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RESEARCH ARTICLE

Some Biochemical Changes in the Brain of Clarias Batrachus during Prolonged Food Deprivation

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ABSTRACT

The present study investigates the biochemical alterations in the brain of the air-breathing catfish, Clarias batrachus, during prolonged periods of food deprivation. Adult Clarias batrachus specimens were subjected to a controlled period of food deprivation extending up to forty days. Brain tissues were extracted at an interval of 10 days for biochemical analysis. Key biochemical markers, including protein, glycogen, cholesterol, and ascorbic acid concentrations, were measured using standard biochemical assays. The findings reveal significant reductions in brain glycogen and ascorbic levels, indicating an adaptive metabolic response to sustain essential brain functions during nutrient scarcity. Additionally, there was a notable alteration in the levels of cholesterol, suggesting a potential impact on the neurophysiological state of the fish. Prolonged food deprivation in Clarias batrachus induces substantial biochemical changes in the brain, highlighting the species' metabolic adaptability to prolonged nutrient stress. These findings provide insights into the neurobiological mechanisms underlying starvation tolerance in fish and may inform strategies for managing fish health in aquaculture settings. The normal values (0 days of starvation) of some biochemical constituents and their depletion after forty days of fasting in the brain were as follows:

Table 1: Level of some biochemical constituents in the nervous tissues of *Clarias batrachus*

Constituents Investigated	Normal value (mg/gm wet weight)		Depletion after 40 days	
	Male	Female	Male	Female
Glycogen	16	19	51%	50%
Cholesterol	48	51	13%	11%
Protein	138	153	3%	6%
Ascorbic acid	43	40	57%	48%

INTRODUCTION

Food deprivation is common in the natural environment, affecting various organisms and triggering a cascade of physiological responses aimed at survival. Among these responses, biochemical changes in the brain play a crucial role in regulating energy levels and maintaining homeostasis.

Understanding the biochemical responses of aquatic organisms to environmental stressors is critical for ecological research and aquaculture practices. The air-breathing catfish, *Clarias batrachus*, known for its remarkable adaptability to adverse conditions, serves as an excellent model to study

physiological and metabolic adjustments during stress. Among various stressors, food deprivation is a significant challenge that aquatic organisms often face in their natural habitats and controlled aquaculture environments.

Previous studies have explored the metabolic adjustments in various fish species under food deprivation, emphasizing changes in energy reserves, enzymatic activities, and hormonal balances. However, the specific biochemical alterations in the brain, a metabolically demanding organ, remain underexplored in *Clarias batrachus*. The brain's role in maintaining homeostasis and survival during nutrient scarcity underscores the importance of understanding its metabolic responses to prolonged food deprivation.

The objective of this study is to examine the biochemical alterations in the brain of both male and female *Clarias batrachus* following an extended period of food scarcity. Adult specimens were subjected to a controlled period of food deprivation for up to forty days, with brain tissues extracted at ten-day intervals for biochemical analysis. The key biochemical markers, like glycogen, cholesterol, protein, and ascorbic acid, were measured using standard biochemical assays.

By providing insights into the neurobiological mechanisms underlying starvation tolerance in *Clarias batrachus*, this study highlights the species' metabolic adaptability to prolonged nutrient stress. These findings have potential implications for managing fish health in aquaculture settings, offering strategies to enhance the resilience of cultured fish populations to environmental stressors.

Understanding the biochemical adaptations in the brain of *Clarias batrachus* during food deprivation is essential for elucidating its survival strategies and can have implications for understanding similar responses in other organisms.

MATERIALS AND METHODOLOGY

Experimental setup

C. batrachus specimens were collected from a nearby freshwater pond with the assistance of fishermen. The specimens were transported to the laboratory in large vessels protected by nets and were then classified using the method described by Shreshtha (1981). The selected fish were initially cleansed meticulously and then rinsed with a 0.1% KMn04 solution in order to eliminate any potential skin infections.

