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Biochemical Adaptations to Prolonged Starvation in Freshwater Catfish: Gonadal Glycogen Dynamics

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A B S T R A C T

Starvation significantly impacts fish species, affecting various organs. This study examines the effect of prolonged starvation on glycogen content in the gonadal tissue of *Clarias batrachus*, a freshwater catfish. Using a calorimetric method, glycogen levels in gonadal tissues were measured over a 40-day period at 10-day intervals. Results showed a gradual decline in glycogen content, likely due to increased gluconeogenesis and heightened rates of deamination. Male specimens exhibited a more pronounced glycogen depletion compared to females. Initially, the reduction was minimal up to 20 days, followed by a sharp decline, resulting in an 80% reduction by day 40. These findings highlight significant metabolic adaptations in Clarias batrachus during prolonged starvation.

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INTRODUCTION

Starvation profoundly impacts the normal metabolic processes of organisms and can ultimately lead to mortality if prolonged. This phenomenon is particularly concerning in underdeveloped and developing nations where food scarcity is prevalent. Organisms experiencing starvation utilize their body reserves to survive, often at significant physiological costs. In fish, starvation-induced declines in various body constituents have been extensively documented. However, most existing research has chiefly focused on mammalian species.

This study aims to address this gap by examining the effects of prolonged starvation on the freshwater catfish, *Clarias batrachus*. This species is noteworthy for its ability to endure extended periods without food, exhibiting unique physiological and biochemical adaptations. Previous studies (Rajyasree and Naidu, 1989; Tripathi and Verma, 2003; Prasad, 2016) have indicated that fish undergo significant changes in physiological status and biochemical composition during starvation, yet the detailed mechanisms remain underexplored.

The objective of this research is to elucidate the impact of a 40-day starvation period on the glycogen content within the gonadal tissues of *Clarias batrachus*. By assessing glycogen levels at ten-day intervals, this investigation seeks to deliver a comprehensive understanding of the biochemical and physiological adjustments that occur in response to prolonged food deprivation. The findings from this investigation will contribute to the broader understanding of starvation biology in fish, with potential implications for fisheries management and conservation in environments prone to food scarcity.

LITERATURE REVIEW

Starvation and its effects on metabolic processes have been extensively studied in various organisms. The fundamental theory behind these studies is the concept of metabolic prioritization, where the body initially utilizes carbohydrate reserves, followed by lipids, and ultimately proteins (Reichsman, 1972). This adaptive mechanism is critical for survival during prolonged periods without food, allowing organisms to manage their energy reserves effectively.

Various researches have shown that fish possess remarkable resilience to prolonged starvation due to their ability to lower basal metabolic rates and utilize environmental resources (Wright, 1976). Studies on different species of fish have documented various physiological and biochemical changes during starvation. For instance, *Clupea harengus*, a species of herring, survived 129 days without food, while *Amia calva* endured 20 months of starvation (Smallwood, 1916).

In studies focusing on glycogen depletion, Fontaine and Hatey (1953) observed a 54% reduction in liver glycogen in *Salmo salar* during spawning migration. Inui and Dshima (1966) noted a slower loss of muscle glycogen compared to liver glycogen in *Anguilla japonica* during starvation. More recent

research by Prasad (2024) reported glycogen depletion in the liver of *Clarias batrachus* reaching 75% in males and 73% in females after 40 days of starvation.

The process of glycogen breakdown and glucose synthesis is crucial during periods of food scarcity. Glycogen is metabolized to glucose, which is then distributed to various organs via the bloodstream (Cahill, 1970; Bell, 1976). The liver primarily facilitates direct glucose synthesis from glycogen, while other tissues contribute through the alanine-glucose cycle and Cori cycle (Cori, 1931; Felig, 1973).

Several studies have documented the reduction of carbohydrate concentration during starvation across different animal species and tissues. Fontaine and Hatey (1953) observed this in *Salmo salar*'s liver, Wallace (1973) in *Carcinus maenas* (shore crab), and Harnath et al. (1984) in *Tilapia mossambica*. Prasad's studies (2014, 2015a, 2015b) further confirmed similar patterns in fish muscle tissues.

Sexual dimorphism in metabolic responses has been observed, indicating that males and females may differ in how they store and utilize energy. Studies by Singh (1981), Singhal et al. (1981), and Prasad et al. (2022) have noted higher levels of glucose and glycogen in females compared to males under both normal and deprived conditions. This difference is explained by the theory of reproductive allocation, which suggests that energy storage in reproductive tissues is prioritized to ensure reproductive success even under nutrient-deprived conditions.

