higher GC level and non-significant change in FCR indicates better nutrient utilization from gelatinized corn than rice and tapioca at higher inclusion level. Schwarz and Kirchgessner (1991) reported better growth in carps fed corn and wheat diets than those fed with tapioca. Best FCR was reported at 35% CP level in the diet of *L. rohita* fry when fed with 40% GC (Mohapatra et al., 2003). This is in agreement with the present study, where lower FCR was recorded in the 40% GC (with 35% protein) fed groups, though it was not significantly different in the corn fed groups. Low FCR at higher protein level was also reported in tilapia (Jauncey, 1982).

Higher PER was observed in *Labeo rohita* fry when GC level in the diet increased upto 40%, any further increase in GC resulted in low PER (Mohapatra et al., 2003). Carbohydrate at its optimum level could be used as a precursor for the amino acid and nucleic acids (Halver, 1976). In this study, the amount of increased carbohydrate in diet was compensated by the decrease in protein level, therefore, PER at 50% GC was higher than 40% GC in corn fed group. However, if the amount of GC in the diet becomes too high the utilization of energy tends to be less efficient affecting growth (Hemre, 2002). Corn fed groups showed better PER and ANPU compared to rice and tapioca fed groups at 50% GC.

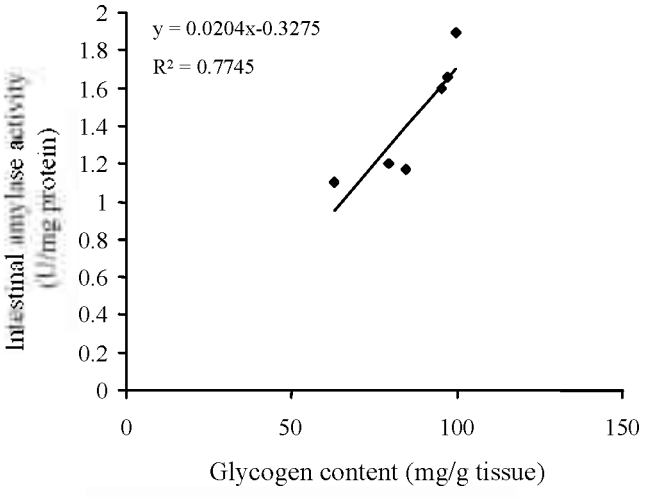

Figure 1. Relationship between liver glycogen content with intestinal amylase activity of *Catla catla* fingerlings.

An increase in hepatosomatic index was observed in 50% GC fed groups for all the carbohydrate sources, which is in agreement with findings of Hilton and Slinger (1983), Hemre et al. (1989), Pfeffer et al. (1991) and Hemre et al. (1995b). The increased hepatosomatic index is due to the deposition of glycogen in the liver. This may correlate with higher liver glycogen content of the fingerlings fed higher levels of GC in the present study. An increase in liver glycogen with increase in dietary gelatinized starch level was reported in rainbow trout, which in turn resulted in enlargement of liver (Kim and Kaushik, 1992). However, different carbohydrate sources at the same inclusion level did not affect the HSI in the present study. The GSI was found to be higher in the corn fed groups at 50% level, which may be due to higher fat deposition in the intestine as it is the major site of fat deposition; but separate study is needed to confirm this.

As compared to initial lipid content of 2.32%, the body lipid content increased to 5.24-8.85% after feeding the fish with carbohydrate-rich diets for 60 d. High carbohydrate diets stimulate lipogenic enzyme activity in omnivorous fish (Likimani and Wilson, 1982; Wilson, 1994) and thus increase lipid deposition in tissue. In corn fed groups, lipid content was considerably higher at 50% GC; a much lower moisture content is also seen in the same group. This indicates that increased gelatinized corn in feed is responsible for lipid accumulation in the body.

Although the corn fed groups had better growth performance, its nutrient digestibility was lower compared to other two groups at 40% GC level. Higher feed intake (data not shown) was observed in the corn fed groups that might have compensated for higher growth in spite of its low nutrient digestibility. Dry matter, body weight and digestion coefficient of dry matter not affected by corn or cassava as source of energy in cows (Chanjula et al., 2004). The protein digestibility was found to be higher in groups fed lower GC level (though not significant in corn fed groups). Decreased protein digestibility at increased dietary carbohydrate level was also reported by Inaba et al. (1963) in rainbow trout. In the present study, the lipid digestibility was not significantly different ($p>0.05$) between the two levels of GC. But among the different sources, tapioca was found to have higher lipid digestibility at both level of GC, which may be correlated to its comparatively higher carbohydrate digestibility. Undigested starch behaves like fibre in fish intestine (Hemre et al., 1995a), which has potential to reduce lipid digestibility (Storebakken, 1985). High level of fibre in other animals have shown to reduce nutrient digestibility (Morel et al., 2005; Tafaj et al., 2005; Wang et al., 2006).

