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# Chronological Changes in Postprandial Blood Glucose Level of *Labeo rohita* (Hamilton, 1822) Fed with Formulated Diet

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## ABSTRACT

In general, fish is considered as a natural diabetic animal. In order to understand how the feed is metabolized to glucose and the time duration for its complete uptake from blood, a study was conducted to estimate the pre and postprandial blood glucose level in Labeo rohita, at two-hour intervals of both fasting and fed state. The blood glucose levels of four replicates at every time point were estimated using glucose oxidase method. The pre-prandial/ fasting blood glucose level was found to be in the range of 10-18 mg/dl. Whereas, two hours post-prandially the blood glucose level had got increased and estimated to be varying between 46-88 mg/dl, which was maximum. Also, it was observed that the sugar levels got reduced back to normal/fasting state after about 10hrs. Time period shows significant inverse relationship with blood glucose level ($p<0.05$). These results will help in establishing and managing a proper feeding schedule for rohu.

**Key words:** Glucose, Pre-prandial, Post-prandial, Fasting blood glucose, Practical diet, Glucose oxidase

## INTRODUCTION

Glucose is a simple sugar or monosaccharide which is preferred as a substrate for energy production for all the cells of the body, and the only energy source for the nervous system. It serves as the key to keeping the body mechanisms in working order and is often referred to as blood sugar. Glucose tolerance is a term that refers to the ability of an organism to rapidly deal with a glucose load. The consequences of glucose intolerance are persistent hyperglycemia and in many cases, reduced growth. Glucose intolerance is a clinical term used in the diagnosis of human insulin-dependent diabetes mellitus. The IDDM and is assessed by glucose tolerance test GTT. A GTT involves administering glucose either orally or intravenously, and if plasma glucose values do not return to baseline within 1-2 h, the person is considered to have impaired glucose tolerance. The GTT has been used by many researchers to study fish glucose tolerance and in all most all cases; persistent hyperglycemia was observed (Moon, 2001). However, the time period of hyperglycemia is species and condition-dependent. Furuichi and Yone (1981) demonstrated the most severe hyperglycemia for longer duration in the omnivorous carp in GTT. According to Wilson (1994) marine species are more tolerant to glucose load than freshwater species, although Garcia-Riera and Hemre (1996 a, b) demonstrated opposite observation. There are differences in tolerance observed between Chinook salmon strains (Mazur *et al.,* 1992). The conditions of the test species are also a factor for its glucose tolerance. Again, Furuichi and Yone (1981) fed different doses of dextrin diet to carp, red sea bream and yellow tail and observed that more the dextrin content there was more hyperglycemia and lower insulin level. Prolonged food-deprivation compared with feeding will generally extend the period of hyperglycemia (Blasco *et al.,* 1996; Legate *et al.,* 2001). And the source of starch can also affect the hyperglycaemic response to a glucose load (Hemre and Hansen, 1998). Although there are many studies on GTT in fish with glucose administration or dextrin in diet, but study of glucose levels with a formulated diet is scarce. In this study we monitored the blood glucose level in Labeo rohita fingerlings after fed with a formulated diet.

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The experiment was conducted at the wet laboratory of ICAR-Central Institute of Fisheries Education (CIFE), Salt Lake, Kolkata Center. 60 numbers of Labeo rohita (Hamilton, 1822) (rohu) fingerlings having an average weight of 12.0 ± 0.25 g procured from the fish farm at Ramsagar, Kolkata. Handling of fish at all stages was done utmost care to give less stress. During the experiment and sampling, all the ethical guidelines of animal care for ICAR-Central Institute of Fisheries Education, Mumbai India were strictly followed. Fishes were treated with 2ppm potassium permanganate to disinfect them following which they were transferred into a 500 litre FRP tank and acclimatized for a few days. After acclimatization, 15 fishes were transferred in each of the four rectangular fibre-reinforced plastic (FRP) tanks (180L capacity). Before blood collection, fish were fasted for 48hrs. After the collection of fasting blood, feeding was carried out with the prepared diet. One hour after feeding, the tanks were siphoned for removal of excess feed and faecal matter before post pandrial blood collection

All fishes were fed with the same diet containing 29% of crude protein, which is optimum protein requirement for carps. The diet was prepared using different common ingredients used for fish feed (Table 1).

**Table 1. Composition of formulated diet (g/100 gm)**

| Ingredients (g 100g-[1]) | In Diet |
|---|---|
| DSBM[a] | 30.00 |
| GNOC[a] | 14.25 |
| Wheat flour[a] | 6.97 |
| DORB[b] | 40.00 |
| Vegetable oil[a] | 3 |
| Fish oil[a] | 2 |
| Vit-min Mix[d] | 2 |
| CMC[c] | 1.50 |
| BHT[c] | 0.03 |
| Choline chloride[c] | 0.25 |

Notes. Quantities of ingredients are expressed in g/100 g. DSBM: de-fatted soybean meal; GNOC: groundnut oil cake; DORB: de-oiled rice bran; CMC: carboxymethyl cellulose; BHT: Butylated Hydroxy toluene.

