Biochemical Impact Of Prolonged Starvation On *Clarias Batrachus*: A Comparative Study Of Liver, Ovary, And Testis

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Abstract:
Starvation poses a significant challenge to organisms, impacting their physiological and biochemical functions. This study investigates the effects of prolonged starvation on the level of protein content in the liver and gonads of *Clarias batrachus*, a fish species known for its ability to adapt to extended periods without food. Over a 40-day starvation period, we measured protein levels at 10-day intervals to understand the biochemical responses to nutrient deprivation. Our results reveal a gradual decrease in protein content across all examined tissues, with the liver showing the highest depletion, followed by the ovaries and the testes. The observed protein reduction is likely due to enhanced gluconeogenesis, inhibited RNA synthesis, and increased rates of deamination and transamination. These findings highlight the differential tissue responses to starvation and provide insights into the adaptive mechanisms employed by *Clarias batrachus* to survive in nutrient-scarce environments.

Keywords: *Clarias*, liver, ovary, protein, starvation, testis

Introduction:

Malnutrition, semi-starvation, and starvation remain some of the most pressing global challenges today, disproportionately affecting underdeveloped and developing nations. According to a recent survey made by the FAO (Food and Agricultural Organization), the food situation in the 21st century is predicted to be bleak, with numerous populations facing ongoing food insecurity. The condition of starvation is not a new phenomenon; since the origin of life on earth, living organisms have continuously faced the threat of food scarcity. Despite this, the drive to survive persists, with organisms often relying on their body reserves to endure prolonged periods without food.

While much research has focused on the effects of starvation in mammals, there is a relative scarcity of studies examining how fish cope with this stressor. Fish, unlike many other organisms, exhibit unique physiological and biochemical adaptations that allow them to withstand extended periods of starvation. These adaptations are particularly evident in species such as *Clarias batrachus*, a fish known for its remarkable resilience to food scarcity (Mustafa, 1983; Prasad *et al.*, 2022).

Starvation in fish often occurs annually due to environmental conditions, affecting their biochemical composition and physiological status (Rajyasree & Naidu, 1989; Tripathi & Verma, 2003; and Prasad, 2014b, & 2015a). Understanding these adaptive responses is crucial, as it provides insights into the survival strategies employed by fish and helps in the management and conservation of aquatic species.

The present investigation aims to find the impact of prolonged starvation on the protein content of hepatic and gonadal tissues in *Clarias batrachus*. By monitoring protein levels over a 40-day starvation period, with measurements taken at 10-day intervals, this research seeks to elucidate the biochemical pathways involved in the adaptive response to starvation. This study addresses a significant gap in the literature by focusing on non-mammalian species, offering a broader perspective on the biological impacts of starvation.

Materials & Methods

For the present study, healthy *Clarias batrachus* fish, each measuring approximately 18.8 cm in length and weighing around 30.4 grams, were collected from a local pond. These fish were acclimatized to laboratory conditions for 20 days, during which they were fed daily to ensure they were in optimal health. After the acclimation period, the experimental phase began by placing 10 fish in each of four separate aquaria.

The experiment spanned 40 days, with fish being dissected at intervals of 10 days—specifically at 0, 10, 20, 30, and 40 days. At each time point, the fish were sacrificed, and their livers and gonads were immediately removed for analysis. The total protein content of these tissues was determined using the method described by Sutherland *et al.* (1949), employing the Folin-Ciocalteu Reagent for accurate measurement.

Table-1: *Protein content of liver, testis and ovary (mg/100 gm wet tissue) of Clarias batrachus during different stages of starvation*

Organs	**0 day**	**10 days**	**20 days**	**30 days**	**40 days**
Liver (male)	98.30 ± 2.0	97.51 ± 1.86	73.11 ± 2.57	62.70 ± 1.36	61.85** ± 2.01
Liver (female)	96.51 ± 1.76	94.05 ± 1.41	69.05 ± 1.63	61.85 ± 2.01	55.26** ± 1.26
Testis	69.34 ± 1.40	69.05 ± 1.41	61.85 ± 2.01	54.40 ± 0.63	52.11 ± 1.73
Ovary	77.70 ± 1.70	75.81 ± 2.22	62.00 ± 1.63	55.26 ± 1.27	53.21** ± 0.75

