

Starvation-Induced Impairment of Metabolism in a Freshwater Catfish

Ghanshyam Tripathi*[§] and Priyanka Verma

Department of Zoology, J. N. V. University, Jodhpur-342001, India

[§] D-41, Sarswati Nagar, New Pali Road, Jodhpur-342005, India. E-mail: drgst@rediffmail.com

* Author for correspondence and reprint requests

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Starvation induced changes in citrate synthase (CS), glucose-6-phosphate dehydrogenase (G6-PDH), lactate dehydrogenase (LDH), DNA, RNA, RNA/DNA ratio and protein were studied in the freshwater catfish *Clarias batrachus*. Starvation gradually decreased the activity of CS, G6-PDH and LDH in brain, liver and skeletal muscle of the freshwater catfish. The maximum reduction in these enzyme activities upto 35–45% was observed after 35 days of fasting. This shows substantial decline in aerobic and biosynthetic capacity during starvation period. DNA, RNA, RNA/DNA ratio and protein contents were also reduced from 40–67% which reflects reduction in an overall capacity of the protein synthesis. Starvation – induced macromolecular changes indicate impairment of metabolism in fish.

Key words: Fish Starvation, CS, G6-PDH, LDH

Introduction

Starvation is experienced in most species of fish during certain periods of every year largely due to environmental conditions and it affects different organs in different ways. Liver and muscle are affected first while brain and heart do not show any depletion. Starvation also affects the physiology and other constituents of fish (Rajyasree and Naidu, 1989; Mukhopadhyaya *et al.*, 1991; Lie and Huse, 1992; Chin and Shin, 1992; Deng *et al.*, 1993). It has been observed that the relative concentration or amounts of different glycolytic enzymes are well conserved during periods of starvation, despite very large decrease in the total activities of the enzymes (Mommensen *et al.*, 1980; Sullivan and Somero, 1983; Lowery *et al.*, 1987; Lowery and Somero, 1990). Starvation – induced changes in red and white muscle of barred sand bass (*Paralabrax nebulifer*) have been demonstrated in relation to the activities of some glycolytic enzymes including lactate dehydrogenase (LDH) (Lowery and Somero, 1990). The activity of LDH is remarkably decreased during the periods of 23 days of fasting. In this case, there occurs smaller decrease initially and its becomes more and more as the period of fasting proceeds. LDH has a higher specific activity and a lower concentration in skeletal muscle and maintains a higher synthesis rate than other glycolytic enzymes

after 5 days of starvation of *P. nebulifer*. Basing on these and other observations, the differential effects of starvation on synthetic rates and stabilization of different enzymes have been suggested.

Prolonged starvation effects on red and white muscles of two freshwater teleost fishes have been studied by Kiran and Talesara (1985). They found a sharp decline in spontaneous activity and metabolic rate during prolonged starvation which was reflected by reduced activities of succinic dehydrogenase and mitochondrial-ATPase in myotomal muscle. Chandra (1982, 1983) reported the effect of starvation on serum acid phosphatase and serum glutamic pyruvic transaminase level. Borah and Yadav (1996) worked on the biochemical and haematological response to starvation in *H. fossilis*. They reported the decrease in the activity of lactate dehydrogenase in both liver and muscles as a function of starvation. The amount of protein, glucose and glycogen also decreased as the period of starvation increased. Letcher *et al.* (1996) showed the size-dependent effects on starvation and mass loss in yellow perch larvae and juveniles.

There is increasing evidence that starvation may be a major cause of mortality in both immature and adult fishes. Fasting also affects metabolic enzymes, RNA/DNA ratio and proteins in fish. It has a great impact on fish growth. Therefore, the present study was planned to determine the starvation

effects in an economically important siluroid species.