Individual healthy fish, with an average length of 18.8 cm and weight of 34.4 grams, were carefully moved one at a time using a small hand net into 40-litre glass aquaria for acclimation. They were acclimatized in laboratory conditions for two weeks before the experiment. The experimental group was subjected to prolonged food deprivation for 40 days, while the control group was fed twice daily with commercial food.

Sampling protocol

In order to make biochemical assessments, a sample of acclimatized, well-fed fish was taken from each sex. The results obtained were then considered to be normal for *Clarias batrachus*. The leftover fish were then sorted into four groups: A, B, C, and D. The fish in batches A, B, C, and D were left undisturbed at room temperature for 10, 20, 30, and 40 days, respectively.

The fish were removed and dissected on appointed days. After carefully cleaning the brain tissue samples by removing any extraneous associated structures and gently blotting them with filter papers, they were promptly immersed in the ice-cold fish saline solution that had been previously prepared using the following ingredients (Young, 1933).

Sodium Chloride	-	5.5 gm
Potassium Chloride	-	0.14 gm

Calcium Chloride	-	0.12 gm
Dechlorinated water	-	1litre

Biochemical assays

Protein, glycogen, cholesterol, and ascorbic acid contents in the brain tissue were quantified using standard biochemical assays.

The protein concentration in the brain tissue homogenates was determined according to the method of Sutherland *et al.* (1949) using the Folin-Ciocalteu reagent.

Total glycogen was estimated using the calorimetric method of Kemp *et al.* (1954) as modified by Krishnaswamy *et al.* (1961).

The total cholesterol content was determined using Sackett's approach (1925), which is a modified version of Bloor's approach (1928).

The technique utilized for extracting and quantifying ascorbic acid in the brain was identical to that employed by Kanungo and Patnaik (1964), which is a modified version of the approach described by Roe (1954).

Statistical analysis

Data were analyzed using appropriate statistical methods to determine significant differences between experimental and control groups at each time point.

RESULTS

The study revealed significant alterations in the biochemical composition of the brain tissues during prolonged food deprivation. The females had higher protein, cholesterol, and glycogen contents in normal and starved conditions. Starvation caused a non-significant drop in the brain protein concentration even after 40 days of starvation. There was no significant reduction in cholesterol levels even after a period of 30 days without food. Following a period of 40 days without food, the protein content of the brain showed the least reduction, with around 3% in males and 6% in females. Conversely, the ascorbic acid level exhibited the maximum depletion, with approximately 57% in males and 48% in females. After 40 days of fasting, the total loss in cholesterol content of the brain was about 13% in males and 11% in females however, the brain glycogen depleted about 51% in males and about 49% in females.

Protein content

During the initial phase of food deprivation (days 0-10), there was a gradual decrease in the protein concentration of brain tissue in *C. batrachus*. However, beyond day 10, protein levels stabilized, indicating a shift towards protein conservation.

Glycogen levels

Glycogen levels showed a rapid decline during the early stages of food deprivation, suggesting the utilization of glycogen stores for energy production. This decline continued throughout the experimental period.

Cholesterol content

Cholesterol levels remained relatively stable initially but increased slightly towards the later stages of deprivation, indicating the mobilization of lipid reserves for energy production.

Ascorbic acid

Ascorbic acid levels exhibited fluctuations during the experimental period, with a transient increase observed at day 10 followed by a gradual decline towards day 40.

Table 2: Brain protein (mg/gm wet weight) of *clarias batrachus*

Sex	Days of Food Deprivation				
	0	**10**	**20**	**30**	**40**
Male	138.27 ± 1.59	138.50 ± 1.51	137.60 ± 1.67	137.58 ± 0.88	133.99 ± 1.43
Female	153.45 ± 0.92	152.08 ± 0.80	151.10 ± 0.85	149.00 ± 0.69	144.15 ± 0.94

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 $Co_{0.01}$ = 9.12

Table 3: Brain glycogen (mg/gm wet weight) of *clarias batrachus*

Sex	Days of Food Deprivation				
	0	**10**	**20**	**30**	**40**
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

