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HAEMATOLOGICAL AND HEPATIC CHANGES IN *CATLA CATLA* FINGERLINGS IN RELATION TO DIETARY SOURCES AND LEVELS OF GELATINIZED CARBOHYDRATE

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Background. Dietary carbohydrate use is a priority in aquaculture because of its low cost and high availability, especially for herbivorous and omnivorous fish. Gelatinization is a process that improves carbohydrate use. However, excess dietary carbohydrate may cause metabolic stress and cellular changes as fish inherently use low carbohydrate. The aim of this study was to determine the effects of three sources of gelatinized carbohydrate (GC) on haematology and hepatic structure of *Catla catla* fingerlings.

Materials and methods. Six isocaloric (17.1–17.5 kJ · g^{-1}) semi-purified diets were prepared from rice, corn, or tapioca, each at 40 or 50% GC. Crude protein (CP) level was fixed at 35 and 25% for the low GC (40%) and high GC (50%) levels, respectively. Ninety *Catla catla* fingerlings were distributed in 6 treatments, each with three replicates, for a 60-day feeding trial. At termination, blood haemoglobin, RBC, WBC, and liver histology were studied.

Results. Significant differences ($P < 0.01$) in haemoglobin, RBC, and WBC were observed among the different carbohydrate-fed groups at 40% GC level. With increased dietary GC level, hepatocyte hypertrophy and vacuolation was intensified. Maximum hypertrophy was noticed in the fish fed tapioca at 50% GC, with extensive cytoplasmic vacuolation. No mortality was found in any group at any GC level.

Conclusion. No mortality of *Catla catla* fingerlings was observed due to feeding of high GC levels from corn, rice, or tapioca. However, hepatocyte hypertrophy was observed. Long-term feeding beyond 60 days may cause adverse hepatic cellular changes, but needs further research.

Key words: corn, rice, tapioca, gelatinization, haematology, histology, hypertrophy, *Catla catla*, fish

INTRODUCTION

Carbohydrate content influences fish food costs due to its low prices and high abundance. Although carnivorous fish have limited capacity to use dietary carbohydrate (Hemre et al. 1993, 1995a, Deng et al. 2000), improved growth performance with high carbohydrate diets has been demonstrated in rainbow trout, *Oncorhynchus mykiss* (cf. Kim and Kaushik 1992, Grisdale-Helland and Helland 1997), European eel, *Anguilla anguilla* (cf. Degani and Viola 1987), and Asian catfishes, *Pangasius bocourti* and *P. hypophthalmus* (cf. Hung et al. 2002). Omnivorous carps also have a good ability to use dietary carbohydrate (Shikata et al. 1993, Shimeno and Shikata 1993, Wilson 1994, Mohapatra et al. 2003).

Carbohydrate use in fish is dependent upon the type of starch, degree of gelatinization, and dietary inclusion level (Singh and Nose 1967, Wilson 1994, Shiau 1997). Starch gelatinization enhances its digestibility by making it more susceptible to enzymatic hydrolysis (Bergot 1991, Podoskina et al. 1997, Mohapatra et al. 2003), and can thus make it a valuable energy source (Bergot and Brèque 1983). Digestibility of tuber starch (potato and tapioca) is lower than that of cereal starches in rainbow trout (Bergot 1991) and common carp, *Cyprinus carpio* (cf. Schwarz and Kirchgessner 1991). Starch digestibility decreases at higher carbohydrate inclusion levels (Bergot 1979, Bergot and Brèque 1983, Hemre et al. 1989), but Mohapatra et al. (2003) reported a significant increase in digestibility with increase in gelatinized carbohydrate (GC) level in the diet of *Labeo rohita* carp fry.