While extensive research has been conducted on the effects of starvation in temperate fish species, there remains a gap in understanding the detailed mechanisms of glycogen utilization in tropical fish species such as *Clarias batrachus*. Most studies have focused on liver and muscle tissues, with limited attention given to gonadal tissues, which play a critical role in reproductive success.

METHODOLOGY

For this study, healthy *Clarias batrachus* specimens, averaging 18.8 cm in length and approximately 30.4 g in weight, were collected and acclimatized under laboratory conditions. The fish were maintained in aquaria and fed daily for a 20-day acclimation period to ensure optimal health and baseline physiological conditions.

Following acclimation, the experiment commenced by placing 10 fish in each of four separate aquaria. The study duration extended from day 0 to day 40, with fish being sacrificed at 10-day intervals. At each specified interval (0, 10, 20, 30, and 40 days), fish were euthanized, and their gonadal tissues were promptly excised.

The glycogen content in the gonadal tissues was quantified using the colorimetric method described by Kemp *et al.* (1954), as modified by Krishnaswami *et al.* (1961). This method provided precise measurements of glycogen levels, facilitating the assessment of metabolic changes throughout the starvation period. The tissues were weighed and then homogenised in 5 ml of ice-cold 10% TCA using a tissue homogenizer. The mixture was subjected to

centrifugation at a force of 500 times the acceleration due to gravity for a duration of 20 minutes. Next, the sediment was thoroughly mixed with an additional 5 ml of TCA and subjected to centrifugation once more. The liquids obtained from both stages of centrifugation were combined. Afterwards, 2 ml of the combined liquid that settled at the top was blended with 6 ml of highly concentrated sulfuric acid. The combination was subjected to heating in a boiling water bath for a duration of 6.5 minutes. Following the establishment of colour, the optical density of the pink hue was quantified at a wavelength of 515 nm using a photoelectric colorimeter. The glycogen content in the unknown sample was quantified by constructing a linear standard curve using glucose, and the values were expressed in milligrams per gram of wet weight.

Standard solution containing 125 µg, 375 µg and 750 µg / ml glucose were prepared along with a blank containing no glucose. The colour was allowed to develop in the same manner as descried above.

RESEARCH RESULT

In this study, a gradual decrease in glycogen content was detected across both the tissue types examined. Male specimens exhibited a more significant depletion of glycogen compared to females in all tissues analyzed. Specifically, both testes and ovaries showed non-significant depletion glycogen content during the first 20 days of hunger. However, after this period, a sharp decline was noted, culminating in approximately 80% glycogen depletion in the gonads after 40 days of starvation.

The collected data's bar notation and analysis of variance (ANOVA) showed a statistically significant drop in glycogen content between each of the following ten-day intervals. Between the intervals of 0 and 10 days, 0 and 20 days, 0 and 30 days, and 0 and 40 days, significant differences were seen. With a P-value of less than 0.01, the glycogen depletion in gonadal tissue between the 30- and 40-day fasting periods was noteworthy.

These results underscore the severe influence of prolonged hunger on the glycogen reserves of *Clarias batrachus*, highlighting a marked metabolic response that facilitates survival under extended periods of food deprivation.

Table-1. Gonad Glycogen Content (mg/gm wet weight) of *Clarias batrachus* during different periods of starvation

Name of organs	Days of Starvation				
	0	**10**	**20**	**30**	**40**
Testis	12.32 ± 0.23	11.80 ± 0.26	10.06 ±0.22	5.28** ± 0.18	1..97** ±0.15
Ovary	19.24 ±0.31	18.95 ±0.44	12.26** ±0.21	7.73** ±0.13	4.05** ±0.11