Table 4: Brain Cholesterol (mg/gm wet weight) of *Clarias batrachus*

Sex	Days of Food Deprivation				
	0	**10**	**20**	**30**	**40**
Male	47.75 ±0.64	46.65 ±0.73	46.25 ±0.67	46.00 ±0.83	41.36** ±0.77
Female	50.86 ± 0.52	50.03 ±0.33	49.87 ±0.76	49.79 ±0.98	45.03** ±0.41

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 $Co_{0.01}$ = 5.15

Table 5: Brain Ascorbic Acid (mg/gm wet weight) of *Clarias batrachus*

Sex	Days of Food Deprivation				
	0	**10**	**20**	**30**	**40**
Male	43.24 ± 1.45	42.48 ± 1.10	39.72 ± 0.94	28.51** ± 0.82	18.54** ± 0.27
Female	40.65 ±0.53	39.60 ± 0.72	37.86 ± 1.01	31.16** ± 0.37	21.23** ± 0.43

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 $Co_{0.01} = 6.95$

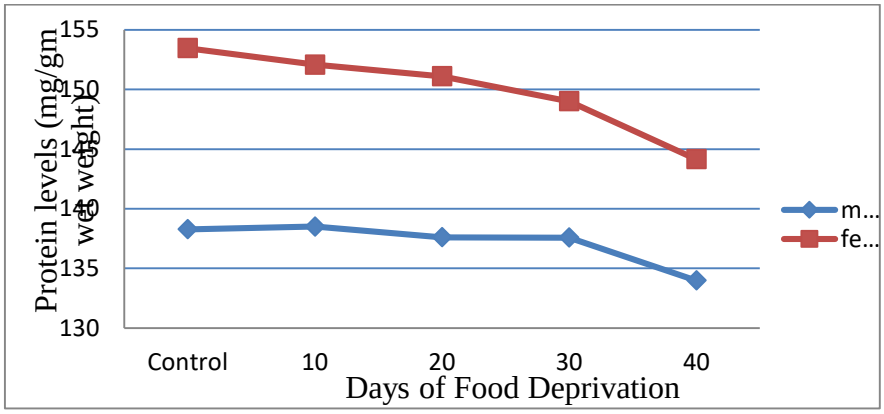

Figure 1. Effect of starvation on the protein concentration of Brain in *Clarias batrachus*.

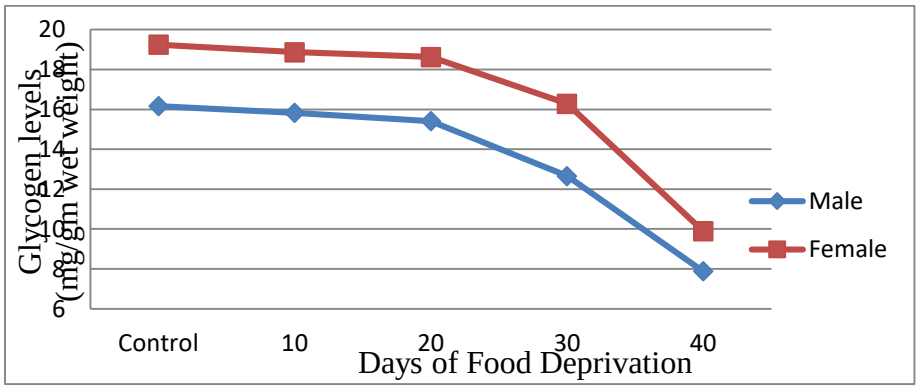

Figure 2. Effect of starvation on glycogen concentration of brain in Clarias batrachus.