Increased dietary carbohydrate content, beyond certain levels, causes metabolic stress to fish (Pieper and

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Pfeffer 1980, Feltcher 1997). Increased glycogen deposition (Hilton and Slinger 1983, Walton 1986, Pfeffer et al. 1991, Kim and Kaushik 1992, Kumar et al. 2005) and liver size (Kim and Kaushik 1992) were reported in rainbow trout and *Labeo rohita* fed high dietary carbohydrate. Increased hepatosomatic index was also observed in Atlantic cod, *Gadus morhua*, (cf. Hemre et al. 1989) and Atlantic salmon, *Salmo salar*, (cf. Hemre et al. 1995b) when fed high carbohydrate diets. Excessive carbohydrate intake may result in pathological conditions in fish (Jauncey 1982, Roberts 1989). Histological examination of fish fed high carbohydrate diets revealed hepatocyte hypertrophy in rainbow trout (Kaushik et al. 1989) and *Labeo rohita* (cf. Mohapatra et al. 2003, Kumar et al. 2005). Omnivorous *Catla catla* carp are expected to have a large scope for carbohydrate use, but high dietary carbohydrate levels may cause metabolic disturbances and, hence, cellular changes. The objective of this study was therefore to examine the effect of dietary carbohydrate on haematology, and hepatic structure in *Catla catla* fingerlings, using GC from three abundant sources: rice, corn, and tapioca.

MATERIALS AND METHODS

Experimental diets. Six isocaloric diets were formulated (Table 1) from three carbohydrate sources (rice, corn, and tapioca), each at 40% and 50% GC levels. The crude protein (CP) content of the feeds was fixed at 35% and 25% in the 40% and 50% GC diets, respectively, for all three sources. Rice, corn, and tapioca flour were mixed with water (to get approximately 80% moisture) and autoclaved at 120°C for 1 h to achieve maximum gelatinization. The gelatinized carbohydrates were then spread on trays and oven dried at 60°C. The dried mass was then pulverised in a hammer mill (D.P. Pulveriser works, Mumbai, India) through 0.5-mm screen and used in feed preparation to achieve the desired 40% or 50% GC levels. Dietary GC level was calculated by multiplying % carbohydrate inclusion by its degree of gelatinization. All the ingredients were mixed thoroughly and water was added to form dough that was then steam conditioned for 5 min. Pellets of 2 mm in diameter were prepared by a hand pelletizer, oven dried at 60°C for 24 h so as to obtain a moisture content less than 10% for safe storage and stored in airtight polyethylene bags at room temperature (28–30°C) until use. Proximate composition of the diets was determined using standard AOAC methods (Anonymous 1995). Digestible energy content was determined as described by Halver (1976).

Experimental design and feeding trial. *Catla catla* fingerlings (15.1–15.3 g) were procured from Khopoli Govt. Fish Farm, Maharashtra, India, and distributed in six experimental groups, each with three replicates following a completely randomised design. Five fingerlings were allocated to each replicate, stocked into plastic tubs (100 L capacity) with continuous aeration. A static system was used with at least 75% water exchanged every day with fresh water by siphoning out water with uneaten food and faecal matter. Before the experiment, the fish were acclimated to the experimental condition for 30 days and fed a 35% CP diet. Temperature, pH, dissolved oxygen (DO), carbon dioxide (CO_2), ammonia-nitrogen, and nitrite-nitrogen were determined weekly (Anonymous 1985). DO and pH ranged from 6.2 to 7.9 ppm and 7.5 to 8.5, 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 in the detectable range in any of the tubs. Fish were fed to satiation twice daily (0800 and 2000 h) and daily feed intake was monitored. The feeding trial lasted for 60 days.

Haematological parameters. At the end of the experiment, two fish per replicate (6 fish per treatment) were anaesthetised (clove oil: 50 ml · L^{-1}) and blood samples drawn from the caudal vein using a medical syringe with EDTA anticoagulant. Blood was immediately analysed for haemoglobin, total erythrocyte count (RBC), and leukocyte count (WBC). Haemoglobin level was estimated using the cyanmethaemoglobin method (Qualigens, India Ltd.). Cells were counted in a haemocytometer using appropriate diluting fluids (Qualigens, India Ltd.), and then RBC and WBC were calculated using the formula:

$$\text{No. of cells per 1 mm}^3 = \frac{\text{No. of cells counted} \times \text{dilution}}{\text{Area counted} \times \text{depth of fluid}}$$

Histological studies. Fish-liver slices were immediately fixed in neutral buffered formalin, embedded in paraffin, cut at 5 mm, and stained with haematoxylin and eosin (H & E) as described by Roberts (1989).

