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Survival Mechanisms of *Clarias batrachus*: Glycogen Utilization During Long-Term Starvation

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Abstract

This study aims to analyze the impact of extended food deprivation on glucose storage in different tissues and organs of the freshwater air-breathing catfish *Clarias batrachus*. The glycogen reserves in the brain, gonads, liver, muscles, and blood of both the male and female *Clarias batrachus* were estimated after forty days of starvation. The total glycogen was determined by a modification of the colorimetric method of Krishnaswami & Srinivasan in collaboration with Kemp and Heijningen. Even though they had to endure the severe deprivation of food, *Clarias batrachus* survived during the entire period of experimentation. Nutrient deprivation due to fasting gradually depletes glycogen reserves to a minimal level in all organs. This is partly caused by increased transamination and deamination processes, partly by the inhibition of RNA synthesis, and perhaps becomes increasingly significant during long-term starvation through gluconeogenesis. Importantly, during the first 20 days of starvation, the concentration of glycogen in the brain did not change noticeably in contrast to the liver, muscles, and gonads, which decreased glycogen stores significantly. The fall in blood glucose levels followed a decline in liver and muscle glycogen stores. Glycogen concentration in the liver was higher than in other solid tissues such as muscle, brain, and gonads. Females were observed to have higher glucose stores in all tissues than males when expressed per unit body mass in normal and starvation conditions. After forty days of starvation, the most substantial decrease in glycogen content was observed in the testes, while the brain exhibited the minimum decrease.

Keywords: Starvation, Glucose Reserves, Glycogen Mobilization, *Clarias batrachus*

INTRODUCTION

The spirit of life and the essence of life have never been depleted, even though malnutrition has been a consistent hazard to living creatures since the inception of life on Earth. The normal metabolic processes of the body are interrupted by prolonged starvation, which may lead to the animal's mortality.

Organisms must protect themselves from food deprivation by depleting their bodily reserves until death occurs. In order to obtain energy, an animal will become entirely dependent on the biochemical components of its tissues when it is famished (Wright, 1976). This leads to the utilization and disintegration of numerous cell components. Lipid reserves are liberated after carbohydrate consumption. Carbohydrates are the initial component to undergo digestion.

The levels of a variety of body constituents in fish were reduced due to the process of experimental starvation, as found by several researchers. This investigation aims to examine the results obtained for the freshwater catfish *Clarias batrachus* following forty days of starvation.

Numerous workers have been employed to gain a comprehension of the facts and causes of famine, as well as the subsequent effects on animals. However, the majority of these workers have concentrated on mammalian fauna. As a result, it is crucial to monitor and disclose the effects of malnutrition on fish, as they have a unique trait that enables them to endure prolonged periods of famine by enduring biochemical and physiological changes (Mustafa, 1983; Prasad, 2014b, 2015a & 2015b). The physiological status and the biochemical elements of fish are both influenced by fasting, as determined by Tripathi & Verma (2003) and Prasad et al. (2024b & 2024c).

The present study was designed to estimate glucose levels in various components of *Clarias batrachus*, a freshwater catfish, during a protracted period of starvation in light of the aforementioned factors. It is a bony fish that breathes water and is prevalent in the region. It is highly valuable in terms of both nutrition and recovery.

MATERIALS AND METHODS

For this investigation, the fish *Clarias batrachus* (Linn.), known as "Mangur" locally, were selected. With the assistance of local fishermen, these were harvested live from a fish pond. The fish were subsequently transported to the lab in sizable clay pots coated with nets and were identified following the guidelines of Srivastava (2006). In order to eradicate any cutaneous infection, they were washed with 0.1% $KMnO_4$ solution. Using a small hand net, healthy fish with average lengths of 18.8 cm and weights of 34.4 grams were gradually moved to 110-litre glass aquariums for acclimatization.

The impact of malnutrition was examined for a maximum of 40 days, as prior research indicated that their moral character remained elevated beyond this time frame. Glycogen levels in the liver, muscles, brain, blood, and gonads were quantitatively evaluated after 0,10, 20, 30, and 40 days of fasting to assess the consequences of starvation.

For biochemical evaluation, a representative sample was taken from both male and female well-nourished and acclimated fish. The obtained values were regarded as the normal values for *Clarias batrachus*. Then, the remaining fish were divided into four groups, labelled A, B, C, and D. The fish from group 'A' were subjected to ten days of food deprivation at room temperature, whereas those from groups 'B', 'C', and 'D' experienced periods of twenty, thirty, and forty days without food, respectively.