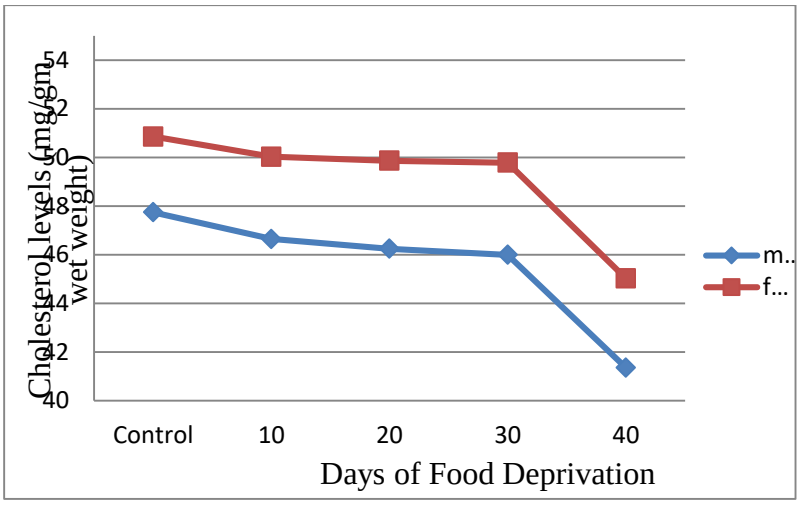

Figure 3. Effect of starvation on cholesterol concentration of brain in Clarias batrachus.

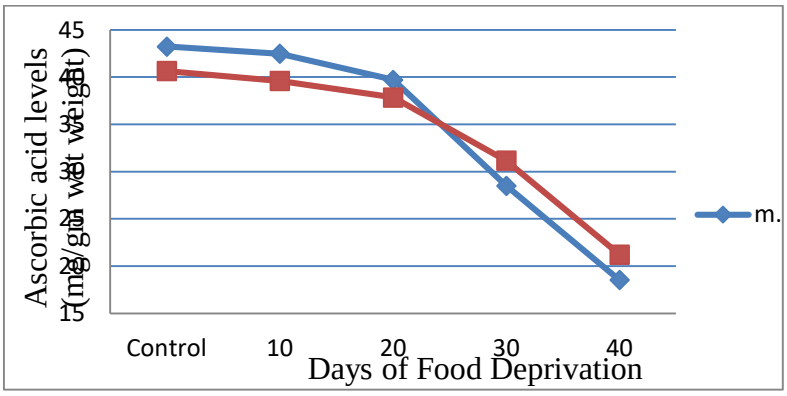

Figure 4. Effect of food deprivation on the ascorbic acid concentration of brain in *C. batrachus*.

DISCUSSION

The observed biochemical changes reflect adaptive responses aimed at maintaining energy homeostasis and ensuring survival under nutrient-limited conditions. These findings enhance our understanding of the physiological mechanisms underlying organismal resilience to environmental stressors and may have implications for conservation efforts. Unlike land-dwelling animals, most fish undergo a significant reduction in their bodily components within a certain period each year. That is why, fishes are usually well adapted to mobilizing their body constituents as fuel for survival during food deprivation (Love, 1980).

During periods of famine, several fundamental processes assist the organism. The basal metabolic rate decreases and the body's calorie need is further diminished due to the elimination of metabolically active tissue components (Weatherley *et al.*, 1981). The main objective is to survive at any cost and so the starving organism becomes more sparing in the expenditures of energy, although the ability to survive depends on many variables, like body size, stores of fat, and surrounding temperature. (Back, 1983). When an organism experiences starvation stress, it faces two conflicting demands. One is the ongoing need for energy-rich substances by important organs such as the brain, heart, and gills. The other is the continuing reduction of materials in the body (Young *et al.*, 1979).

In order to understand how the body can utilize its internal resources to survive without food, it is necessary to examine its chemical requirements. The main requirement, undoubtedly, is fuel to provide energy for essential tasks. Typically, glucose serves as the primary fuel source, generated through the breakdown of glycogen stores. The brain is the most crucial consumer of glucose, as it is just as vital to the brain as oxygen (Young, *et al.*, 1979).