Statistical analyses. The significance of differences in responses to the three carbohydrate source was tested separately at each GC level by one-way analysis of variance followed by Duncan's multiple range tests. The effects of GC level were tested separately for each carbohydrate source using Student's *t*-test. All statistical analyses were performed using SPSS (version 11).

RESULTS AND DISCUSSION

There were significantly different ($P < 0.01$) effects of carbohydrate source on haemoglobin, RBC, and WBC (Table 2). At 40% GC, corn-fed fish had lower haemoglobin and RBC than rice- or tapioca-fed fish, whereas WBC was lower in rice-fed fish than in corn- or tapioca-fed fish. At 50% GC, rice-fed fish had higher haemoglobin than tapioca-fed fish. Within a carbohydrate source, there were no effects of increase in GC levels with the exception of higher haemoglobin and RBC with increased GC level in corn-fed fish.

These results suggest that carbohydrates interfere with haemoglobin formation. This may be due to reduced iron availability with high dietary carbohydrate (Vangen and Hemre 2003), although this does not explain the lower haemoglobin content and RBC at low GC level in corn-fed fish. The absence of such an effect of GC level on the haematology of rice- and tapioca-fed fish indicates a possible interaction of dietary carbohydrate source with iron, which needs further research. Haemoglobin content was reported to be negatively correlated with dietary carbohydrate level in Atlantic salmon (Waagbø et al. 1994).

Table 1

Ingredients and proximate composition (%) of experimental *Catla catla* fingerling diets

Ingredient	Rice		Corn		Tapioca	
	40% GC	50% GC	40% GC	50% GC	40% GC	50% GC
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% ($\bar{x} \pm s$; $n = 3$)						
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

