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# Effect of Different Levels of Dietary Carbohydrate on Growth and Metabolic Enzyme Activity in *Clarias batrachus* (Linnaeus, 1758)

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**A B S T R A C T**

A 60-days feeding trial was conducted to assess the growth and metabolic enzyme activity in *Clarias batrachus* fingerlings fed with graded levels of digestible carbohydrate (DC). Ninety fingerlings (90) of *Clarias batrachus* (Av. Wt. 5.5g) were stocked in three distinct experimental groups in triplicates following completely randomized design. Three experimental diets viz. 20 % DC (T1), 30% DC (T2) and 40% DC (T3) were fed to *Clarias batrachus* fingerlings. The lowest weight gain was observed in the group fed with 20% DC (T1). Specific growth rate (SGR) and protein efficiency ratio (PER) were significantly ($p<0.05$) higher in T2 and T3 groups than the T1 group. Activities of intestinal amylase and liver Glucose - 6 - phosphate dehydrogenase (G-6-PDH) enzymes were significantly higher ($p<0.05$) in T3 group. Both T2 and T3 groups showed higher aspartate amino transferase (AST) and alanine amino transferase (ALT) activities in both liver and muscle tissues. No significant change was observed in the hexokinase activity in the liver and muscle tissues ($p>0.05$). Hence, from the findings of the present study it can be concluded that the dietary inclusion of 40% DC can be used effectively in aqua feed formulation without compromising the growth of *C. batrachus.*

## Introduction

Fish feed represents around 40-50% of total aquaculture production costs. Carnivorous and omnivorous fishes have high protein requirements than herbivorous fishes in their diet, which results in high feed costs (Buhler and Halver, 1961). Also, fish preferentially use protein energy for metabolism. Thus, protein in diet mainly goes for metabolism rather than growth. But it has been already found in many studies that increasing digestible carbohydrate in the diet can be used to spare protein in diet (Hemre *et al.*, 1989; Shiau *et al.*, 1993; Azaza *et al.*, 2013). Carbohydrates are easily available and inexpensive source in formulated feeds, which are efficiently utilised in several fish species (Gao *et al.*, 2010). Carbohydrate utilization of fish varies between fish species and carbohydrate sources (Wilson, 1994; Moon, 2001; Krogdahl *et al.*, 2005). Di- and monosaccharide are more digestible to catfishes with maximum protein retention and energy retention rate (Erfanullah and Jafri, 1999). Also, physical treatments like extrusion, cooking etc, gelatinise carbohydrate thus easing the process of fish digestion (Tester and Morrison, 1990). Prepared feeds for carnivorous fish usually

contain less than 20 percent soluble carbohydrate, while feeds for omnivorous species usually contain 25 to 45 percent (Buhler and Halver, 1961). The ability of carnivorous fish species to hydrolyze or digest complex carbohydrates is limited due to the weak amylotic activity in their digestive tract (Spannhof and Plantikow, 1983). However, reduced growth rates have been observed in some species when fed carbohydrate-free diets (Degani *et al.,* 1986). Alternatively, feeding excessive dietary carbohydrate to fish has been shown to adversely affect an array of morphometric and physiological parameters used to measure growth and nutrient utilization or physiological function (Hilton and coworkers, 1983; Dixon and Hilton, 1985; Hemre *et al.*, 1989). This implies higher level of carbohydrate than required is not going to spare protein contributing to growth. Thus, it is evident to optimize the level of carbohydrate for each species according to their utilisation capacities. Therefore, the present study was conducted with an objective to assess the different levels of dietary carbohydrate on growth and metabolic enzyme activity in *Clarias batrachus* fingerlings (Linnaeus, 1758).

