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The sequential mobilization of glucose reserves in various tissues and organs of freshwater catfish, *Clarias batrachus* during starvation.

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Abstract

The present work has been selected to know the impact of starvation on glucose reserves of various tissues and organs of a freshwater teleost, Clarias batrachus. The level of glucose reserves has been estimated in liver, gonads, muscles, brain and the blood in both the sexes of Clarias batrachus, which were subjected to starvation for 40 days. The total glycogen content was estimated according to the calorimetric method of Kemp & Heijningen (1954) as modified by Krishnaswami & Srinivassan (1961). *Clarias batrachus* adapted well to starvation stress and survived all throughout the experimental period.

During starvation, the gradual decrease of glucose reserves has been noticed in all types of tissues which may be related to blocking of RNA synthesis, enhanced gluconeogenesis and increased rate of transamination and deamination. Liver, muscle, and gonad glycogen decreased sharply but the brain glycogen did not show significant depletion up to 20 days of starvation. The blood glucose level decreased gradually like the glycogen stores of liver and muscle. The glycogen content of liver was high in comparison to the other solid tissues (muscle, gonad & brain). The females had higher values of glucose reserves in all the tissues investigated in comparison to males both in normal and starved conditions. The testes showed maximum drop while the brain tissues with least drop in glycogen content after 40 days of starvation.

***Key words**: Clarias batrachus, glucose, mobilization, starvation.*

1. Introduction

Since the emergence of life on the earth, starvation is commonly hanging before the living beings but the essence of life as well as sprit of living has never exhausted. Prolonged starvation affects the normal body metabolism and may even cause death of the animal. Organisms facing starvation fight it at the cost of their own body-reserves till death. During starvation the animal becomes entirely dependent on the biochemical constituents of its own tissues for obtaining energy (Wright, 1976). This causes the breakdown and mobilization of different cellular constituents. The carbohydrates are the first constituent to be consumed and thereafter lipid reserves are mobilized.

A decline in various body constituents of fish, following experimental starvation, have been reported by various authors. This paper deals with the results obtained for the fresh-water catfish, *Clarias batrachus*, following starvation up to 40 days.

To know the facts and causes of starvation and their consequent impacts on animals have been carried out by many workers but most of the works have been confined to mammalian fauna. So, it is pertinent to see and reveal the effect of starvation on fish as there is a unique feature of fish to withstand prolonged starvation through physiological and biochemical changes (Mustafa, 1983). In fact starvation affects the physiological status and biochemical constituents of fish (Rajyasree and Naidu, 1989; and Tripathi & Verma, 2003).

In the light of above facts, the present work has been designed to carry out glucose estimation under prolonged starvation on various tissues and organs of a fresh water teleost *Clarias batrachus.* It is a common air breathing fish of the area with high nutritive and convalescence value.

2. Materials & Methods:

The fish *Clarias batrachus* (Linn.) locally called "Mangur" were chosen for the present study. These were collected live form a local fish pond with the help of fishermen. The fish were then brought to the laboratory in large earthen pots covered with mosquito net. They were identified according to Srivastava (2006) and rinsed with 0.1% $KMnO_4$ solution to get rid of any dermal infection. Healthy fish of an average length (18.8 cm) and weight (34.4 gm) were transferred one by one with the help of small hand net to 110 litre glass aquaria for acclimation.

The effect of starvation was studied up to 40 days, since it was observed earlier that their morality was high beyond this period. To evaluate the effects of starvation, quantitative estimation of glycogen in liver, muscle, gonad and brain were made after 0, 10, 20, 30 & 40 days of starvation.

Biochemical estimation were made by taking a sample from each sex of acclimatized and well fed fish and the values obtained were taken as normal value for *Clarias batrachus*. The remaining fish were divided into four batches named A, B, C & D. The fish of batch A were kept without food at room temperature for 10 days, that of batch B for 20 days, C for 30 days and D for 40 days.

On scheduled dates, the fish were dissected out and their liver, gonad, brain and muscles were removed and placed immediately in the previously prepared ice-cold fish saline.