[a]Procured from local retail shop. [b]Purchased from Vaighaiagro products, India. [c]Purified ingredients procured from HImedia ltd., India. dComposition of vit.-min. premix (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; VitaminB6, 1,000 mg; Vitamin B12, 6 mg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000mg; 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 50 ppm.

Proximate composition of diets was analysed according to the standard methods of AOAC (1995). The experimental diet was dried to constant weight at 100 ± 2°C in hot air oven to determine the moisture content. Crude protein content was determined using the micro-Kjeldahl method (Kelplus, PELICAN, India), whereas ether extract was calculated by the Soxhlet extraction method (SOCS plus, SAS-AS 08; PELICAN). The ash content was estimated using muffle furnace and fibre approximation was done in Fiber tech (Tulin Equipments) apparatus and further ashing was done using a muffle furnace at 550°C for 5 hr. The proximate composition of experimental diets is given in table 2.

**Table 2. Proximate composition of experimental diet (% dry matter)**

| Proximate composition | Diet (% dry matter) |
|---|---|
| Dry matter | 91.05 ± 1.45 |
| CP[1] | 29.02 ± 0.41 |
| EE[2] | 7.29 ± 0.07 |
| CF[3] | 6.56 ± 0.24 |
| NFE[4] | 48.18 ± 0.15 |
| TA[5] | 8.95 ± 0.03 |

Data are expressed as mean ± SE; (n = 3)

[1]CP: crude protein. [2]EE: ether extract; [3]CF: crude fibre; [4]NFE: nitrogen free extract; [5]TA: total ash; NFE (nitrogen-free extract) = 100 - (crude protein + crude lipid + crude fiber +ash).

Each fish was anesthetized with clove oil (50µl of clove oil per litre of water) before taking blood from them. Blood was taken from caudal vein using a medical syringe (N-22) of 2 ml and then immediately centrifuged in a micro-centrifuge (5 minutes at 6000 rpm) for plasma collection.

In order to monitor the changes in blood glucose level, the fishes were examined in quadruplicates after two hours of fasting and then at the interval of two hours postprandial. Each time after blood collection, the fish were transferred to another common tank and a set of four new fishes from the four experimental replicate tanks were used in the next time for blood glucose estimation, i.e. at every intervals four new fish are choosen for blood collection to minimise the stress.

The glucose level in plasma was determined using Erba diagnostic kit (Transasia Biomedicals Pvt. Ltd., India). In the assay, glucose is oxidized to gluconolactone with concomitant reduction of the flavin adenine dinucleotide (FAD)-dependent enzyme glucose oxidase. The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide reacts with 3, 5-dichloro-2- hydroxybenzene sulfonic acid and 4-amino antipyrine to generate a pink dye with an optimal absorption at 514 nm.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \text{Concentration of standards (mg/dl)}$$

Data of the current experiment were analysed by regression analysis using SPSS program, 22.0 version. Second degree Polynomial equation showed the best fit. R square and P values were calculated.

The pre-prandial/ fasting blood glucose level in the absence of food for 48 hours was found to be in the range of 10-18 mg/dl. Two hours post-prandial blood glucose level was observed to be varying between 46-88 mg/dl (Table 3), which was the maximum estimated blood glucose level, thus indicating the rise in circulating blood glucose by absorption immediately after the digestion of feed and it is reduced back to normal range or fasting blood glucose levels after 10hr (Fig. 1).

**Table 3. Blood glucose concentration of replicate**

| Time | R1(mg/dl) | R2(mg/dl) | R3 (mg/dl) | R4(mg/dl) | Average ±S.E. |
|---|---|---|---|---|---|
| Fasting -2 hr | 18 | 16 | 10 | 12 | 14.00±1.83 |
| After feeding +2hr | 65 | 88 | 46 | 74 | 68.25±8.80 |
| +4hr | 50 | 42 | 42 | 52 | 46.50±2.63 |
| +6hr | 31 | 27 | 23 | 34 | 28.75±2.39 |
| +8hr | 29 | 19 | 17 | 18 | 20.75±2.78 |
| +10 hr | <10 | 15 | 11 | <10 | 10.75±1.55 |

Data presented examine blood glucose level changes in rohu (Labeo rohita) at two hrs interval before and after feeding, a commercially cultured fish species. R1-R4 represents four replicates