## Materials and Methods

### Experimental animals and experimental set up

Asian catfish (Magur), *C. batrahus* (Linnaeus, 1758) were procured from Powarkheda centre of Central Institute of Fisheries Education (CIFE), Madhya Pradesh, India. The experiment was conducted for a period of 60 days in the wet laboratory of Central Institute of Fisheries Education. The setup consisted of 9 plastic rectangular tubs (57 X 36 X 47cm, 75L capacity) covered with perforated lids. Ten fishes with initial weight ranging from 5.2-5.8g (Av. wt. 5.5g) were stocked in each plastic tub with 50 L chlorine free bore well water. The total volume of water was maintained throughout the experimental period. Round the clock aeration was provided. To provide shelter to fingerlings plastic hide outs was placed at the bottom of each tub. Fishes were fed up to satiation two times a day for 60 days. Fortnightly, measurements of fish weight were taken.

### Experimental diets

Three iso-nitrogenous (38.2%) and iso-lipidic diets (8%) with different levels of gelatinised starch and dextrin as carbohydrate sources were prepared. The level of carbohydrate in three diets were T1 (20% CHO), T2 (30% CHO) and T3 (40% CHO). Composition of diet is given in Table 1.

### Diet preparation

Pre- weighed required ingredients as per formulation were mixed with gelatinised starch to form dough. Then dough was then cooked for half an hour in a pressure cooker. When the steamed dough was completely cooled, the calculated concentration of the oils, vitamin and mineral mixture were incorporated in it and mixed well and pressed through a semi-automatic pelletizer to get uniform sized pellets, which were air dried and packed in airtight polythene bags were labelled according to the treatments.

### Sampling and analysis of samples

### Proximate analysis

Proximate composition of the fish and diets were analysed following standard methods of AOAC (1995). Moisture was determined by drying the sample at105˚C to a constant weight. The nitrogen content of the sample was analyzed using Kjeltec system (2200 Kjeltec Auto distillation, Foss Tecator,

Sweden) and crude protein was calculated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was determined using Soxtec system (1045 Soxtec Extraction Unit, Foss Tecator, Sweden) using diethyl ether (boiling point, 60 - 80˚C) as a solvent and the ash content was estimated by incinerating sample in a muffle furnace at 600˚C for 6 h. The fibre was estimated in FIBRO tech extraction apparatus by giving acid followed by alkali digestion. Total carbohydrate was calculated by differences as carbohydrate % = 100-(CP%+EE%+Ash%). The digestible energy (DE) value of experimental diets and tissue was calculated as described by Halver (1976).

## Enzyme assays

At the end of the experiment (T1, T2 and T3), 3 fishes were collected from each tank and anesthetized with clove oil (50 $\mu L^{-1}$). Fishes were then dissected and the tissues *viz.*, liver, intestine and muscle, were immediately removed. A 5% tissue homogenate was prepared in chilled 0.25M sucrose solution by Teflon coated mechanical homogeniser (MICCRA D-9, Digitronic, Germany). The whole procedure was followed in ice cold condition. Homogenized samples were centrifuged at 5000 rpm for 10 min at 4ºC. The supernatant was collected in 5ml plastic vials and stored in a deep freezer (-20ºC) for enzyme assay. Total protein content was analyzed from the supernatant (Lowry *et al.*, 1951) for calculating enzyme activities. All the colorimetric assays were carried out using UV–VIS spectrophotometer (E-Merck, Germany).

## Digestive enzymes

Amylase (E.C. 3.2.1.1) activity was measured by estimating the reducing sugars produced due to the action of α-amylase on starch (Rick and Stegbauer, 1974). A 2% starch solution was prepared in phosphate buffer (pH 7.0). The reaction mixture, consisting of starch solution, buffer and tissue homogenate, was incubated at 37 °C for 30 min. Then dinitrosalicylic acid was added to stop the reaction and kept in boiling water for 5 min. After cooling, the reaction mixture was diluted with distilled water and absorbance recorded at 540 nm. Activity was determined from the maltose standard curve and expressed as mole of maltose released from starch/min/mg protein at 37 °C.