The fish were dissected according to a predetermined schedule. Their muscles, liver, gonads, and brain were promptly removed. These were kept in a pre-prepared fish saline solution chilled to a very low temperature.

The brain, muscles, gonad, liver, and other related components were extracted and thoroughly washed before being immersed in saline solution. The muscles were dissected from the lateral side using a knife and scissors, and the nerves were purified. Before usage, the tissues were meticulously cleaned using filter sheets. A plastic syringe was employed to draw blood via the cauda dorsalis. EDTA was used as the anticoagulant. The researchers adopted the calorimetric approach developed by Kemp and Heijningen in 1954, which was later updated by Krishnaswami and Srinivasan in 1961, to determine the total glycogen concentration.

RESULTS

Solid tissue glucose stores were quickly released. Up to 20 days of hunger, there was no discernible loss of brain glycogen. However, there was a dramatic decline in the glycogen stores in the liver, muscles, and gonads. When both normal and hungry, the female's glycogen content was greater than that of males. Of all the solid tissues, the liver has the largest concentration of glycogen. Similar to the liver's and muscles' glycogen stores, the blood glucose level steadily dropped.

Brain.......... 51% in male & 50% in female;

Gonad......... 84% in males & 79% in females;

Liver.......... 74% in males & 73% in females;

Muscle........ 82% in males & 81% in females;

Blood.......... 63% in males & 69% in females.