Feeding higher levels of carbohydrate in the diets resulted in an increased deposition of glycogen in the liver. High hepatic amylase activity was found due to feeding of higher GC (50%) from rice and tapioca. This value corroborates the glycogen content in liver indicating the storage of excess glucose as glycogen in the liver. However, in the corn fed groups, no significant difference was found in amylase activity between the two levels of GC. Higher amylase activity was also found in higher GC fed groups in the intestine. But there was no difference ($p>0.05$) in intestinal amylase activity among the different sources of carbohydrate at same level of GC. In general, higher amylase activity was observed in liver than the intestine, which is in agreement with findings in carps (Hidalgo et al., 1999). A positive correlation ($Y = 0.0204X-0.3275$; $r^2 = 0.77$) was found between intestinal amylase activities and the liver glycogen deposition (Figure 1). Higher amylase activity in intestine increases carbohydrate digestion and consequently absorption of glucose, which was deposited as glycogen in the liver. Hidalgo et al. (1999) reported higher protease activity in the intestine than the liver. Same trend was observed in the present study. Chymotrypsin and trypsin are two important enzymes in digestion and breaking down protein and their activities do not vary with the change of dietary protein and crude protein digestibility in rabbits (Lei et al., 2004).

Glucose-6-phosphate dehydrogenase catalyzes the rate-limiting reaction of pentose phosphate metabolic pathway, which provides NADPH required for lipid synthesis. Higher G6PDH activity in 50% GC fed groups than the 40% GC (except tapioca) indicates an increase in reductive biosynthesis e.g. fatty acid synthesis. Increased G6PDH activity with the increase in carbohydrate content in the diet was also reported in other fish species (Likimani and Wilson, 1982; Shimeno and Shikata, 1993; Meton et al., 1999; Borrebaek and Christophersen, 2000; Borrebaek and Christophersen, 2001). The blood glucose level may

indicate the G6PDH activity, because blood glucose may be used to provide NADPH for lipogenesis. Rice fed groups showed the lowest blood glucose as well as the lowest G6PDH activity at both the levels. The AST and ALT enzyme activities are quantitatively the most important aminotransferases in the teleostean fish liver (Cowey and Walton. 1989). The higher activity of AST in the 40% GC fed groups level may be due to the higher protein content (35%) in the diet. Highest AST activity was observed in fish fed on high protein-low carbohydrate diet (Meton et al., 1999). No defined trend was observed for ALT activity in *Catla catla* fingerlings. AST or ALT activity depends on species rather than diet as reported by Nagai and Ikeda (1973), Cowey and Walton (1989) and Fynn-Aikins et al. (1995). Hexokinase is a glucose phosphorylating enzyme which converts glucose to glucose-6-phosphate. Glucose-6-phosphatase catalyzes the reverse of this reaction and is important in gluconeogenesis. The liver hexokinase activity was not affected by the levels of GC and source. This suggests that energy requirement of the fingerlings were satisfied at both level of GC from all the sources for which there was no need of break down of hexoses via glycolysis. No significant difference in hexokinase activity was observed in Atlantic salmon when dietary starch was gradually increased (Borrebaek et al., 1993). The activity of glucose-6-phosphatase, which is regulated inversely with hexokinase (Borrebaek and Christophersen. 2001), did not differ significantly between different experimental groups. This indicates that sufficient availability of glucose did not stimulate the gluconeogenic enzyme.

In summary, the results suggest that 50% gelatinized corn in the diet of *Catla catla* fingerlings improved PER and APU, whereas both GC levels of corn had good FCR and SGR. But in case of tapioca and rice, FCR was higher at higher GC level indicating poor nutrient utilization. Rice was poorly utilized by fingerlings and the order of nutrient utilization at 50% GC level was corn>tapioca>rice. At 40% GC level, corn and tapioca were comparable and more efficiently utilized than rice. Though nutrient digestibility of tapioca fed groups was higher, but overall nutrient utilization was higher in corn fed groups. However, high GC in the diet induced more liver-glycogen. More deposition of body lipid was due to more feeding of gelatinized corn. Moreover, species specificity for carbohydrate utilization from different carbohydrate sources remains as suggestive rather than conclusive and need to be elucidated.

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