Changes in protein

Protein is the chief structural and functional component of the body and it maintains the integrity of the body (Cahill, 1970). Structural proteins make the framework or fabric of the body whereas functional proteins (enzymes & hormones) guide, regulate and control the body's metabolism and physiology (Navarro *et al.* 1995).

In animals exposed to starvation, after the utilization of carbohydrate and lipid stores, protein mobilization begins. Smith (1982) concluded that the glycogen and lipids stores are insufficient to provide the glucose needed by the nervous tissue and other glycolytic tissues. The additional glucose requirement is not mainly through the formation of glucose from amino acids mobilized from protein breakdown. In the present investigation, slow mobilization of protein was observed in the brain tissues in comparison to glucose and cholesterol contents. The decrease in the protein content of the brain showed a non-significant drop even after 40 days of starvation.

Various researchers have reported a reduction in the protein level in the various tissues of animals that are experiencing starvation, such as Nandi *et al.* (1990) in *Heteropneustes fossilis*; and Magnoni *et al.* (2007) in rainbow trout.

In the present investigation, the impact of starvation on the level of protein is in conformity with that of Kiran *et al.* (1985). The slow depletion of the protein content during starvation is due to the strong measures taken by the starving body to prevent protein loss. Cahill (1970) suggested an alternative pathway of metabolism in starving mammals, which is somewhat analogous to the Cori cycle for lactate. It may be suggested that the same cycle might be operating in the fish. This cycle only recycles a set supply of glucose and provides an effective way to transfer nitrogen from the amino acids released during muscle protein breakdown to the liver (Young *et al.*, 1976). Approximately two-thirds of the glucose that is in circulation in the body is used by the brain, with the majority of the remaining third going to the voluntary muscles and RBCs (Grande, 1964). Since the body is deprived of food, the cellular components are broken and this leads to the differential decline in the glycogen, fat and ultimately the protein contents.

The decrease in brain protein content during the early stages of food deprivation may reflect increased protein catabolism to meet energy demands. However, the stabilization of protein levels suggests a shift towards the conservation of protein reserves during prolonged fasting.

Changes in Glycogen/Glucose

Carbohydrate metabolism is crucial during periods of food scarcity since carbs are the primary source of fuel for energy generation. Glucose molecules undergo continuous oxidation to liberate energy (Almeida *et al.*, 1997). During periods of hunger, glycogen is metabolized to generate glucose, which is then transported via the bloodstream to the organs that require it. During periods of hunger, the elevated concentration of glucagon drives the production of glucose from glycogen (Chaudhary *et al.*, 1981).

Various researchers, including Prasad (1980), Mustafa *et al.* (1979), Chaudhary *et al.* (1981), and Nandi *et al.* (1990), have observed a reduction in carbohydrate content in starving organisms across different tissues and animal species. For example, Prasad (1980) studied *Clarias batrachus*,

Mustafa *et al.* (1979) examined *Schizodactylus monstrosus* brain, and Chaudhary *et al.* (1981) focused on *Heteropneustes fossilis*.

Females had larger levels of glycogen compared to males in both normal and deprived situations. This discovery of a disparity between the sexes aligns with the research conducted by Singh (1981) and Singhal *et al.* (1981).

The rapid depletion of glycogen stores in the brain indicates a reliance on glycogen as a primary energy source during the initial phase of food deprivation. The continuous decline in glycogen levels highlights the sustained metabolic demand under prolonged fasting conditions (Polakof *et al.,* 2012).

Changes in Cholesterol

Cholesterol is a crucial constituent of the sterol category of lipids. It is found in every cell, both in the cell membrane and cytoplasm. With the exception of cerebrospinal fluid, all biological fluids include cholesterol, but its presence in cerebrospinal fluid is insignificant (Bell *et al.*, 1976). The level of cholesterol in any tissue is approximately directly related to its level of activity (Chatterjee, 1980).

