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Sex-Specific Responses to Prolonged Fasting in Clarias Batrachus: a Glycogen Study

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Abstract: Glucose is stored as glycogen. It is a polysaccharide molecule with many branches that acts as an animal's primary energy store. This study examines the alterations in glycogen levels in the brain tissues of Clarias batrachus fish that were exposed to a lengthy period of fasting lasting 40 days. The glycogen level was determined using the calorimetric method devised by Kemp et al. (1954), which was subsequently revised by Krishnaswamy and Srinivasan (1961). Initial findings indicated that there was no significant decrease in brain glycogen levels in males or females after a period of up to 20 days of starvation. However, there was a noticeable decrease afterwards, with males showing a more significant decline compared to females. Following a period of 40 days without food, the levels of glycogen in the brain tissues were reduced by around 51% in males and 50% in females compared to their initial values. Although experiencing a decrease in resources, Clarias batrachus exhibited exceptional resilience to the effects of fasting, managing to survive for the whole duration of the trial. This study offers valuable information about the physiological and biochemical reactions of fish to famine. It specifically focuses on the differences between males and females in how their bodies break down glycogen after extended periods of fasting.

Keywords: Biochemical Estimation, Brain, Colorimetric Method, Clarias batrachus, Glycogen, Physiological Adaptation, Prolonged Fasting.

Introduction

Starvation is a common challenge many fish species face annually due to environmental fluctuations, affecting their normal metabolic processes and potentially leading to mortality. During periods of food scarcity, organisms rely on their body reserves, which deplete progressively until death ensues. Numerous studies have documented the decrease in various constituents of the body under experimental starvation in fish. This study specifically examines the freshwater catfish, *Clarias batrachus*, subjected to a 40-day starvation period.

The primary objective of this research is to evaluate the effects of extended starvation on the amounts of glycogen that are present in the brain tissues of *Clarias batrachus*. While similar studies have predominantly focused on mammalian fauna, there is a notable paucity of research on starvation-induced effects in fish, particularly in the context of Nepal. The physiological status and biochemical composition of fish are significantly affected by starvation, as documented by Tripathi and Verma (2003), and Prasad (2024). Fish possess unique physiological and biochemical adaptations that enable them to withstand extended periods of starvation, as noted by Mustafa (1983).

This study aims to elucidate the levels and variations of brain glycogen content in *Clarias batrachus* over a prolonged starvation period of 40 days, with measurements taken at 10-day intervals. By providing a detailed analysis of the glycogen depletion patterns, this research contributes to a deeper understanding of the biochemical and physiological adaptations of fish to starvation stress.

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Materials and Methods

- **Sample Collection and Preparation**: Healthy live specimens of *Clarias batrachus*, averaging 18.8 cm in length and 34.4 g in weight, were collected from a local fish pond using fishermen's nets. Large earthen pots and mosquito nets were used to transport the fish that had been captured to the laboratory. This was done to ensure that the fish arrived in a secure manner.

Upon arrival, the fish were identified based on Srivastava's "Fishes of UP and Bihar" (2006). To eliminate any dermal infections, the fish were treated with a 0.1% $KMnO_4$ solution for five minutes.

The healthy fish were then transferred individually to a large glass aquarium with a capacity of 110 litres, measuring about 30 inches x 12 inches x18 inches. The fish were acclimated in controlled laboratory circumstances for a period of 20 days. During this, they were fed with a commercial fish meal twice a day. Feeding was stopped 24 hours before the experiment to clean the gut.