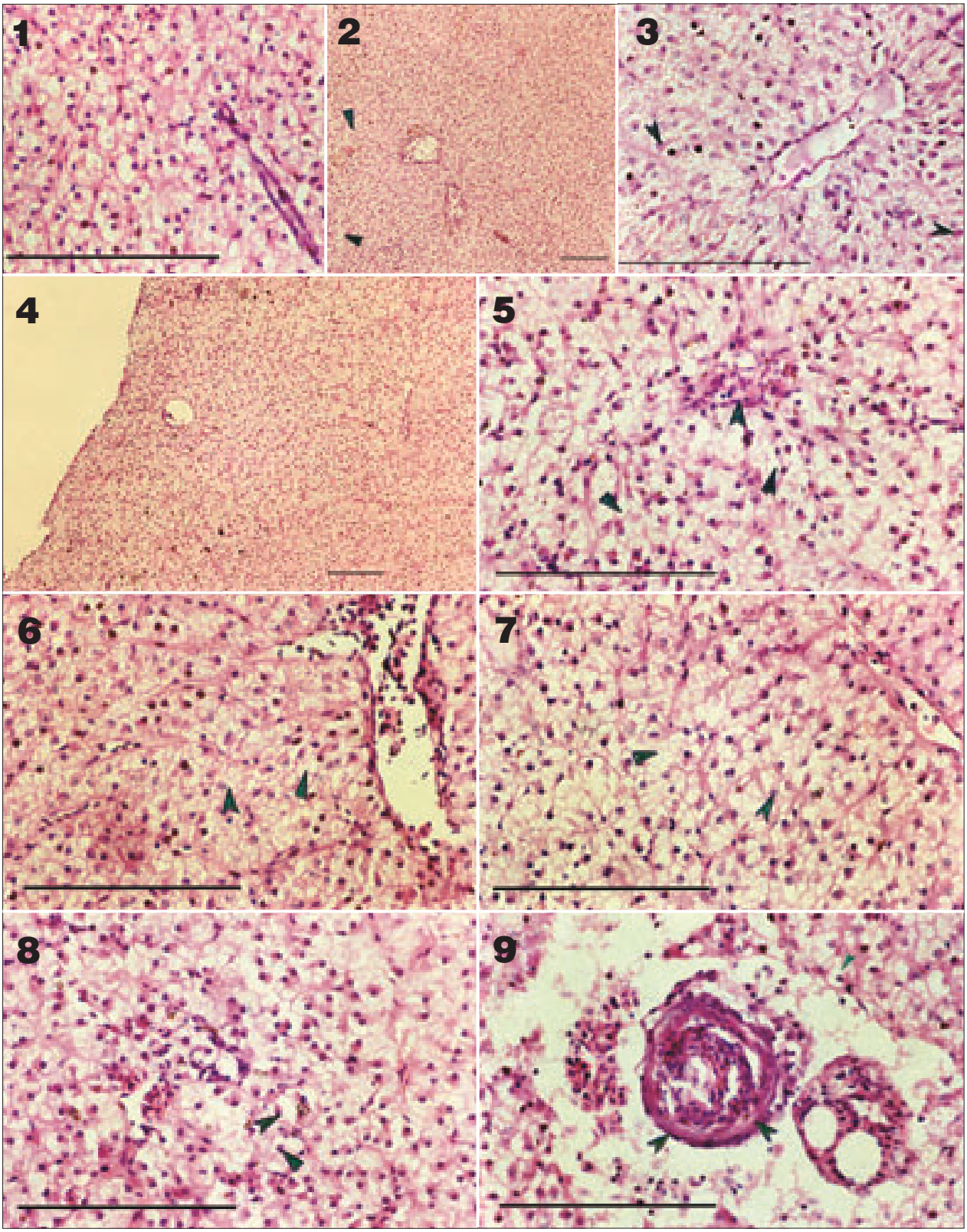
[1]Casein fat free: 75% CP (Himedia Ltd., India); [2]Gelatin: 96% CP (Himedia Ltd., India); [3]Procurred from local market, Mumbai, India; [4]Sd Fine Chemicals Ltd., India; [5]Composition of vitamin mineral mix (Agrimin, India) (quantity · kg^{-1}): vitamin A 625 000 IU; vitamin D_3 62 500 IU; vitamin 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^{-1}): 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; the antioxidant butylated hydroxy toluene (Himedia Ltd., India) was added at 0.02% of the added oil

Table 2

Haematological effects of different experimental diets in *Catla catla* fingerlings ($\bar{x} \pm s$)

Source	GC level	Haemoglobin (g/100 mL)	RBC (×10^{6}cells/mm)	WBC (×10^{3}cells/mm)
Rice	40	$7.5^{y} \pm 0.6$	$1.7^{y} \pm 0.1$	$5.6^{x} \pm 0.3$
	50	$7.8^{Y} \pm 0.5$	1.5 ± 0.1	6.1 ± 0.6
Corn	40	$5.6^{ax} \pm 0.6$	$0.9^{ax} \pm 0.2$	$8.2^{y} \pm 0.9$
	50	$7.2^{bXY} \pm 0.8$	$1.4^{b} \pm 0.2$	8.1 ± 1.3
Tapioca	40	$6.9^{y} \pm 0.0$	$1.3^{y} \pm 0.1$	$8.4^{y} \pm 0.4$
	50	$6.1^{X} \pm 0.4$	1.5 ± 0.1	6.7 ± 1.2
ANOVA			P value	
Among sources at 40 GC		0.007	0.008	0.002
Among sources at 50 GC		0.044	0.660^{ns}	0.128^{ns}

Figures in the same column having the same superscript letter do not differ significantly ($P > 0.05$); ^{ab} between two levels within the source; ^{xyz} among different sources at 40 GC; ^{XYZ} among different sources at 50 GC; ns, not significant; $n = 6$