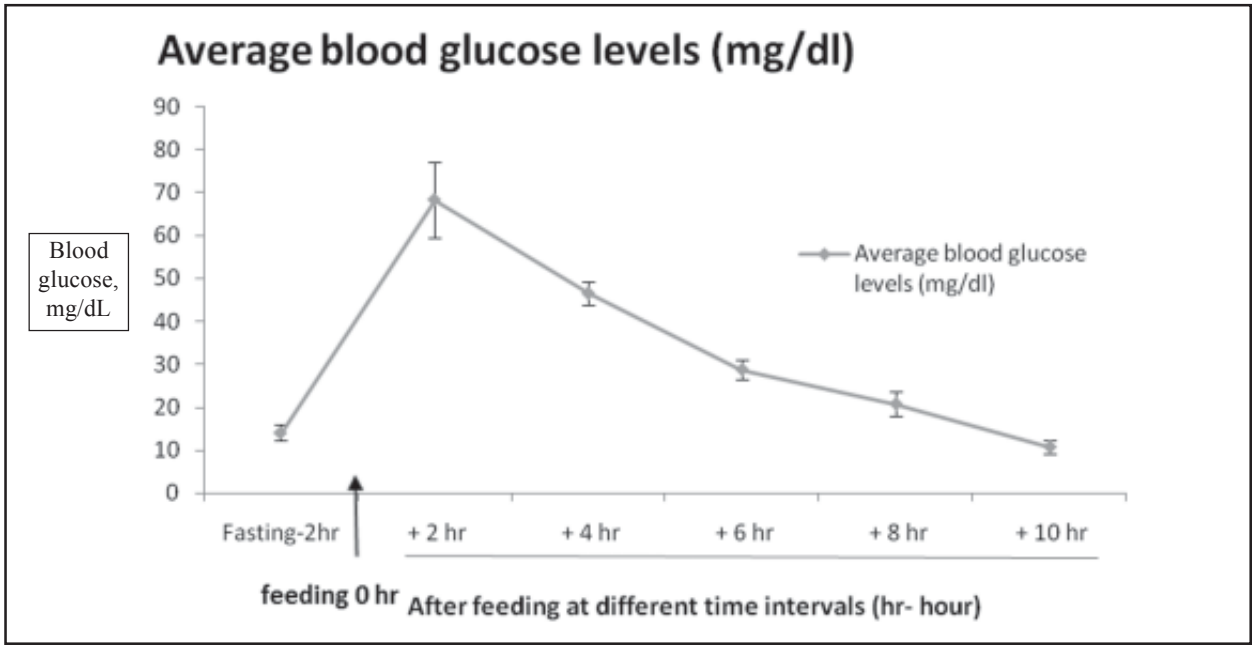


**Fig. 1. Pre and post prandial blood glucose level**

This result re-establishes the claim that fish is a diabetic animal (Malik, 2020). As per Legate *et al.* (2001) and Falkmer (1961), the pre-prandial glucose level reported in fish blood is 25 mg/ dl to 90 mg/dl while the post-prandial glucose level is 300 mg/100 ml. After every meal, the blood sugar levels increase and in response, insulin, a pancreatic hormone is secreted to reduce the sugar levels until it becomes normal. But, clearance of the blood glucose is very slow in fishes; it takes up to 6 hrs to 7 hrs, while in human beings it takes only 30 min. However, exceptionally in some fishes it takes up to 24 hr for its clearance. The glycaemic changes that occurred clearly indicate the sensitivity of blood glucose levels. Consistent with this, modifications in glucose metabolism after feed intake are often the reasons for these changes, suggesting that glucose is an important substrate that is mobilized as a result of a variety of physiological, metabolic and environmental challenges. Later on, glycaemic decreases were observed with food deprivation. These alterations in blood glucose suggests that glucose metabolism in fish is important. Normal blood glucose levels of fish are within 40-90 mg/dl (Polakof *et al.,* 2012) and the

data recorded also lies approximately within the optimum range within four hrs of feeding. But after that there is gradual decrease in blood glucose level that is as low as 10mg/dl. Regression analysis of the level of glucose vs time gives the best fit curve (glucose level = 93.85 - 14.16 time + 0.594 time*time) has a R-square vale of 99.79%, showing very good fit of the curve with the data. The p-value of 0.004 for the regression model signifies that the predictor variable, i.e. time, significantly varies the response variable, i.e. glucose level.

All the previous studies were based on either pure glucose or dextrin diet base studies. As per our knowledge, this is the first report of such study in Labeo rohita, in which after feeding formulated diet blood glucose has been estimated chronologically. After 8 hrs the level of blood glucose was very low, so feeding of rohu should be done at 8hrs interval.

The study establishes the fact that the fasting blood glucose level of fish can go below 20mg/dl and in this particular study, 10-18 mg/dl is the observed fasting blood sugar level in fish. It takes about 8-10 hrs to fully absorb the blood sugar post feeding. However, further study with other fish species is necessary to validate these findings.

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