Materials and Methods

Fish

The freshwater catfish *Clarias batrachus* weighing 75–80 g and having a body length of 18–19 cm were collected from fish markets. They were acclimatized to laboratory condition in container having tap water (pH 7.6–7.9) at least for two weeks prior to experimentation. They were fed on minced goat liver on alternate day. After acclimatization for two weeks, healthy and adult specimens of *C. batrachus* were divided into control and fasting groups. Each group contained five individuals. Control was fed *ad libitum* with chopped goat liver. However, the food was withheld from the fasting group. Fish were sacrificed after 7, 14, 21, 28 and 35 days of starvation for enzymatic and other biochemical studies.

Chemicals

Acetyl CoA, nicotinamide adenine dinucleotide (β -NADH), nicotinamide adenine dinucleotide phosphate (β -NADP⁺), oxaloacetate, sodium pyruvate, glucose 6-phosphate and other chemicals were procured from Sisco Research Laboratory, Mumbai, (India).

Enzyme extraction

The specimens of *C. batrachus* were sacrificed by decapitation. The brain, liver and caudal white skeletal muscle were removed and washed in 0.6% saline. They were cleaned and weighed rapidly. A 10% homogenate (w/v) was prepared in tris-HCl buffer (0.1 M tris-chloride, pH 7.5) using Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate was centrifuged at $700 \times g$ for 15 min in a high speed refrigerated centrifuge. The supernatant was decanted and centrifuged at $12,000 \times g$ for 20 min to get the mitochondrial pellet. The resulting supernatant was taken as the cytoplasmic fraction for the assay of glucose 6-phosphate dehydrogenase (G6-PDH) and lactate dehydrogenase (LDH). The mitochondrial pellet was washed twice in 0.1 M tris-HCl buffer (pH 7.5) and each washing was followed by centrifugation at $12,100 \times g$ for 15 min. The pellet was resuspended

in the above buffer and homogenized at high speed. The homogenized suspension was recentrifuged at $21,000 \times g$ for 15 min to remove particulate matter. The resulting supernatant was taken as mitochondrial fraction for the assay of citrate synthase (CS). The marker enzyme, lactate dehydrogenase (LDH), was assayed in the mitochondrial fraction to test cytoplasmic contamination, if any. The procedure employed for subcellular fractionation and extraction of the mitochondrial enzyme was based on Foster and Moon (1986).

Assay procedure

The procedures adopted for assay of citrate synthase (CS) and lactate dehydrogenase (LDH) were those of Foster and Moon (1986). The enzyme glucose 6-phosphate dehydrogenase (G6-PDH) was assayed according to the method of Mommsen *et al.* (1980).

Nucleic acid and protein estimation

DNA and RNA contents were estimated according to the method of Schneider (1957) using diphenylamine and orcinol reagents, respectively. The concentration of protein was estimated by Lowry method (1951) using Folin-Ciocalteu reagent.

Statistical analysis

Analysis of variance (ANOVA) and Newman-Keul's multiple range test were performed to ascertain the level of significance.

Results

The activity of CS decreased gradually in brain, liver and skeletal muscle of the freshwater catfish in response to fasting. The changes in enzyme activity were statistically significant ($P < 0.05$) in relation to the changes in fasting period. The maximum effect on the enzyme activity was obtained after 35 days of starvation. The decrease in CS activity in all the three tissues was approximately 46% (Fig. 1). The activity of G6-PDH declined gradually in brain and liver of the freshwater fish in response to starvation for 35 days. The changes in enzyme activity were statistically significant ($P < 0.05$) with respect to the changes in fasting duration. The decrease in G6-PDH activity in both the tissues (brain and liver) was about 49%. However,