## Metabolic enzymes

### Hexokinase

Hexokinase (HK; E.C. 2.7.1.1) activity was measured by the method of Easterby and O'Brien (1973). A reaction cocktail was prepared by mixing Tris–HCl buffer (50 mM), glucose (50 mM), adenosine triphosphate (30 mM) and MgCl2 (200 mM). The pH was adjusted to 7.6 at 30 °C.

The final reaction mixture consisted of reaction cocktail, β-NADP (1 mM) and G6PDH (500 U/mL). One unit of enzyme is defined as the amount of enzyme activity that phosphorylates 1.0 µmol of D-glucose/ min at 30 °C.

### Glucose -6-Phosphate Dehydrogenase (G-6-PDH)

The G-6-PDH activity in different tissues was assayed by the method of De Moss (1953). Total 3ml of the reaction mixture was comprised of 1.5ml of 0.1M Tris buffer (pH 7.8), 0.2ml of 2.7mM NADP, 0.1ml of tissue homogenate, 1.05ml of distilled water and 0.1ml of 0.02M glucose-6-phosphate (G6P). The reaction was started by adding G6P as substrate. The OD was recorded at 340nm at 15second interval against distilled water. The G6PDH activity was expressed as unit mg

protein$^{-1}$ min$^{-1}$. One unit was equal to Δ0.01 OD min$^{-1}$ ml$^{-1}$ at 25ºC.

## Alanine amino-transferase

Alanine amino-transferase (ALT; E.C. 2.6.1.2.) was assayed with 200 mM DL-alanine and 2 mM α-ketoglutarate in 40 mM phosphate buffer (pH 7.4), and estimated at 540 nm (Wootton, 1964). Aspartate amino-transferase (AST; E.C. 2.6.1.1.) was assayed by the same procedure as for ALT except for the substrate, 200 mM DL-aspartic acid instead of alanine (Wootton, 1964). The enzyme activity was expressed as nanomoles oxaloacetate formed/min/mg protein at 37 °C.

## Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) via SPSS v.22 software. Duncan's multiple range test was used for post hoc comparison of means ($P<0.05$).All data presented in the text, figures and tables are means ± standard error and statistical significance for all statistical tests was set at $P<0.05$.

## Results and Discussion

## Physio-chemical parameters of water

The water temperature of the different experimental groups ranged from 27.2ºC to 27.8ºC throughout the experimental period. The pH values were recorded within the range of 7.5 to 8.0. The dissolved oxygen concentration of all the experimental groups was recorded within the range of 5.6 to 7.8mgL$^{-1}$. The free carbon dioxide was not detected in the cultured water. Carbonate hardness was found to be 228-245mgL$^{-1}$. The total ammonia content of all the experimental groups was found to be in the range of 0.14 to 0.27mgL$^{-1}$. The nitrite-N content was found to be in the range of 0.001 to 0.005mgL$^{-1}$. The nitrate-N content was found to be in the range of 0.02 to 0.06mgL$^{-1}$, during the experimental period. All the physio-chemical parameters of the source water used for the present study were found to be in the optimum range (Jhingran, 1991). Therefore, the variation in the finding can be attributed to the dietary factors applied.

## Proximate composition of diet

Proximate composition of the experimental diets is presented in Table 2. Moisture content in the diet was observed between 7.10% and 7.33% and crude protein in the diet varied from 37.97% to 38.10%. The ether extract (EE) content varied from 7.77% to 7.96%, whereas the ash of the diet varied from 2.08% to 2.37%.