During periods of hunger, the body initially uses its carbohydrate reserves to produce energy. Once these reserves drop below a certain threshold, the body starts to break down lipids for energy. Cholesterol is efficiently used, particularly in the brain (Bell *et al.* 1976). During the process of breakdown, the alcohol component of the lipids undergoes conversion into glucose (Chatterjee, 1980). The reduction in cholesterol levels during hunger has been documented by researchers like Shreni (1979), Mosin *et al.* (1982), and Weber *et al.* (1995).

During the current study, it was found that the brain showed minimal movement of cholesterol for up to 30 days of hunger. This indicates that the brain is able to resist the effects of famine at a biochemical level, as shown by the analysis of several components (Table IV and Fig. 3). The current observations on the reduction of cholesterol levels during hunger align with the discoveries made by Shreni (1979) and Mosin *et al.* (1982). The discrepancy in cholesterol levels between males and females aligns with the findings of Shreni (1979) and Prasad (2015b & 2016).

The maintenance of cholesterol levels during food deprivation suggests a regulated lipid metabolism in the brain of *C. batrachus*. The slight increase in cholesterol depletion towards the later stages of deprivation may indicate the mobilization of lipid reserves for energy production.

Changes in Ascorbic Acid

Ascorbic acid is thought to function as a catalyst in oxidation-reduction systems, although little is known about the metabolic reactions that call for it (Bal and Kalyani 1960). It is thought to have anti-oxidant and fatigue-retarding properties (Paratheswararao, 1967). According to Siddiqui (1967), ascorbic acid is essential for the synthesis of tissues and the repair of wounds. It is necessary for the healthy development of the precursor cells of different tissues as well as the preservation of the normal state of intercellular substances in cartilage, bones, teeth, skin, and connective tissues, such as mucoprotein and collagen (Prosser, 1984).

Almost all vertebrates convert hexoses, including glucose, into ascorbic acid. Humans, monkeys, and guinea pigs are the only known species that rely on dietary sources of ascorbic acid (Prosser, 1984). Like other animals, fish have tissues that contain and manufacture ascorbic acid. The ascorbic acid levels found in *Clarias batrachus*'s brain are consistent with those found by Prasad (2015a), and Bal & Kalyani (1960). After 10 days of fasting, the content of ascorbic acid in the nervous tissues of *Clarias batrachus* decreases significantly and keeps declining as the days of famine go by (Table V and Fig. 4).
It is well known that animals use the tissues in their bodies as food when they are starving

(Wright, 1976). For the synthesis of ascorbic acid, the animal is dependent on the dietary intake of hexoses (Briggs, 1962). When an animal is starving, it consumes carbs quickly, which prevents it from getting enough hexoses. Therefore, the brain's ascorbic acid content drops during hunger, indicating a decrease in ascorbic acid production. It is noteworthy to note that the trend of decreased ascorbic acid concentration during famine is similar to that of decreasing glycogen concentration (Navarro & Gutiérrez, 1995).

The fluctuations in ascorbic acid levels suggest dynamic antioxidant activity in response to oxidative stress induced by food deprivation. The transient increase in ascorbic acid may represent an adaptive response to mitigate oxidative damage during the early stages of fasting (Iwama & Thomas, 1991).

The findings revealed significant reductions in brain glycogen and ascorbic acid levels, indicating an adaptive metabolic response to sustain essential brain functions during nutrient scarcity. Additionally, notable alterations in cholesterol levels were observed, suggesting a potential impact on the neurophysiological state of the fish.

CONCLUSION

The biochemical changes observed in the brain of *C. batrachus* during prolonged food deprivation reflect adaptive responses aimed at maintaining energy homeostasis and ensuring survival. The utilization of protein and glycogen reserves, coupled with dynamic antioxidant activity and regulated lipid metabolism, highlights the remarkable resilience of *C. batrachus* to adverse environmental conditions. Further research is needed to elucidate the molecular mechanisms underlying these adaptive responses and their significance in the broader context of organismal survival strategies.

Understanding the biochemical changes occurring in the brain of *C. batrachus* during prolonged food deprivation is essential for elucidating its survival strategies and may have broader implications for understanding similar responses in other organisms.

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