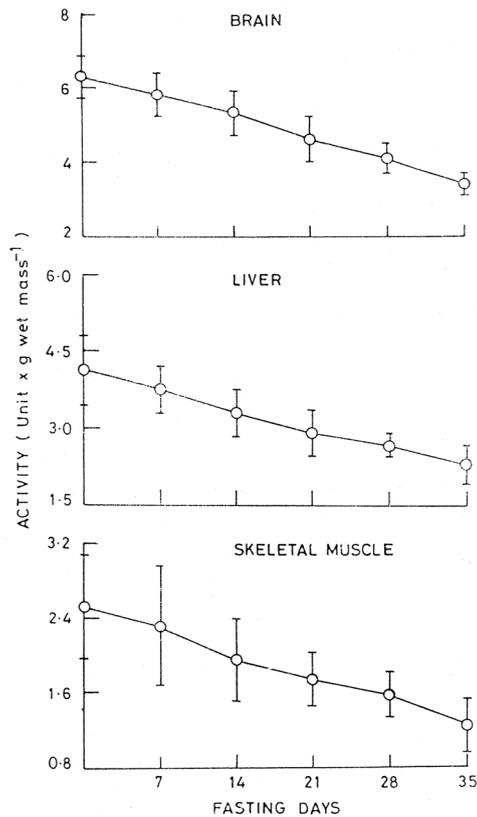


Fig. 1. Effect of starvation on the activity of CS in the brain, liver and skeletal muscle of the freshwater catfish, *Clarias batrachus*.

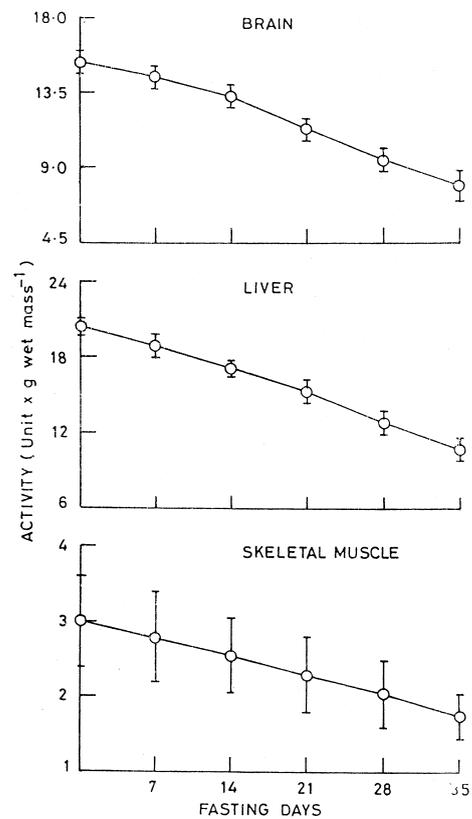


Fig. 2. Effect of starvation on the activity of G-6 PDH in the brain, liver and skeletal muscle of the freshwater catfish, *Clarias batrachus*.

in case of skeletal muscle it did not produce any significant change ($P > 0.05$) as a function of fasting period. The maximum reduction in G6-PDH activity was 43% (Fig. 2). The LDH activity decreased gradually in all the three tissues of *C. batrachus*. The changes in enzyme activity were statistically significant ($P < 0.05$) in relation to the changes in fasting period. The maximum effect on the enzyme activity was noticed after 35 days of starvation. The decrease in enzyme activity was approximately 40% in all the three tissues (Fig. 3). RNA, RNA/DNA ratio and protein contents decreased gradually in brain, liver and skeletal muscle of the freshwater catfish in response to fasting. The alterations in RNA, RNA/DNA ratio and protein contents were statistically significant ($P < 0.05$) with respect to variations in fasting period (Fig. 4–6). The maximum effects on RNA, RNA/DNA ratio and protein were observed after 35

days of fasting. The decreases in these macromolecular concentration ranged from 36–60%.

However, the variation in DNA content was not significant ($P < 0.05$) in response to starvation. Pickering and Pottinger (1955) have suggested that the total quantity of DNA per cell remains constant in normal somatic tissues and is not altered by starvation and other forms of stress. However, the quantity of RNA within a cell is highly variable and reflects the activity of protein synthesis. Hence low RNA/DNA ratio represents rapid decline in protein synthesis.