Before transferring the liver, gonad, muscles and brain into saline, they were properly cleaned by removing other attached tissues. The muscles were removed from the lateral side with the help of scalpel and scissor and cleaned off the nerves. Before use, the tissues were nicely blotted with filter papers. The blood was drawn from the cauda dorsalis with the help of plastic syringe. EDTA was used as the anticoagulant.

The total glycogen content was estimated to the calorimetric method of Kemp & Heijningen (1954) as modified by Krishnaswami & Srinivassan (1961).

3. Results

The glucose reserves of solid tissues were mobilized fast. Muscle, liver, and gonad glycogen decreased sharply but the brain glycogen did not show significant depletion up to 20 days of starvation. The females had higher values of glycogen content both in normal and starved conditions. The glycogen content of liver was highest among the solid tissues. The blood glucose level decreased gradually, like the glycogen stores of liver and muscle (Table 1). In comparison to normal values, the depletion of glucose reserves after 40 days of starvation in different tissues was as follows:

Liver………. 74% in male & 73% in female;
Muscle…….. 82% in male & 81% in female;
Gonad…….. 84% in male & 79% in female;
Brain……… 51% in male & 50% in female;
Blood……… 63% in male & 69% in female.

***Table 1**. Liver Glycogen Content (mg/gm wet weight) of Clarias batrachus*

Tissues & Organs	Sex	Days of Starvation				
		Control	10	20	30	40
Liver	Male	93.60 ± 1.09	58.30** ± 1.10	44.99 ± 1.71	30.85** ± 0.77	23.76** ± 0.65
	Female	101.5 ± 0.81	74.44 ± 1.61	52.11 ± 0.30	41.66** ± 0.44	27.53** ± 0.69
Gonad	Male	12.32 ± 0.23	11.80 ± 0.26	10.06 ± 0.22	5.28** ± 0.18	1.97** ± 0.15
	Female	19.24 ± 0.31	18.95 ± 0.44	12.26** ± 0.21	7.73** ± 0.13	4.05** ± 0.11
Muscle	Male	11.44 ± 0.12	10.17** ± 0.37	5.86** ± 0.32	4.66** ± 0.54	2.07** ± 0.46
	Female	13.77 ± 0.22	11.44** ± 0.24	7.71** ± 0.18	5.42** ± 0.18	2.62** ± 0.17
Brain	Male	16.17 ± 0.19	15.83 ± 0.22	15.41 ± 0.18	12.66** ± 0.20	7.88** ± 0.19
	Female	19.25 ± 0.28	18.87 ± 0.20	18.63 ± 0.45	16.29** ± 0.34	9.90** ± 0.15
Blood	Male	88.62 ± 0.83	72.37** ± 1.42	58.51** ± 0.95	43.13** ± 1.07	32.16** ± 0.84
	Female	106.01 ± 1.34	78.50 ± 1.57	63.62** ± 1.43	46.17** ± 1.34	32.95** ± 1.06

Values are mean of 8 samples of both male & female ± SE
** Significant

4. Discussion

The maintenance of biological functions requires a steady supply of energy. Since the cell maintains itself by utilizing energy it must have one or more mechanisms for the input of energy and must have a number of energy transducers, capable of changing energy into work. These mechanisms operate continuously in every organism but during stress like toxicity, temperature fluctuations, fear and food deprivation a number of additional changes expected to work (Davison & Dobbing, 1961).

The fishes get maximum support from their environment which enables them to conduct their activities without recourse to their body constituents and so their basal energy consumption is very low. For this reason, fish are able to withstand astonishingly long periods of starvation such as, *Amia calva* survived for 20 months without food (Smallwood, 1916), *Clupea harengus* survived for 129 days and above all *Anguilla anguilla* managed to live without food for 15 days (Boetius, 1967).