- **Experimental Design**: The research was carried out between May 2009 and June 2013. The fish were split into four experimental groups (A, B, C, and D) after they had time to acclimate to their surroundings. Each batch consisted of ten fish (5 males and 5 females). A control group continued to be fed and was kept submerged in tap water. The experimental batches, group A, group B, group C, and group D, were starved for varying periods as 10, 20, 30, and 40 days.
- **Biochemical Estimation of Glycogen**: The glycogen content in the brain tissues was evaluated using the calorimetric technique developed by Kemp *et al.* (1954) and modified by Krishnaswamy and Srinivasan (1961). At intervals of 10 days (0, 10, 20, 30, and 40 days), fish from each group were euthanized, and their brains were promptly removed and kept in fish saline that had been chilled on ice. Before the examination, the brain tissues were thoroughly cleansed to eliminate any adhering tissues and subsequently dried by blotting with filter paper.
- **Homogenization and Extraction**: Using a tissue homogenizer, a predetermined volume of **brain** tissue was mixed with 5 millilitres of refrigerated 10% trichloroacetic acid (TCA). For twenty minutes, the mixture was centrifuged with a force 500 times the acceleration caused by gravity. The sediment was mixed again in an additional 5 ml of trichloroacetic acid (TCA) and subjected to centrifugation once more. The liquids obtained from both centrifugations were combined.
- **Colorimetric Analysis**: In order to measure the amount of glycogen, 2 ml of the supernatant liquid that settled at the top was combined with 6 ml of highly concentrated sulfuric acid. The solution was subjected to incubation in a water bath at boiling temperature for a duration of 6.5 minutes in order to facilitate the development of the colour. The pink colour's optical density was measured using a photoelectric colourimeter at a wavelength of 515 nm. The glycogen content in the samples was determined by calculating the quantity of glycogen (in milligrams per gram of wet weight) using a standard curve that was created using glucose. This ensured that the relationship between the amount of glycogen and the amount of glucose was linear.
- **Statistical Analysis:** The analysis of variance test (ANOVA) was used to assess the relevance of glycogen depletion over time. Bar notations were used to depict the variations in glycogen levels at different time intervals (10 days, 20 days, 30 days, and 40 days). The disparity of glycogen levels between 30 and 40 days of fasting exhibited a remarkably substantial significance at the 1% probability level ($P < 0.01$).

Results

The present study investigated the influence of prolonged hunger on glycogen contents in the brain tissues of *Clarias batrachus* over a 40-day period. The results revealed sex-specific differences in the amount of glycogen under both the normal and starving circumstances.

- **Glycogen Content Under Normal Conditions:** Under normal feeding conditions, female *Clarias batrachus* exhibited higher glycogen levels in brain tissues compared to males.
- **Glycogen Content During Starvation:** Neither the male nor the female *Clarias batrachus* showed any apparent reduction in glycogen levels in brain tissues after 20 days of deprivation.

Nevertheless, there was a noticeable decrease in glycogen levels after 20 days. Glycogen levels in brain tissues dropped about 50% in females and 51% in males after 40 days of fasting compared to normal values.

- **Statistical Analysis:** Glycogen levels significantly decreased between each subsequent period, according to the analysis of variance (ANOVA) (0 & 10 days, 0 & 20 days, 0 & 30 days, 0 & 40 days). At the 1% probability level, the glycogen depletion that occurred between 30 and 40 days of fasting was very significant ($P < 0.01$).

Table No.- 1 Glycogen content (mg/gm wet weight) in the brain of *Clarias batrachus*

Sex	Control	Period of Starvation			
		10 days	20 days	30 days	40 days
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

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

** Significant

P 0.01 between 0 & 10, 0 & 20, 0 & 30, 0 & 40 and among 10, 20, 30, 40.