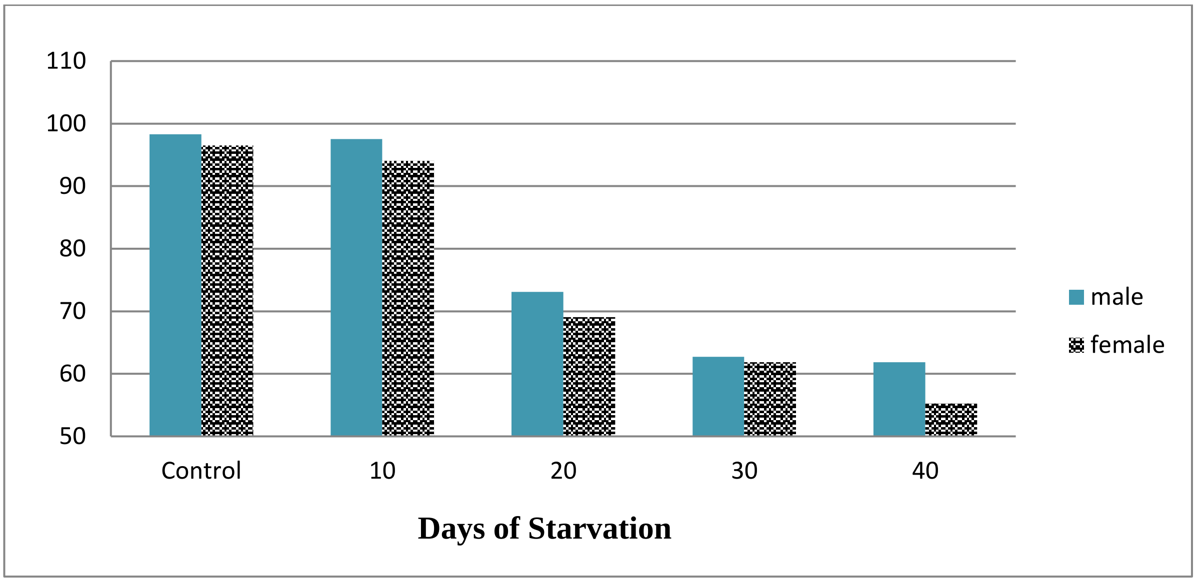

Fig. 1. Effect of Starvation on protein level of Liver in *Clarias batrachus*.

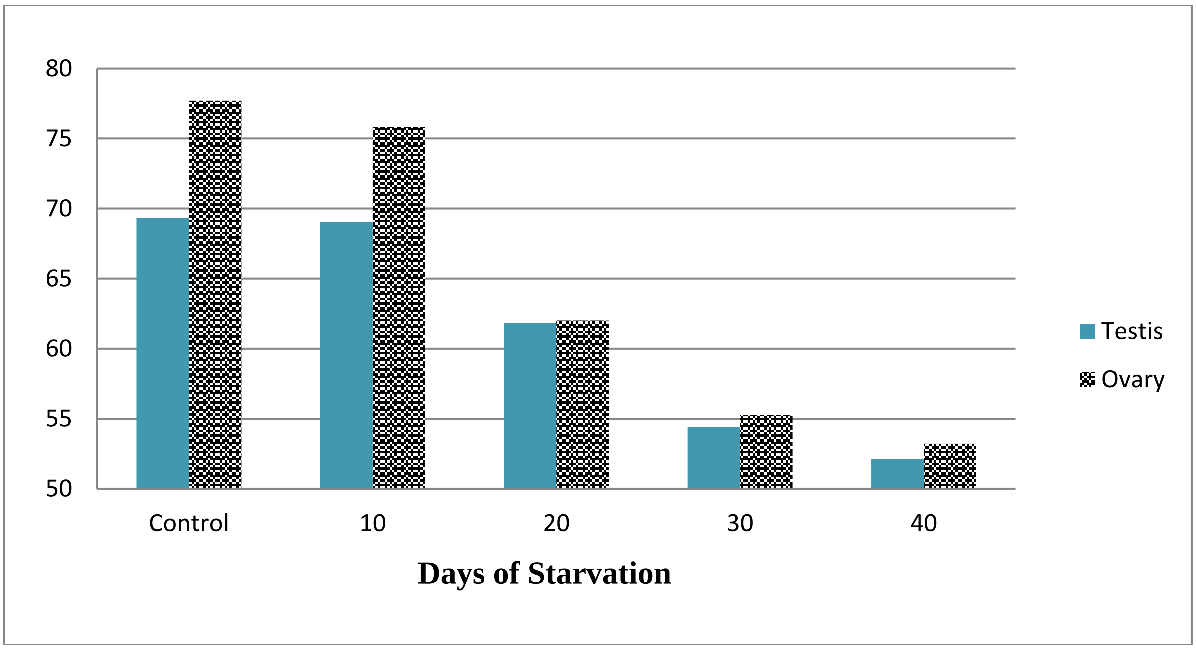

Fig.3 Effect of Starvation on protein level of Gonads in *Clarias batrachus*.

Observation:

In the present study, a gradual reduction in protein content was detected across all the examined tissues. The liver exhibited the most significant depletion, followed by the ovary, and the testis showing the least amount of depletion. Notably, after 10 days of starvation, the observed protein depletion was minimal, suggesting that protein is the last biochemical component to be exhausted during prolonged starvation.