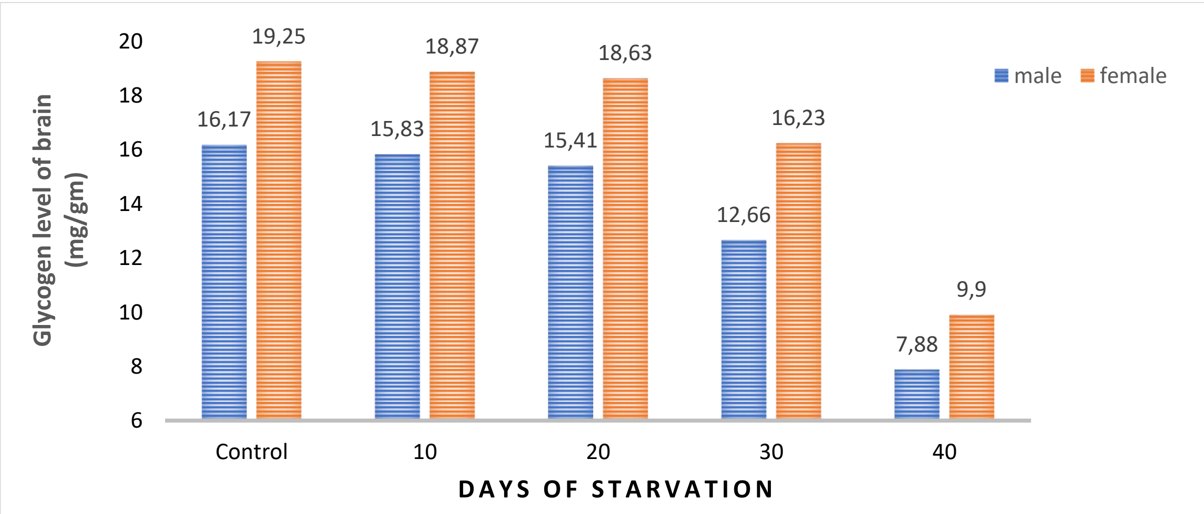

Fig. 1. Deployment of glycogen in the brain during food deprivation

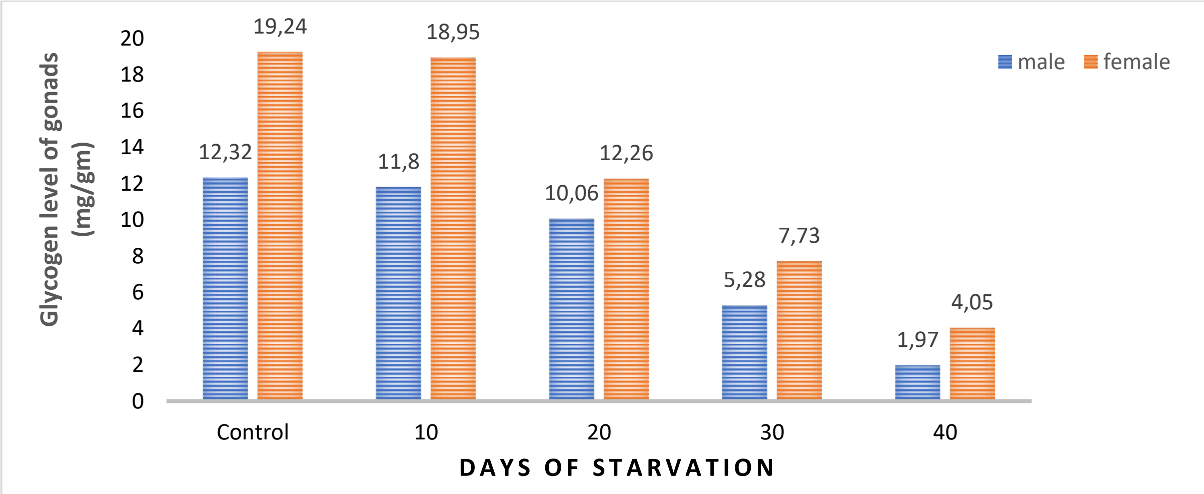

Fig. 2. Deployment of glycogen in the gonads during food deprivation

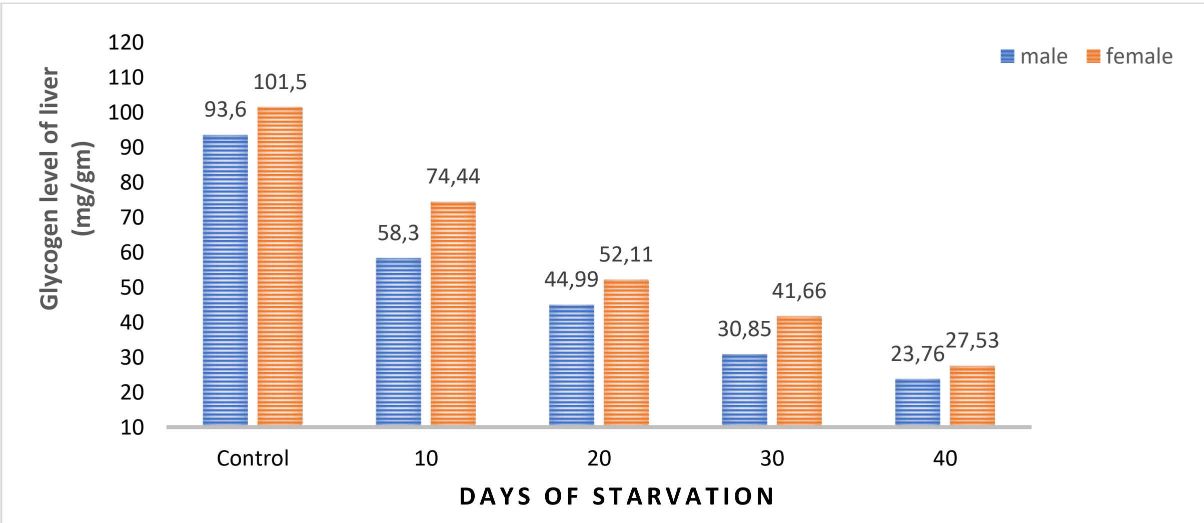

Fig. 3. Deployment of glycogen in the liver during food deprivation

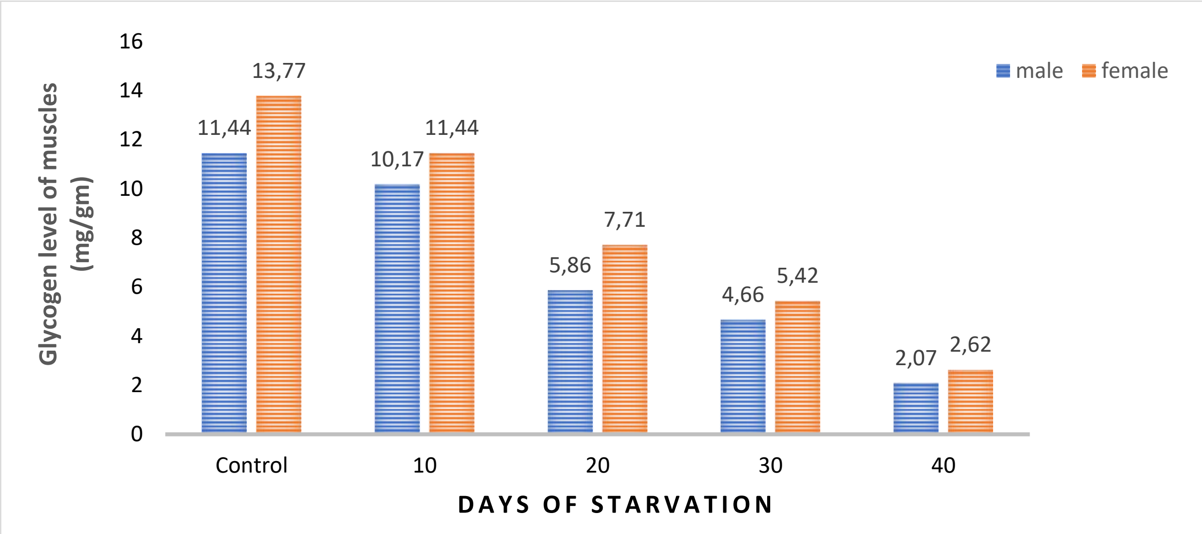

Fig. 4. Deployment of glycogen in muscles during food deprivation

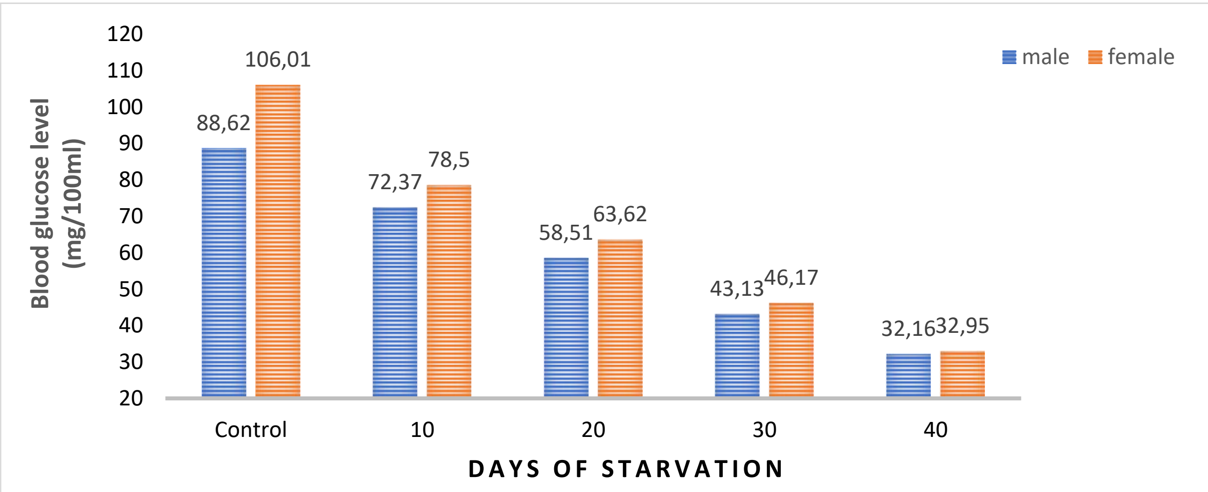

Fig. 5. Deployment of blood glucose during food deprivation

DISCUSSION

All living organisms need a continuous energy supply to maintain various biological activities. The cell can't stay alive without energy. Therefore, it needs a way to take in energy and a bunch of transducers to convert that energy into work. While these processes are always at work in all living things, they are expected to undergo many additional modifications in response to stress, such as exposure to harmful substances, extreme temperature changes, anxiety, or hunger (Davison & Dobbing, 1961).

These mechanisms operate continuously in all species. Nevertheless, several further changes are expected to occur in times of stress, such as exposure to toxins, fluctuations in temperature, feelings of dread, and limited food availability (Davison & Dobbing, 1961). Fishes benefit greatly from their environment, enabling them to perform their functions without excessive dependence on their physiological resources. Consequently, their basal metabolic rate is modest. Fish have the extraordinary capacity to withstand prolonged periods of starvation. Amia calva showed a remarkable ability to survive for 20 months without consuming any food, as documented by Smallwood in 1916. Similarly, *Clupea harengus* persisted for a duration of 129 days without food, as reported by Boetius in 1967. *Anguilla anguilla*, on the other hand, managed to sustain itself for 15 days without consuming any food, also observed by Boetius in 1967.