Analysis of variance test and bar notation $Co_{0.01} = 1.63$

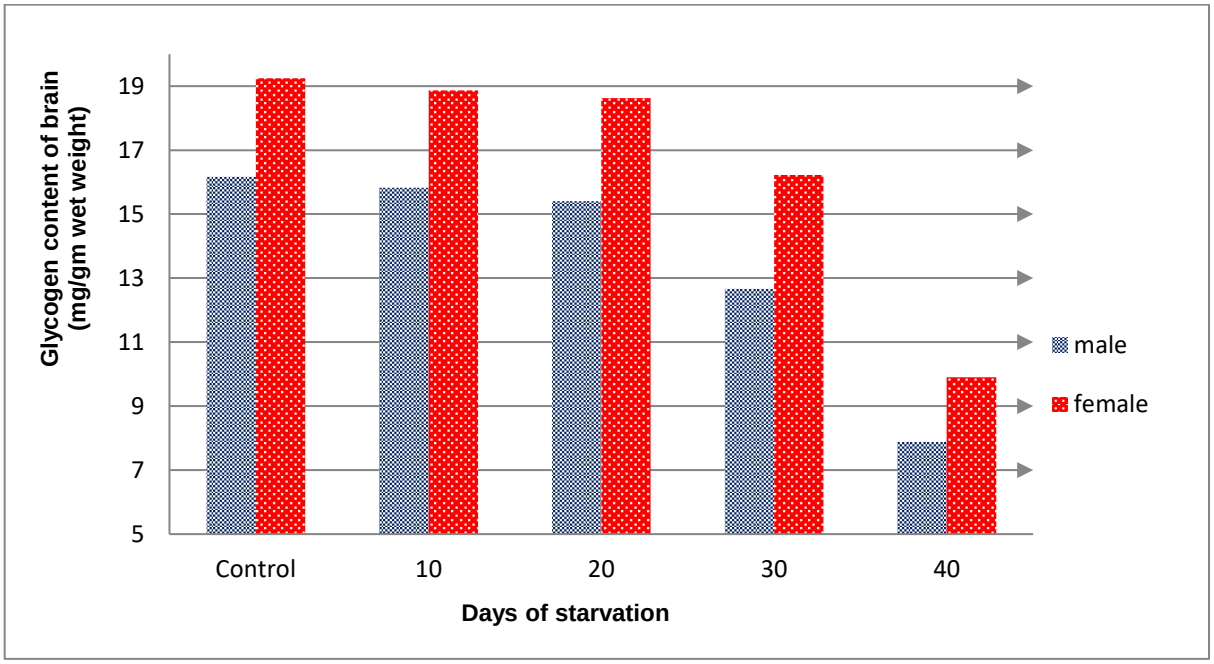

Fig. 1. Deployment of glycogen content in the brain of male and female *C. batrachus*.

Discussion

Fish benefit greatly from their aquatic habitat, allowing them to carry out their daily activities with limited dependence on internal energy stores, resulting in low basal energy expenditure. As a result, fish are capable of enduring exceptionally lengthy durations without food. Amia calva, for example, has been seen to endure a period of 20 months without consuming any food (Smallwood, 1916).

Carbohydrate metabolism is essential during times of food shortage since carbs act as the main source of energy. The process of constantly oxidizing glucose molecules is used to produce energy. During periods of hunger, glycogen undergoes hydrolysis to liberate glucose, which is subsequently delivered through the bloodstream to organs requiring energy. During periods of famine, the increased levels of glucagon in the body promote the process of gluconeogenesis, which involves the conversion of glycogen stores into glucose. This phenomenon has been seen in studies conducted by Cahill in 1970;

Chaudhary & Mandal in 1981; and Prasad in 2024. Glucose synthesis takes place primarily from liver glycogen reserves and secondarily from other tissues such as muscles, gonads, and brain through metabolic processes such as the alanine-glucose cycle and the Cori cycle (Cori, 1931; Felig *et al.*, 1969; and Mauro, 1970).

Extensive research has been conducted on the effects of both long-term and short-term starvation, with a primary focus on temperate fish species. Fontaine and Hatey (1953) reported a significant decrease of 54% in the amount of glycogen stored in the liver of *Salmo salar* during the period of hunger that coincided with their migration for spawning. Inui & Oshima (1966) reported that glycogen depletion in muscle occurred at a slower pace as compared to the liver in fasting *Anguilla japonica*.

Multiple researchers have observed reductions in the amount of carbohydrates in animals that are experiencing starvation, occurring in various tissues and species. For instance, Fontaine & Hatey (1953) observed this phenomenon in the liver of *Salmo salar*. Prasad (1980), and Chaudhary & Mandal (1981) made similar observations in the brain of *Schizodactylus monstrosus*. Haranath *et al.* (1983) in *Tilapia mossambica*, and Prasad (2024) reported similar findings in the liver of *Clarias batrachus*.

The current study found that the brain glycogen levels of *Clarias batrachus* did not exhibit a notable decline for up to 20 days of hunger. Nevertheless, subsequent to this time frame, a significant decrease in glycogen levels was noted. The results align with the research conducted by Freminet & Lilliane (1981); Juniper *et al.* (2006); and Prasad *et al.* (2022).

Furthermore, females consistently exhibited larger quantities of glycogen and glucose as compared to males, regardless of whether they were in normal or deprived settings. This observed sexual disparity corresponds with the findings published by Shreni (1979) and Singhal *et al.* (1981). The findings of this research indicate that females may possess a higher ability to mobilize and utilize glycogen throughout extended periods of food restriction, which could be seen as an advantageous adaptation for sustaining hunger.

Conclusion

This study provides valuable insights into the physiological and biochemical adaptations of *Clarias batrachus* under prolonged starvation conditions. The findings indicate that while both male and female fish experience significant glycogen depletion in brain tissues during extended periods of food deprivation, males show a slightly higher depletion rate than females.

The ability of *Clarias batrachus* to survive a 40-day starvation period suggests that this species has effective adaptive mechanisms to cope with prolonged food scarcity. These adaptations likely involve mobilizing energy reserves and altering metabolic processes to maintain essential physiological functions.