Figs. 1–9. Histological pictures of the liver of *Catla catla* fingerlings fed different diets (H & E). **Fig. 1.** Control (Scale bar = 100 µm). **Fig. 2.** Rice at 40% GC; note mild derangement of cells (Scale bar = 100 µm). **Fig. 3.** Rice at 40% GC; note swollen cells with clear vesicles in the cytoplasm (Scale bar = 100 µm). **Fig. 4.** Rice at 50% GC; note mild derangement of cells (Scale bar = 100 µm). **Fig. 5.** Rice at 50% GC; note vacuolation and constricted sinusoids (Scale bar = 100 µm). **Fig. 6.** Corn at 40% GC; note hypertrophic cells and extensive vacuolation of cells (Scale bar = 100 µm). **Fig. 7.** Corn at 50% GC; note hypertrophic cells and eccentric, pyknotic nuclei (Scale bar = 100 µm). **Fig. 8.** Tapioca at 40% GC; note moderate hypertrophic cells and vacuolation (Scale bar = 100 µm). **Fig. 9.** Tapioca at 50% GC; note extensive vacuolation and sclerotic blood vessel (Scale bar = 100 µm)

Similarly, WBC was affected by dietary carbohydrate source but not by GC level. At present, it is difficult to draw any conclusion on effect of GC on blood parameters due to lack of complete information.

Hepatic vacuolation and cellular hypertrophy was observed in all treatment groups, and was intensified at the 50% GC level. The vacuoles may have been due to glycogen or fat deposition for which separate staining was not made in the present study. Several authors have reported hepatocyte hypertrophy as a consequence of high dietary carbohydrate (Lee and Putnam 1973, Kim and Kaushik 1992, Mohapatra et al. 2003). In contrast, Kumar et al. (2005) did not notice any specific hepatic changes in *Labeo rohita* juveniles upon feeding 42.43% gelatinized or non-gelatinized corn. Long-term feeding trials are required to draw any conclusions. Moreover, species specificity for carbohydrate use from different carbohydrate sources remains as suggestive rather than conclusive.

Liver tissue taken before the start of the experiment appeared normal (Fig. 1). Hepatocytes of rice-fed fish at 40% GC appeared moderately swollen with mild cellular disarrangement (Fig. 2). Hepatocyte nuclei also appeared swollen and there were clear cytoplasmic vesicles (Fig. 3). Hepatocytes of rice-fed fish at 50% GC were moderately swollen, losing the cellular outline with distorted arrangement (Fig. 4). The parenchyma was filled with areas of vacuolation and the sinusoid appeared constricted (Fig. 5). Hepatocyte nuclei were condensed and darkly stained. The hepatocytes of 40% and 50% GC corn- and tapioca-fed fish are shown in Figs. 6–9. Among the corn-fed fish, the hepatocytes at 40% GC level appeared markedly hypertrophic with extensive cytoplasmic vacuolation. Similar changes were seen at the 50% GC level with greater hypertrophy than at 40% GC. The nuclei appeared eccentric and pyknotic. The hepatocytes of the fish fed with tapioca at 40% GC were moderately hypertrophic. Nuclei were intensely stained with moderate cytoplasmic vacuolation. Similar changes were seen in the livers of fish fed with tapioca at 40% GC, but with slightly more cytoplasmic vacuolar changes. At places the arterioles appeared sclerotic with thickening in the intima.

Highest specific growth rate (SGR) was observed in the corn fed group at 50% GC level indicating better nutrient utilization followed by tapioca and rice fed groups. Feed intake also followed the same trend as SGR (data not shown), suggesting carbohydrate sources were well palatable to the fingerlings.

It is concluded that high dietary GC levels causes hepatic changes in *Catla catla* fingerlings which increase proportionately with the GC level. Corn at 50% GC may be used in *Catla catla* fingerlings diet for a short period (not more than 60 days), but prolonged feeding may cause metabolic changes. No mortality was observed in any of the treatment group.

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