Effect of prolonged as well as short term starvation has been extensively studied, mostly on temperate fish. Fontaine & Hatey (1953) observed 54% drop in liver glycogen content of *Salmo salar* during spawning migration starvation. Inui & Oshima (1966) observed slower rate of glycogen depletion in muscle than that of liver in starving *Anguilla japonica.*

The carbohydrate metabolism plays a primordial part during food deprivation as carbohydrates are the principal fuel for the energy production. Glucose molecules are constantly oxidized to release energy. During starvation glycogen is broken to produce glucose which in turn is supplied through blood to the needy organs. The increased level of glucagon during starvation (Cahill, 1970; Bell, *et al.* 1976; and Chaudhary & Mandal, 1981) stimulates the synthesis of glucose from glycogen. The glucose production occurs directly from the glycogen stores of liver and indirectly through other tissues like muscle, gonad and brain through the Cori-cycle and alanine glucose cycle (Cori, 1931 and Felig, 1973).

The decrease in the carbohydrate content in the starving organism was observed by several workers in different tissues of different animals such as Fontain & Hatey (1953) in the liver of *Salmo salar,* Bellamy (1968) in the liver of *Rooseveltielia nattereri*, Narasimhan & Sundararaj (1971) in fish muscle, Rivera & Jesus (1974) in muscles of rhesus monkey, Matsumoto *et al.* (1980), Prasad (1980), Chaudhary & Mandal (1981) in the brain of *Schizodactylus monstrosus*; and Saini & Purohit (1981) in *Mus musculus.*

In the present investigation, the glycogen content of liver and muscle showed sharp and significant depletion. After 40 days of starvation more than 70% of the liver glycogen was lost whereas the loss in the muscle was more than 80% (Table 1; Fig. 1 & 3). The glycogen stores of the gonad and brain did not show significant drop up to 20 days of starvation (Fig. 2 & 4). When the glycogen stores of liver and muscle dropped below 50% of the normal, the glycogen stores of gonad started to drop sharply. After 40 days of starvation gonad showed glycogen depletion almost equivalent to that of muscle. The glycogen content of brain showed minimum depletion in comparison to the liver, muscle and gonad. The blood glucose level seemed to follow gradual pattern of depletion like liver and muscle glycogen (Fig. 5).

The present findings are in conformity with the findings of Fontain and Hatey (1953), Freedland (1967), Rivera & Jesus (1974), Chaudhary & Mandal (1981), Ottolenghi *et al.* (1981), and Freminet & Lilliane (1981).

The value of glycogen and glucose was found higher in females than males under both normal and starved condition. This finding of sex difference is in conformity with Singh (1981) and Singhal *et al.* (1981).

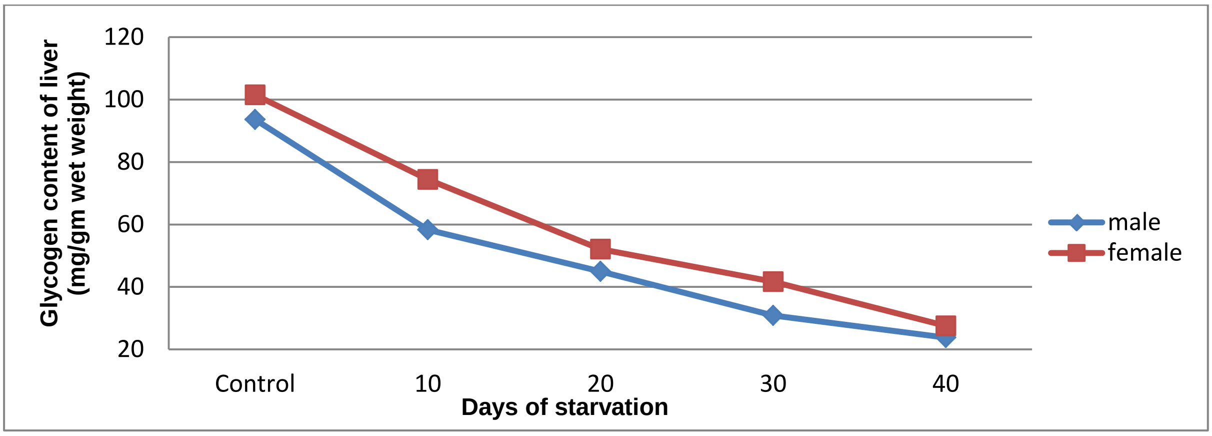

Fig. 1. Mobilization of glycogen content of liver during starvation in *C. batrachus*.