## Growth Parameters

The data pertaining to growth parameters are presented in Figures 1 to 4. Weight gain, specific growth rate and protein efficiency ratio were significantly lower ($p<0.05$) in 20% carbohydrate fed group (T1) than the other two treatments. Weight gain and specific growth rate were observed similar ($p>0.05$) in 30% and 40% carbohydrate fed group (T2 and T3 respectively). Similar results was seen in finding of Jantrarotai *et al.*, (1994) who found that weight gain hybrid Clarias catfish (*Clarias macrocephalus* × *C. gariepinus*) fed a diet containing 36%, 44%, 49% carbohydrate did not differ significantly ($P<0.05$). This may be due to the fact that when dietary energy is more than required level, it may reduce diet digestibility and growth. Even, it was observed in rainbow trout fed a diet containing 30% starch that growth and feed efficiency were improved by increasing gelatinized, starch but reached a plateau at around 40% which supports the finding of the present study (Jeong *et al.*, 1992). The feed conversion ratio was

significantly higher ($p<0.05$) in the T1 group while it was found similar T2 and T3 groups ($p>0.05$). This may be due to low level of gelatinised starch (20 %) in T1 diet compared to T2 (30 %) and T3 (40 %). It is supported by findings of Banos *et al.,* (1998), it was found that even if there is a supplementation of the extruded carbohydrate source of higher inclusion level (even of 37%) in diet, rainbow trout did not show slower growth than fishes fed with 20% raw starch. This result was found in correlation to the findings of several authors (Mohapatra *et al.,* 2003; Yengkokpam *et al.,* 2007) which demonstrated that gelatinization improves the carbohydrate digestibility and thus can incorporate more carbohydrate in the diet of the fish. Also, Erfanullah and Jafri (1998) observed highest weight gain, FCR, SGR and protein retention in *C. batrachus* fingerlings fed with diet 27% white dextrin and 8% lipid diet. There was no significant ($p>0.05$) increase in weight in T3 (40% CHO) compared to T2 (30% CHO) with the increase in the percent of gelatinised carbohydrate.

## Digestive and metabolic enzymes

### Amylase

The amylase activity is shown in Table 3. The amylase activity was found significantly lower ($p>0.05$) in T1 whereas, its activity was similar inT2 and T3 groups ($p>0.05$). In the present study, there was significant ($p<0.05$) increase in amylase activity from T1 to T3 fed groups (Table 3). This is in accordance with the findings of Mukhopadhyay (1976), who also reported the increase in amylase activity in *C. batrachus* fed with high carbohydrate.

**Table.1** Composition of the experimental diets

| Ingredients | T1 (20%) | T2 (30%) | T3 (40%) |
|---|---|---|---|
| Casein | 35 | 35 | 35 |
| Gelatin | 7 | 7 | 7 |
| Dextrin | 10 | 15 | 20 |
| Pre-gelatinised starch | 10 | 15 | 20 |
| Cellulose | 25.6 | 15.6 | 5.6 |
| Cod liver oil | 4 | 4 | 4 |
| Sunflower oil | 4 | 4 | 4 |
| CMC | 1 | 1 | 1 |
| Vitamin and mineral mix * | 2 | 2 | 2 |
| Choline chloride | 0.88 | 0.88 | 0.88 |
| Betaine hydrochloride | 0.5 | 0.5 | 0.5 |
| BHT | 0.02 | 0.02 | 0.02 |

*Composition of vitamin mineral mix (PRE-EMIX PLUS) (quantity/kg) –Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L- lysine, 10 g; DL- Methionine, 10 g; Selenium, 125 mg.

Abbreviations: CMC- Carboxymethyl cellulose; BHT- Butylatedhydroxy toluene

**Table.2** Proximate composition of the different experimental diets (% DM basis)

| Treatments | T1 | T2 | T3 |
|---|---|---|---|
| Dry matter | 92.99±0.15 | 92.66±0.16 | 92.76±0.23 |
| Crude protein | 38.10±0.10 | 38.36±0.22 | 37.97±0.12 |
| Ether extract | 7.95±0.05 | 7.77±0.10 | 7.96±0.09 |
| Total ash | 2.20±0.06 | 2.37±0.16 | 2.08±0.02 |
| Crude fibre | 25.11±0.31 | 15.06±0.08 | 5.53±0.05 |
| Nitrogen free extract (NFE) | 19.54±0.29 | 29.11±0.05 | 39.23±0.48 |
| DE (Kcal/100g) | 302.14±0.79 | 339.82±0.92 | 380.43±0.87 |

Data expressed as mean ± SE, n=3.