An extensive analysis has been conducted on the effects of both prolonged and brief periods of famine, with a primary focus on fish species that thrive in moderate climates. Fontaine & Hatey (1953) observed a 54% reduction in the liver glycogen levels of *Salmo salar* following bouts of starvation during their spawning and migration. Researchers Inui and Oshima (1966) found that when *Anguilla japonica* fish were fasting, their muscle glycogen depletion rate was lower than their liver glycogen depletion rate.

During food shortages, carbohydrate metabolism is crucial since carbohydrates are the primary fuel for producing energy. In order to release energy, glucose molecules undergo oxidation indefinitely. During the period of hunger, glycogen is broken down to produce glucose, which is then transported to the organs that require it through the circulatory system.

Studies conducted by Cahill (1970) and Chaudhary & Mandal (1981) have shown that during times of hunger, there is an increased level of glucagon in the body. This raised concentration of glucagon stimulates the conversion of glycogen into glucose. Cori (1931) and Felig (1973) reported that glucose is generated from glycogen reserves in the liver as well as from other tissues, including gonads, muscles, and brain, through the alanine glucose cycle and Cori-cycle.

Researchers have discovered a decrease in the amount of carbohydrates in numerous tissues of different animals that were fasted. For example, in 1953, Fontaine and Hatey noted this occurrence in the hepatic tissues of *Salmo salar*. Similar findings were also reported by Chaudhary & Mandal (1981) in the brain tissues of *Schizodactylus monstrosus*.

The present investigation unveiled a significant reduction in glycogen concentrations in both the hepatic and muscular tissues. The glycogen contents in the brain and the gonads did not show a significant reduction up to 20 days of starvation, as seen in Figures 2 and 4. After 40 days of fasting, the liver glycogen levels declined by more than 70%, while the muscle glycogen levels decreased by more than 80% (Fig. 2 & Fig. 4).

As the glycogen reserves in the muscles and liver dropped to less than 50% of their usual levels, the glycogen stores in the gonads started to decline rapidly. Following a period of 40 days without meals, the gonad exhibited a degree of glycogen depletion that closely resembled the depletion reported in the muscle. The brain had the smallest decrease in glycogen levels in comparison to the gonads, muscles, and liver. Similar to the breakdown of glycogen in the liver and muscles, the level of glucose in blood also decreased gradually (Fig. 5). The present outcomes are consistent with the research conducted by Freedland (1967), Chaudhary & Mandal (1981), and Freminet & Lilliane (1981). In both normal and deprived situations, females had elevated amounts of glycogen and glucose in comparison to males. This finding of sexual dimorphism is consistent with the studies undertaken by Singh (1981), Singhal et al. (1981), Prasad (2015, 2016, 2018, 2019), and Prasad et al. (2022, 2023, 2024a).

CONCLUSION

This investigation provides a detailed comparative analysis of tissue glucose depletion in starved male and female *Clarias batrachus* over forty days. The research highlights several key findings regarding the metabolic adaptations and sex-specific responses to prolonged food deprivation in this species.

The data indicate a significant depletion of glycogen reserves across various tissues, with notable differences in the rate and extent of depletion among the liver, muscles, gonads, and brain. The liver and muscles showed the most substantial decreases in glycogen content, with reductions exceeding 70% and 80%, respectively, after 40 days of starvation. The gonads also experienced marked glycogen depletion, particularly in the testes, which displayed the most significant reduction among all tissues. Conversely, the brain exhibited the slightest decrease in glycogen levels, maintaining relative stability until twenty days of starvation.

The study also underscores the sex-specific variations in glucose metabolism during starvation. Female *Clarias batrachus* consistently demonstrated higher glucose and glycogen reserves in all examined tissues compared to males, both under normal and starving conditions. This gender difference is consistent with prior research and suggests that females may possess a greater capacity to endure prolonged periods of food scarcity, possibly due to differences in hormonal regulation and energy storage mechanisms.

Overall, the findings contribute to our understanding of the physiological and biochemical responses of *Clarias batrachus* to starvation. They emphasize the critical role of carbohydrate metabolism in sustaining energy production during food deprivation and highlight the adaptive strategies employed by fish to survive extended periods without food. Future research should further explore the underlying mechanisms driving these sex-specific differences and investigate the long-term impacts of starvation on reproductive fitness and overall health in *Clarias batrachus*.

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