Overall, this research contributes to a deeper understanding of how prolonged starvation affects fish, with implications for fisheries management, aquaculture practices, and conservation efforts. Further studies are recommended to explore the underlying mechanisms of glycogen metabolism and the broader physiological impacts of starvation in different fish species.

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References

1. Cahill, G. F. Jr. (1970). Starvation in man. *New England Journal of Medicine*.
2. Chaudhary, D. K., & Mandal, S. (1981). Fluctuations of glycogen content in brain during post-embryonic development in *Schizodactylus monstrousus*: Effect of starvation stress and corpora allatectomy. *Indian Journal of Experimental Biology*, 19, 532-534.
3. Cori, C. F. (1931). Mammalian carbohydrate metabolism. *Physiological Reviews*, 11, 143-175.
4. Felig, P., Owen, O. E., Wahren, J., & Cahill, G. F. Jr. (1969). Amino acid metabolism during prolonged starvation. *Journal of Clinical Investigation*, 48, 584.
5. Fontaine, M., & Hatey, J. (1953). Contribution to the study of glucose metabolism in the Salmon (*Salmo salar*) at different stages of its development and of its migration. *Physiologia Comparata et Oecologia*, 3, 37-52.
6. Freminet, A., & Lilliane, L. C. (1981). Effects of fasting on liver and muscle glycogen in rats and pigs. *Journal of Physiology (Paris)*, 76(8), 877-880.
7. Haranath, V. B., Bhaskar, M., Rao, M. V. C., Rani, P. S., Reddanna, P., & Govindappa, G. (1983). Effect of long-term starvation on branchial tissue glycolysis of freshwater fish, *Tilapia mossambica* (Peters). *Indian Journal of Fisheries*, 30(2), 340-343.
8. Inui, Y., & Oshima, Y. (1966). Effect of starvation on metabolism and chemical composition of soles. *Bulletin of the Japanese Society of Scientific Fisheries*, 32, 492-501.
9. Juniper, D. T., Phipps, R. H., Jones, A. K., & Bertin, G. (2006). Effect of starvation and refeeding on glycogen content in tissues of Atlantic salmon (*Salmo salar*). *Aquaculture*, *254*(1-4), 713-720.
10. Kemp, A., & Heijningen, V. K. (1954). A colorimetric micromethod for the determination of glycogen in tissues. *Biochemical Journal*, 56, 646-646.
11. Krishnaswamy, S., & Srinivasan, V. V. (1961). A simple method of determination of glycogen content of marine animals. *Current Science*, 30, 353-353.
12. Mauro, F. (1970). The biochemistry of starvation. *The American Journal of Clinical Nutrition*, *23*(8), 1059-1068.
13. Mustafa, S. (1983). Changes in biochemical composition in starving catfish, *Heteropneustes fossilis*. *Japanese Journal of Ichthyology*, 29, 4.
14. Prasad, B. K. (1980). Effect of starvation on the carbohydrate content of tissue of some teleosts (Ph. D. Thesis). Patna University, Patna, India.
15. Prasad, N.K. (2014). Effects of prolonged starvation on glycogen content of muscles in *Clarias batrachus*. *Himalayan Biodiversity*, Pokhara, 2(1), 52-55.
16. Prasad, N.K. (2024). Gender disparities in hepatic glycogen depletion during prolonged starvation in *Clarias batrachus*. *African Journal of Biological Sciences*, 6(5), 75-79.
17. Prasad, N.K., Shachi, K., & Sahani, S.K. (2022). Nutritional stress and ascorbic acid dynamics: Gender perspectives in *Clarias batrachus*. *Journal of Advanced Zoology*, 43(1), 914-918. https://doi.org/10.53555/jaz.v43i1.4728
18. Shreni, D. K. (1979). Influence of starvation on the brain and liver cholesterol levels of catfish, *Heteropneustes fossilis* (Bloch). *Proceedings of the Animal Sciences*, 88(3), 205-208.
19. Singhal, P. C., Gupta, R. K., & Joshi, L. D. (1981). Effect of sex on blood sugar levels in experimental animals. *Indian Journal of Experimental Biology*, 19, 1201-1202.
20. Smallwood, W. M. (1916). Twenty months of starvation in Amia calva. *Biological Bulletin of the Marine Biological Laboratory*, 31, 453-464.
21. Srivastava, G. J. (2006). *Fishes of U.P. and Bihar* (11th ed.). Vishwavidyalaya Prakashan.
22. Tripathi, G., & Verma, P. (2003). *Starvation-induced impairment of metabolism in a freshwater catfish*. *Zeitschrift für Naturforschung C*, 58, 446-451.