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 Co0.01 = 2.85

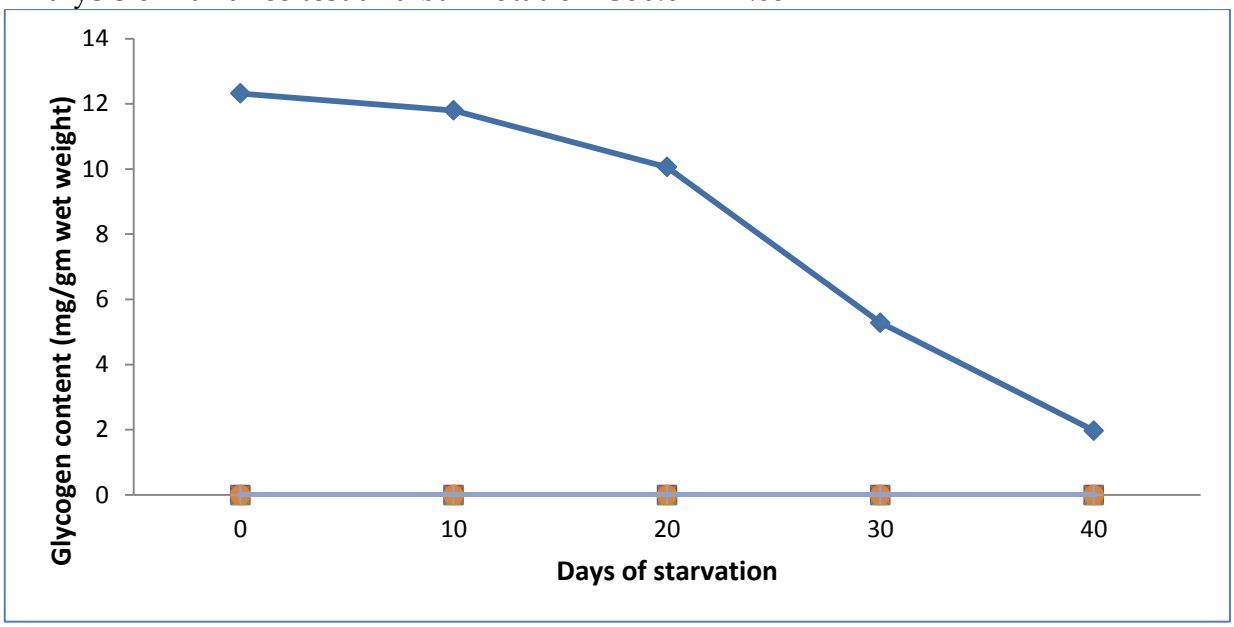

Fig. 1. Glycogen content in testis of Clarias during different periods of starvation

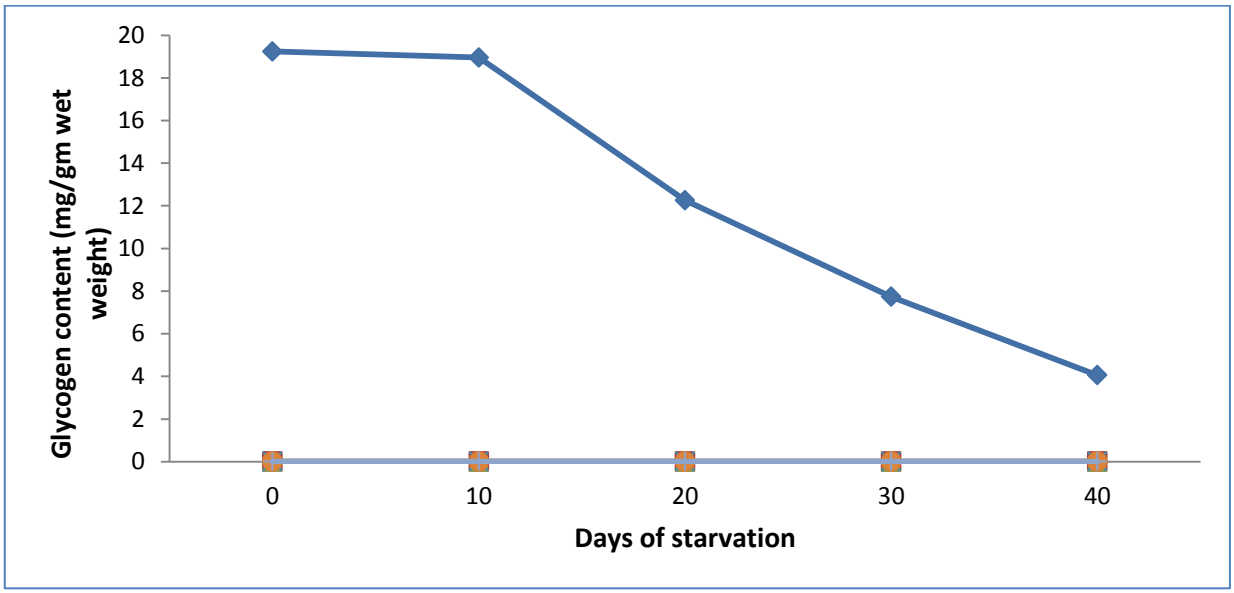

Fig. 2. Glycogen content in ovary of Clarias during different periods of starvation