**Table.3** Activity of metabolic enzymes in sampled fishes fed with different diets

| Treatments | T1 | T2 | T3 |
|---|---|---|---|
| Amylase | $1.26^{a}$±0.20 | $2.25^{b}$±0.13 | $2.41^{b}$±0.32 |
| Hexokinase (Liver) | 0.50±0.08 | 0.57±0.05 | 0.64±0.05 |
| Hexokinase (Muscle) | 2.31±0.66 | 2.74±0.24 | 2.5±0.35 |
| G-6PDH (Liver) | $0.97^{a}$±0.12 | $1.12^{a}$±0.03 | $1.63^{b}$±0.05 |
| G-6PDH (Muscle) | $1.32^{a}$±0.06 | $1.64^{a}$±0.05 | $2.13^{b}$±0.11 |
| AST (Liver) | $14.59^{a}$±0.50 | $17.41^{b}$±0.22 | $18.52^{b}$±0.19 |
| AST (Muscle) | $20.06^{a}$±0.25 | $24.55^{b}$±0.18 | $25.32^{c}$±0.19 |
| ALT(Liver) | $18.74^{a}$±0.24 | $23.67^{b}$±0.42 | $26.09^{b}$±0.27 |
| ALT(Muscle) | $24.17^{a}$±0.25 | $29.90^{c}$±0.33 | $27.17^{b}$±0.24 |

Data expressed as mean ± SE, n=3; Mean values in the same column with different superscript differ significantly (P<0.05). Units: Amylase (µm of maltose released/min/mg protein), AST (nM oxaloacetate formed/min/mg protein), ALT (nM pyruvate formed/min/mg protein), Hexokinase (Milli units/min/mg protein), G-6-PDH (µg of phosphorus released/min/mg protein).

**Fig.1** Percentage weight gain of the *Clarias batrachus* fingerlings fed with different experimental diets. Bar bears the same superscript do not differ significantly (p>0.05)

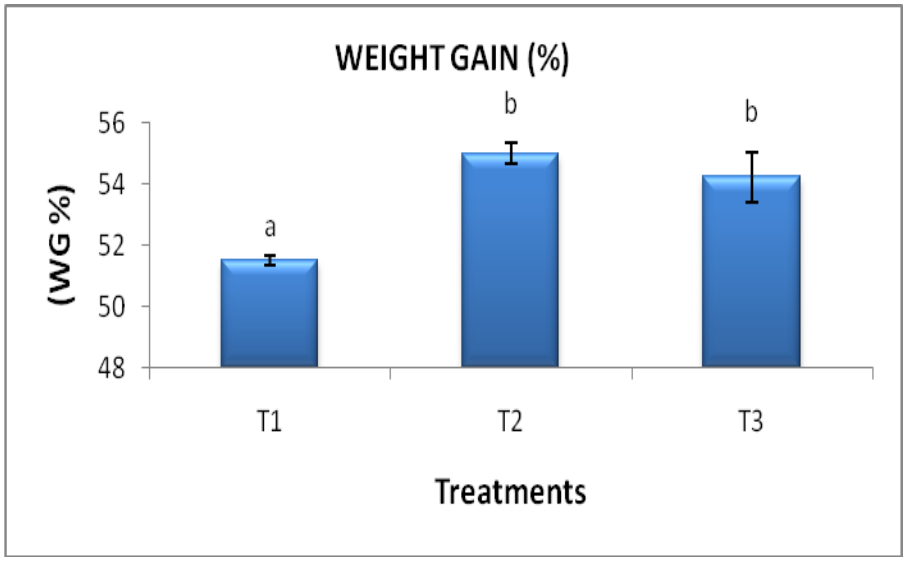


**Fig.2** Specific growth rate (SGR) of *Clarias batrachus* fingerling fed with different experimental diets. Bar bears the same superscript do not differ significantly (p>0.05)