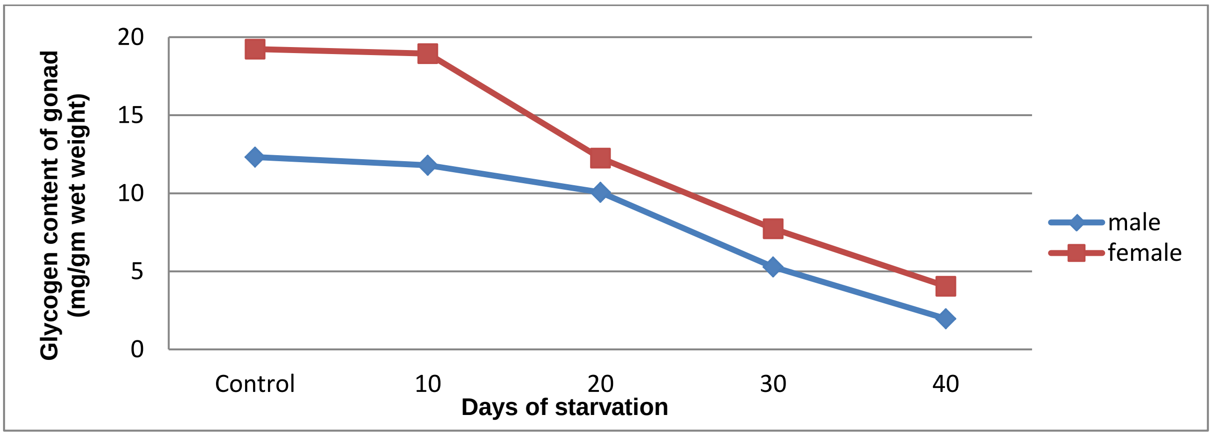

Fig. 2. Mobilization of glycogen content of gonad during starvation in *C. batrachus*.

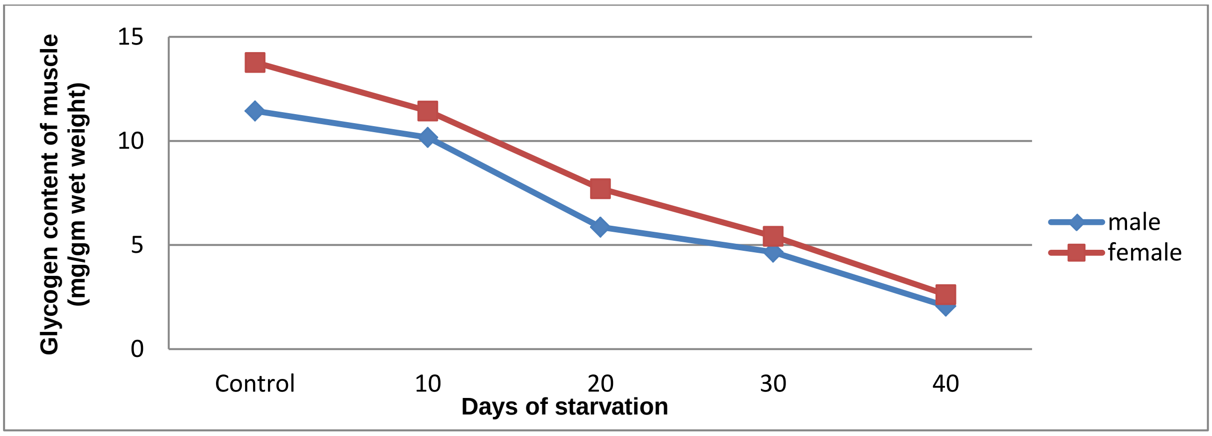

Fig. 3. Mobilization of glycogen content of muscle during starvation in *C. batrachus*.

