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Nutritional Stress And Ascorbic Acid Dynamics: Gender Perspectives In *Clarias Batrachus*

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Abstract

The current study investigates the impact of starvation on the levels of ascorbic acid in the brain and hepatic tissues of both male and female *Clarias batrachus*. Male individuals exhibited a significantly greater concentration of ascorbic acid compared to female individuals. Under typical circumstances, the brain contains higher levels of ascorbic acid (43.24mg/100gm in males and 40.65mg/100gm in females) compared to the liver (17.59mg/100gm in males and 13.32mg/100gm in females). Extended nutritional stress leads to a gradual decrease in the level of ascorbic acid in all types of tissues, which could be associated with an increase in gluconeogenesis. The liver exhibited a higher level of depletion (73% in males and 78% in females), whereas the brain showed a lower level of depletion (57% in males and 48% in females) following a period of 40 days of hunger.

Key words: *ascorbic acid, brain, Clarias batrachus, liver, stress*

AUTHORS' CONTRIBUTIONS
This work was carried out in collaboration among all authors. Author NKP designed the study, and wrote the protocol and the first draft of the manuscript. Authors KS and SKS managed the analysis of the study. Authors NKP and KS managed the literature searches. All authors read and approved the final manuscript.

1. Introduction

The three basic needs of all living things are food, shelter, and reproduction. Since the beginning of time, a vast majority of people have been suffering from food insecurity. According to the WHO, starvation is the single biggest threat to the global biota. Animal biology is impacted by food scarcity on a number of levels, most notably the biochemical makeup of different tissues and organs. In actuality, starving organisms fight malnutrition with the resources in their bodies until they perish.
Most fish species suffer from starvation at specific times of the year, primarily as a result of environmental factors that have varying effects on different organs. Fish physiology and other components are also impacted by starvation (Rajyasree & Naidu, 1989; Mukhopadhyaya et al., 1991; Chin & Shin, 1992; Deng et al., 1993).

Kiran & Talesara (1985) examined the effects of prolonged hunger on the red and white muscles of two freshwater teleost fish species. They reported that decreased activities of succinic dehydrogenase and mitochondrial ATPase in myotomal muscle were seen following extended fasting, which was reflected in a dramatic drop in spontaneous activity and metabolic rate. Yadav and Borah (1996) studied the haematological and biochemical reactions of *H. fossilis* to hunger. They discovered that hunger caused a reduction in lactate dehydrogenase activity in the muscles and liver. With an increase in the fasting time came a drop in the amounts of protein, glucose, and glycogen. There is mounting evidence that fish death, in both immature and adult forms, may be primarily caused by malnutrition. Fish proteins, the RNA/DNA ratio, and metabolic enzymes are all impacted by fasting. It significantly affects the growth of fish. In order to ascertain the effects of starvation in a siluroid species that is significant to the economy, the current study was designed.

In this study, the common air-breathing catfish, *Clarias batrachus*, were selected as experimental material due to their easy availability in nearby ponds and rivers. These can withstand famine for an extended amount of time. The purpose of this study is to learn more about the causes, facts, and effects of malnutrition on animals. Numerous researchers have conducted numerous comparable studies; however, the majority of their efforts have only focused on the mammalian fauna. Research on the impact of hunger on fish has not been conducted much in Nepal. Due to fish's special ability to endure extended hunger through physiological and biochemical alterations, it is important to observe and disclose the effects of fasting on fish (Mustafa, 1983).

In the light of aforementioned information, the goal of the current study is to determine the ascorbic acid level in the brain and liver tissues of *Clarias batrachus* throughout a 40-day protracted period of starvation by estimating the ascorbic acid constituents every 10 days.