Discussion

Proteins are essential components of protoplasm and are crucial in determining both the form and function of living organisms. The mobilization and loss of proteins during the periods of stress, such as starvation, vary significantly among different organisms and tissues. Previous research has shown that the effect of starvation on protein content is more pronounced in active fish compared to lethargic ones (Drilhon, 1954). Studies on various species have reported a considerable reduction in serum protein concentration following periods of starvation. For example, Cordier *et al.* (1957) found a significant decrease in serum protein of *Scyllium canicule* after 15 days of starvation, while similar trends were observed in various other cases (Prasad, 2014a, 2015b). Additionally, Thomas and McCrimmon (1964) noted changes in the electrophoretic patterns in *Petromyzon marinus* under starvation conditions, and Kosmina (1966) documented a decrease in blood serum albumin in starving *Lota lota* after an initial rise.

When examining solid tissues, it has been observed that fish, like mammals, deplete contractile proteins more rapidly during starvation as compared to connective tissue proteins (Love, 1970). Collagen, a major protein found in connective tissue, remains relatively stable and its quantity increases proportionally during periods of starvation due to its low mobilization (Fisher and Griminger, 1963). Research conducted on rats during a seven-day fasting period revealed that various tissues contributed to total protein loss in differing proportions. Muscles and skin accounted for the largest share at 62%, followed by the liver at 16%, and the alimentary canal, pancreas, and spleen collectively contributing 14%. The kidney accounted for 1% of protein loss, while the heart contributed 0.5%. Blood accounted for about 6% of the total protein loss, with the remaining organs collectively contributing 0.5% (Best & Taylor, 1961). The complexities of protein metabolism during starvation have been the focus of numerous studies, with researchers noting changes in both the quality and pattern of protein utilization (Prasad, 2019). Sobel *et al.* (1967) reported the mobilization of collagen from the skin in mice, while Robertson *et al.* (1963) observed epidermal atrophy in *Salmo gairdneri*.

In the present investigation, a gradual reduction in protein content was detected in the liver and gonads of *Clarias batrachus* during a 40-day starvation period. The liver exhibited the highest protein levels under normal conditions but also showed the most significant depletion after 40 days of starvation. This maximum depletion in hepatocytes, followed by the ovary and testis, aligns with the findings of earlier research indicating that liver tissue is more susceptible to protein loss during starvation. The minimum depletion observed during the first 10 days of starvation suggests that protein is the last biochemical constituent to be depleted, as initial energy demands are met through the utilization of carbohydrates and fats.

The observed slow proteolysis in gonadal tissues compared to the liver indicates that these tissues are more resistant to protein loss. In female gonadal tissue, a significant drop in protein content was noted after 20 days of famine, whereas the depletion was non-significant during the first 10 days. This pattern highlights the differential rates of protein mobilization across tissues.

The significant decline in liver protein content can be attributed to the obstruction of RNA synthesis during famine. Initially, the exhaustion of carbohydrate and fat reserves results in minimal protein loss, but as starvation progresses and these reserves are depleted, proteins become the primary energy source. In the later phase of starvation observed in this study, there was a substantial depletion of protein. This significant loss is likely attributed to impaired carbohydrate metabolism, which intensifies the processes of protein deamination and transamination to satisfy the energy requirements of the body (Prasad *et al.* 2022).

In conclusion, *Clarias batrachus* exhibits a distinct pattern of protein depletion during prolonged starvation, with liver tissue being the most affected, followed by the ovaries and testes. These findings deliver valuable insights into the biochemical and physiological adaptations of fish to starvation, emphasizing the critical role of liver metabolism and the relative resilience of gonadal tissues.

Conclusion

The present study elucidates the significant effect of prolonged food deprivation on the protein content of hepatic and gonadal tissues in *Clarias batrachus*. Our findings indicate a clear and progressive depletion of protein reserves in these tissues over 40 days, with the liver showing the highest degree of protein loss, followed by the ovaries, and the testis exhibiting the least depletion. This gradient of protein depletion underscores the differential utilization and conservation strategies employed by various tissues during periods of nutritional deficit.

The pronounced reduction in liver protein content suggests a critical role of hepatic tissue in maintaining metabolic homeostasis during starvation, likely due to its involvement in gluconeogenesis, transamination, and deamination processes. The relative preservation of protein content in the testis highlights potential adaptive mechanisms to protect reproductive functions, which could be crucial for the survival of the species under adverse conditions.

These observations provide valuable insights into the physiological and biochemical adaptations of fish to prolonged periods of starvation, emphasizing the liver's role in energy management and the gonads' resilience to nutritional stress. Future research should aim to explore the molecular mechanisms underlying these adaptations and investigate the long-term effects of starvation on fish health and reproductive success.

In conclusion, *Clarias batrachus* demonstrates a remarkable ability to endure extended starvation through strategic protein conservation and utilization across different tissues, offering a broader understanding of starvation biology in aquatic organisms.

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