DISCUSSION

Starvation induces a metabolic state where organisms resort to utilizing internal energy reserves, leading to the breakdown of various cellular components (Wright, 1976). This phenomenon is explained by the theory of metabolic prioritization, where the body sequentially consumes carbohydrates, lipids, and ultimately proteins, reflecting an adaptive strategy to sustain vital functions during food scarcity (Reichsman, 1972).

Our findings align with previous research on starvation-induced metabolic changes in fish. The significant depletion of glycogen in the gonadal tissues of *Clarias batrachus* over the 40-day starvation period underscores the

severe impact of prolonged food deprivation on this species. Initially, both male and female gonadal tissues exhibited non-significant changes in glycogen levels up to 20 days, indicating a period of metabolic adjustment. However, a sharp decline in glycogen content was observed thereafter, culminating in an approximately 80% reduction by day 40. This pattern suggests a critical threshold at which the body shifts from utilizing other energy reserves to tapping into glycogen stores in reproductive tissues.

The pronounced glycogen depletion in male gonadal tissues compared to females can be understood through the lens of sexual dimorphism in metabolic responses. Previous studies have reported higher baseline glycogen levels in females, which may provide them with a buffer against starvation (Singh, 1981; Singhal et al., 1981; Prasad et al., 2022). This sexual dimorphism could be an evolutionary adaptation to ensure reproductive success even under adverse conditions.

The theory of reproductive allocation posits that organisms prioritize energy storage in reproductive tissues to enhance reproductive success despite nutrient deprivation. Our data support this theory, showing that gonadal glycogen levels remained relatively stable until other energy reserves, such as liver and muscle glycogen, were substantially depleted. This adaptive strategy may enhance the likelihood of survival and reproductive success during periods of prolonged starvation.

The findings of this study are consistent with earlier research on the metabolic adaptations of various fish species to starvation. For example, Fontaine and Hatey (1953) reported significant glycogen depletion in the liver of *Salmo salar* during migratory fasting. Similarly, Inui and Dshima (1966) observed slower muscle glycogen loss in *Anguilla japonica* compared to liver glycogen. Prasad (2024) also documented substantial glycogen depletion in the liver of *Clarias batrachus* under prolonged starvation.

The observed metabolic shifts during starvation are further explained by increased gluconeogenesis, inhibited RNA synthesis, and heightened rates of deamination and transamination, as noted in our results. These biochemical processes reflect the body's efforts to maintain glucose homeostasis and provide energy to essential organs and tissues.

In summary, this study highlights the significant metabolic adaptations in *Clarias batrachus* during prolonged starvation, particularly the marked depletion of glycogen in gonadal tissues. These findings contribute to the broader understanding of starvation biology in fish and have potential implications for fisheries management and conservation in environments prone to food scarcity. Future research should explore the molecular mechanisms underlying these metabolic changes and investigate the long-term effects of repeated starvation episodes on reproductive success and population dynamics.

CONCLUSIONS AND RECOMMENDATIONS

This study provides valuable insights into the physiological and biochemical responses of the freshwater catfish, *Clarias batrachus*, to prolonged starvation. The observed significant depletion in glycogen content across different tissues, particularly the gonadal tissues, underscores the critical

metabolic adjustments that fish undergo to endure extended periods of food scarcity. The sharp decline in glycogen stores after 20 days, following initial stability, highlights the prioritized utilization of glycogen reserves, with gonadal tissues being the last to be depleted significantly.

The findings align with previous studies on various species, affirming that carbohydrates serve as the primary energy source during starvation, followed by lipids and, finally, proteins. The sex-specific differences observed, with females maintaining higher glycogen levels than males under both normal and starving conditions, add an important dimension to understanding metabolic strategies in fish.

These results emphasize the resilience and adaptability of fish to environmental stressors, such as food shortages, by modulating their energy reserves and metabolic pathways. This study not only contributes to the broader understanding of starvation physiology in fish but also offers a comparative basis for future research on other aquatic and terrestrial species. Further studies on molecular mechanisms governing these adaptations could pave the way for better management and conservation strategies in aquaculture and natural ecosystems.

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