Discussion

It is evident from the starvation studies that the fasting decreases significantly the activity of CS, G6-PDH and LDH by approximately 44% in brain, liver and skeletal muscle of the freshwater

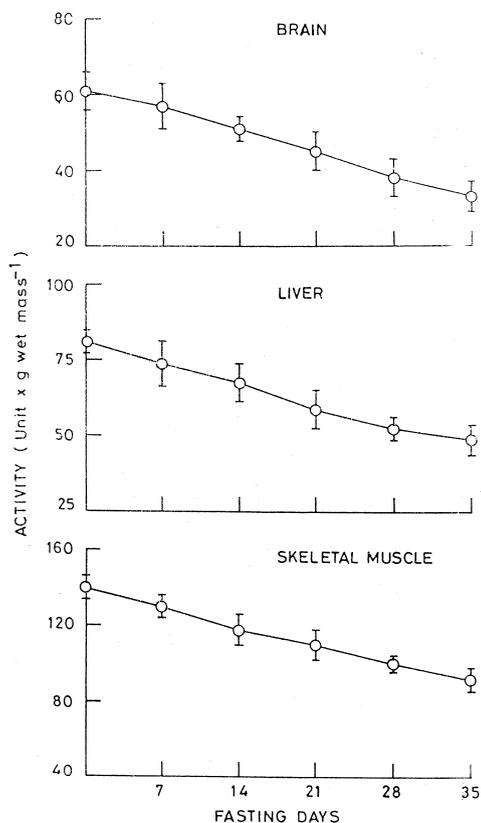


Fig. 3. Effect of starvation on the activity of LDH in the brain, liver and skeletal muscle of the freshwater catfish, *Clarias batrachus*.

catfish, *C. batrachus*. This shows starvation-induced substantial decline in aerobic and biosynthetic capacity of the fish. Similarly, Lowery and Somero (1990) have demonstrated changes in several glycolytic enzymes in the skeletal muscle of barred sand bass (*Paralabrax nebulifer*). They suggested that the starvation in the fish is accompanied by complex changes in the synthesis rates of different classes of proteins in tissues. Starvation-induced enzymatic changes have also been observed in tissues of other species of fishes (Woo and Cheung, 1980; Sullivan and Somero, 1983; Kiran and Talesara, 1985; Black and Love, 1986; Borah and Yadav, 1996; Sounderapandian *et al.*, 1997). The relative activities of white muscle glycolytic enzymes remain conserved despite a significant loss in an overall glycolytic capacity (Lowery and Somero, 1990). The starvation-induced decreases in RNA, RNA/DNA ratio and protein (29–60%) in brain, liver and skeletal muscle of the freshwater catfish indicates substantial reduction in synthesis of these macromolecular constituents.

The decrease in rate of protein synthesis could result from reduced protein synthesis capacity brought about by reduction in the concentration of ribosomes. Since ribosomes compose the bulk of cellular RNA, changes seen in the total concentration of RNA primarily reflect changes in ribosomal RNA concentration. In other words, a decrease in the ribosomal activity brought about

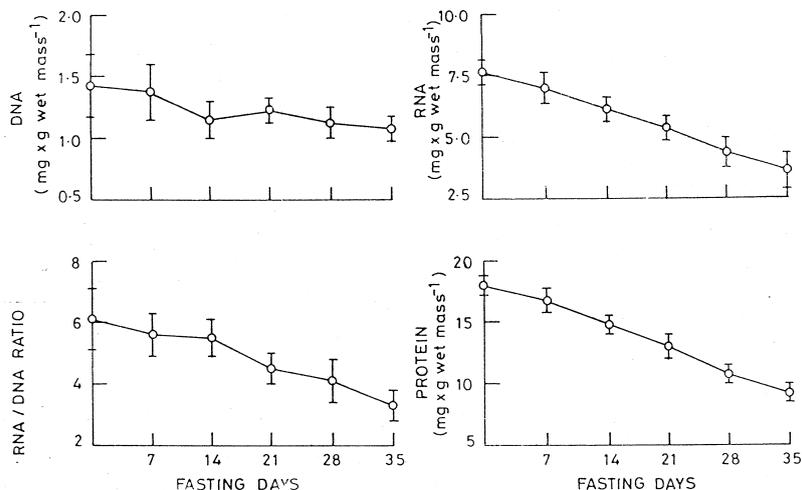


Fig. 4. Effect of starvation on DNA, RNA, RNA/DNA ratio and protein contents in the brain of the freshwater catfish, *Clarias batrachus*.