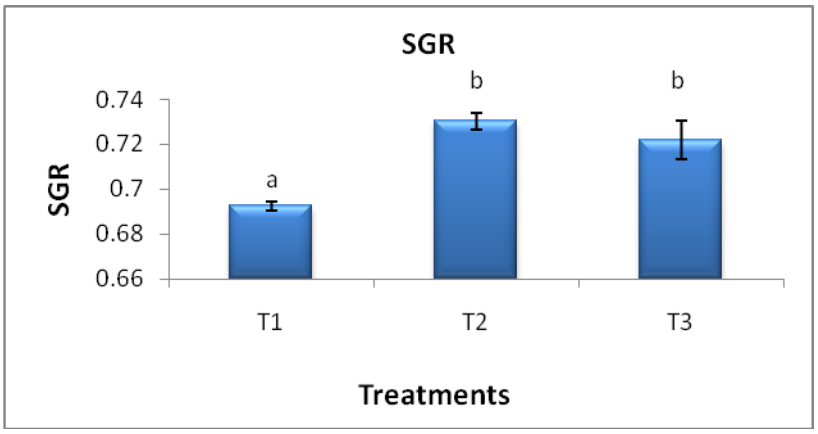



**Fig.3** Feed conversion ratio (FCR) of *Clarias batrachus* fingerling fed with different experimental diets. Bar bears the same superscript do not differ significantly (p>0.05)

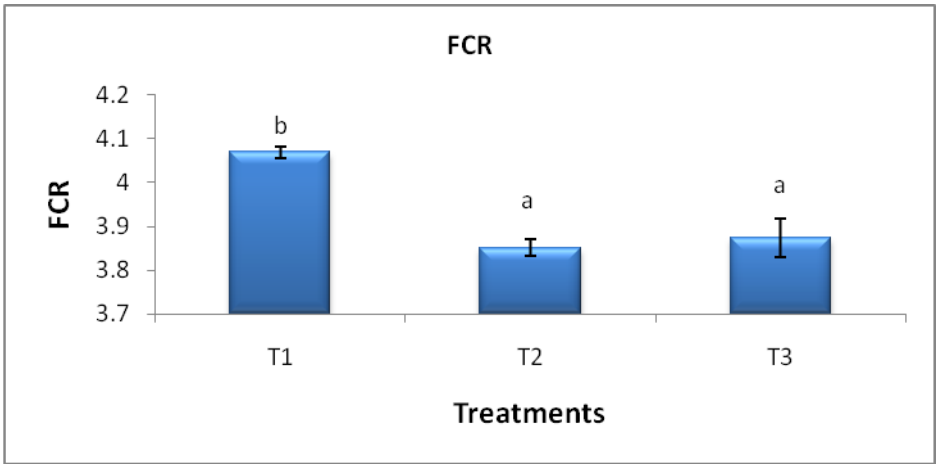
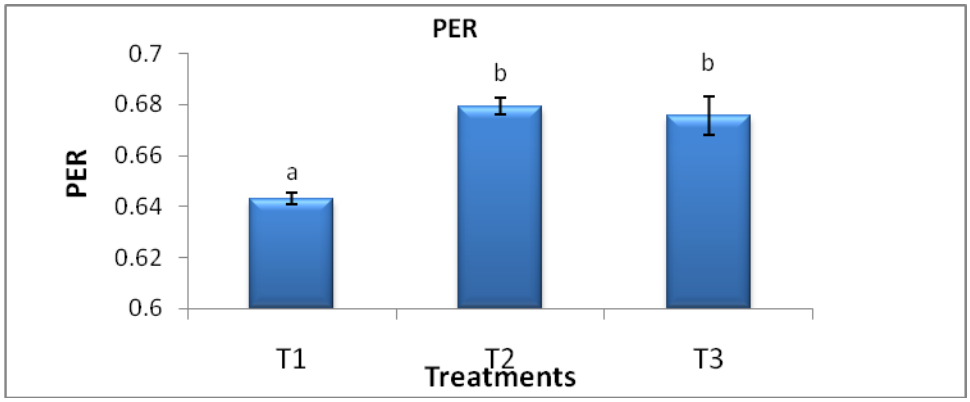