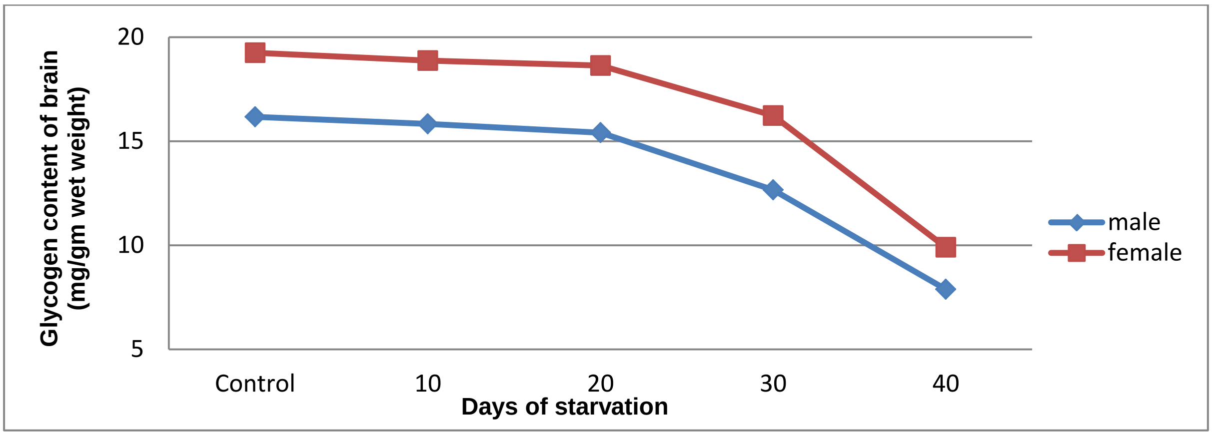

Fig. 4. Mobilization of glycogen content of brain during starvation in *C. batrachus*.

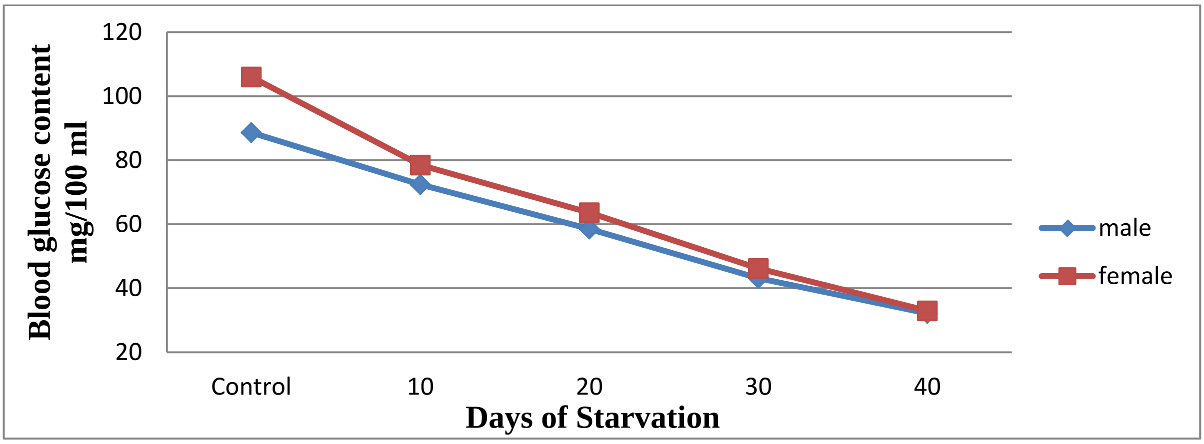

Fig. 5. Mobilization of blood glucose during starvation in *C. batrachus*.

5. Conclusion

From this study, it is quite evident that when *Clarias* was subjected to starvation stress, the animal successfully overcame the stressful period and survived all throughout the starvation. The level of glucose reserves in various tissues and organs have been found higher in females than that of males under both the normal and starved conditions. The main cause for the depletion in glucose reserves was the effect of starvation stress as indicated by the control group. After 40 days of starvation, testis showed highest depletion while the brain tissues with least drop in glycogen content.

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