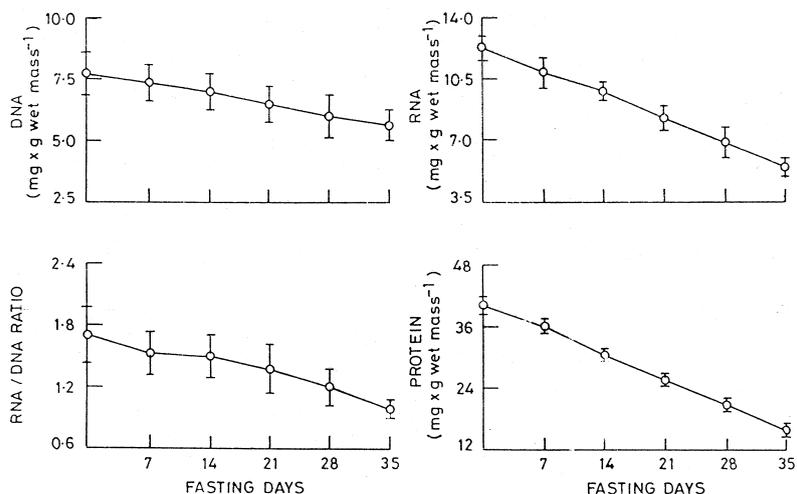


Fig. 5. Effect of starvation on DNA, RNA, RNA/DNA ratio and protein contents in the liver of the freshwater catfish, *Clarias batrachus*.

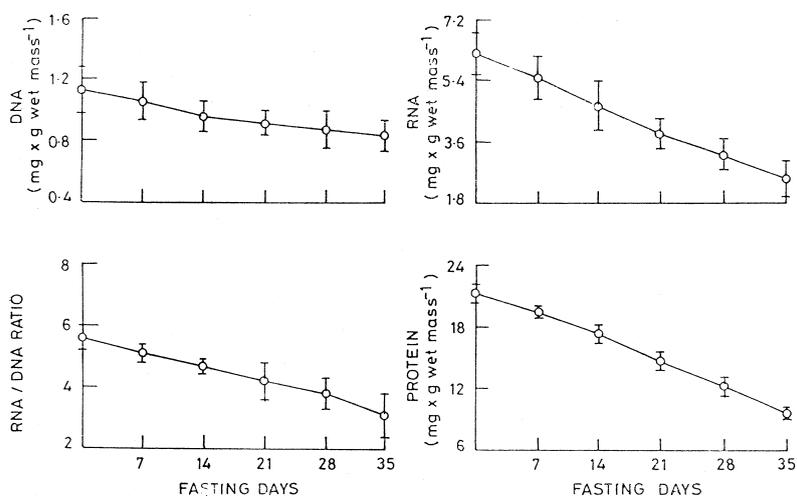


Fig. 6. effect of starvation on DNA, RNA, RNA/DNA and protein contents in the skeletal muscle of the freshwater catfish, *Clarias batrachus*.

by reduced rates of translation of mRNA without a decrease in concentration of ribosome could result in reduced protein synthesis (Timoshina, 1970; Henshaw *et al.*, 1971; Leech *et al.*, 1979). In this study a decreased ratio of RNA concentration to DNA concentration was used as an indicator of changes in the overall capacity for protein synthesis *i.e.*, a change in the number of ribosomes per cell. The changes in rates of protein synthesis prior to any reduction in the RNA/DNA ratio could therefore indicate a change in the ribosomal activ-

ity. In the present experiment the starvation-induced decrease in RNA/DNA ratio is in agreement to the reports of Lowry and Somero (1990) and Pickering and Pottinger (1995).

The starvation-induced changes in CS, G6-PDH, LDH, DNA, RNA, RNA/DNA ratio and protein in brain, liver and skeletal muscle tissues reflect starvation associated decline in metabolic enzymes and protein in the freshwater catfish. It may lead to reduction in capability of various activities including sprinting away from predators or striking at prey.

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