**Fig.4** Protein efficiency ratio (PER) of *Clarias batrachus* fingerling fed with different experimental diets. Bar bears the same superscript do not differ significantly (p>0.05)



### Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The ALT and AST activity of muscle and liver tissue of different treatments were recorded in the Table 3. The AST and ALT activity in the muscle and liver were significantly lower ($p<0.05$) in the T1 group compared to other treatment groups. The activity of these enzymes showed no significant difference ($p>0.05$) between T2 and T3 groups in liver tissue. In muscle, AST activity was significantly higher in T3 than T1 and T2 groups. However, ALT activity was significantly ($p<0.05$) higher in the T2 group than T3 treatment group. Transaminases are the enzymes that redistribute amino nitrogen among the amino acids, forming new amino acid with the amino group from the pre-existing ones. Both the aspartate and alanine aminotransferase acts as a link between carbohydrate and protein metabolism by catalyzing the inter conversion of compounds like α- ketoglutarate and alanine to pyruvic acid and glutamic acid (Knox and Greengard, 1965). The pyruvate and oxaloacetate thus formed are responsible for the synthesis of non-essential amino acids like alanine, asparagine, glutamine etc. which in turn help in protein synthesis and hence the growth of the animal. In the present study, the AST and ALT activity was studied in liver and muscle tissue. In liver higher AST activity ($p<0.05$) was found in T2 and T3 than T1, whereas in muscle highest activity ($p<0.05$) was seen in T3 group. ALT activity in muscle was highest in T2 (Table 3). This shows that a higher activity of these enzymes is responsible for growth by increasing the supply of non-essential amino acids for protein synthesis (Sanchez-Muros *et al.,* 1998).

### Hexokinase

Hexokinase activity (Table 3) in both liver and muscle tissues did not vary significantly ($p>0.05$) among different treatment groups. Hexokinase is a glucose phosphorylating enzyme which converts glucose to glucose-6-phosphate. In the present study, there was not any significance ($p>0.05$) difference in hexokinase activity among the treatment groups (Table 3). This shows that glycolytic pathway is not affected by dietary carbohydrate up to the 40 % inclusion. A similar result was reported by Legate *et al.*, (2001), where he found no observable change in the activity of enzyme in muscle with the difference in diet composition.

### Glucose-6-phosphate dehydrogenase (G-6-PDH)

The G-6-PDH enzyme activity of the different experimental groups is presented in the Table 3. The G-6-PDH enzyme activity was significantly higher ($p<0.05$) in the T3 treatment group for liver and muscle tissues compared to other two treatment groups. There was no significant difference ($p>0.05$) observed between the other two groups (T1 and T2). Glucose-6-phosphate dehydrogenase is an enzyme of the pentose phosphate pathway which play role in fatty acid biosynthesis. In this experiment, the enzyme activity was significantly higher ($p<0.05$) in T3 fed groups as compared to T1 and T2 (Table 3). It is in accordance with the reports of Likimani *et al.,* (1982), who observed that the G-6-PDH activities were increased with higher levels of gelatinized carbohydrate in diet of channel catfish. Therefore, the present study, indicates the preferred anabolic use of carbohydrate by fish up to a level of 40 % inclusion.

Carbohydrate utilization is the key concern for economic feeding in aquaculture. The growth and related digestive and metabolic enzymes, assessed in *C. batrachus* fingerlings, fed with different dietary carbohydrate levels (20% -$T_1$, 30% -$T_2$and

40% -$T_3$) for 60 days indicated the better utilization ability of carbohydrate by the species at higher inclusion level of dietary carbohydrate. Therefore, it can be concluded that dietary inclusion of 40% carbohydrate is economic in aquafeed formulation of *C. batrachus* for better growth without any adverse impact on metabolism.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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