2. Materials and Procedures

For this investigation, robust living fish were acquired from a nearby fish pond with the assistance of a fisherman. The fish were transported to the laboratory in spacious ceramic containers that were supplied with covers made of mosquito netting. The individuals were identified using the techniques outlined by Srivastava (2006) and then treated for five minutes with a 0.1% KMnO4 solution to remove any potential skin infections. Using a compact handheld net, fish in optimal condition, with an average length of 18.8 cm and a weight of around 34.4 g, were individually moved to a spacious glass aquarium with a capacity of around 110 litres. The aquarium has approximate measurements of 75 cm in length, 30 cm in width, and 45 cm in height. A twenty-day interval was allocated to allow them to acclimate to the laboratory setting. To alleviate malnutrition, the fish were given commercial fish meal twice a day throughout this time frame. To prepare the digestive tract, food intake was stopped 24 hours before to the start of the trial. The study was conducted between May 2009 and June 2013. Samples collected from both male and female well-nourished, acclimated fish were subjected to biochemical analysis. The obtained values were found to be within the typical range for C. batrachus. The control group used tap water. From the remaining fish, a total of ten were chosen, comprising an equal number of males and females, namely five of each gender. The remaining fish were categorized into four groups: A, B, C, and D. The fish from batch A were maintained at room temperature for a period of 10 days without being provided with any sustenance. The fish from batches B, C, and D were subjected to identical circumstances for twenty, thirty, and forty days, respectively. The fish were dissected at regular intervals of 10 days, beginning on the first day and continuing until the fortieth day. The ascorbic acid content of the brain and liver was extracted and quantified using the same procedure described by Kanungo and Patnaik [9], which is a modified version of the Roe method. The liver, muscles, gonads, and brain were divided into measured portions and pulverized using 5 ml of 6% (w/v) trichloroacetic acid (TCA) in a tissue homogenizer that had been pre-chilled and contained chemically pure sand that had been washed with acid. The homogenates underwent centrifuging at a speed of 3000 revolutions per minute (rpm). After decanting the liquid layer, the procedure was performed twice more using the leftover solid and a solution consisting of five millilitres of trichloroacetic acid (TCA) with a concentration of six percent. To initiate oxidation, a small quantity of bromine water was added to the liquid portion containing ascorbic acid. After undergoing vigorous stirring, the mixture was subsequently filtered. The extra bromine was eliminated by aerating the filtrate. To assess the concentration of ascorbic acid, two 2 ml samples of the filtrate were collected in duplicate using Roe's 2-4 dinitro-phenyl-hydrazine method. After introducing the concentrated sulphate acid and hydrazine-thiourea reagent, a colour developed and was subsequently measured at a wavelength of 520 μm using a colourimeter. The level of ascorbic acid in each sample of brain and liver homogenates was measured using the standard method.

3. Findings

According to the current study, under normal circumstances, males have comparatively higher levels of ascorbic acid than females. The concentration of ascorbic acid in the brain was greater than in the liver. Among the solid tissues under investigation, the female *Clarias's* liver had the lowest ascorbic acid content value. Considerable depletion was only seen in the male liver after 10 days of deprivation, but in the female liver, considerable depletion was seen after 20 days. Up to 20 days of hunger, there was no discernible depletion in the brain; but, after that, there was a noticeable and severe depletion. Table 1; and Figures 1 and 2 present the ascorbic acid amounts in the brain and liver of both the male and female *Clarias batrachus*, respectively.

4. Discussion

The ascorbic acid, a variant of Vitamin C is one of the most naturally occurring chemical compounds that possesses antioxidant properties. Limited information is available regarding metabolic reactions that rely on ascorbic acid. The antistress impact of antioxidant vitamin C is well-established and widely acknowledged (Azad et al., 2007; Misra et al., 2007). The antioxidant activity of ascorbic acid is derived from its ability to interact with free radicals. It is considered to be a substance that reduces fatigue and acts as an antioxidant (Paratheswararao, 1967). Ascorbic acid is vital for the creation of connective tissues and for the process of wound healing (Siddiqui, 1967). Ascorbic acid concentration has been seen to increase during the summer and autumn and decrease during the winter (Shanta and Motelica, 1962). Formative cells in many tissues rely on it for proper functioning, and it is necessary to preserve the normal condition of intracellular substances including collagen and mucoprotein in the connective tissues, cartilage, bone, skin, and teeth. (Prosser, 1984).

The ascorbic acid is produced by most vertebrates through the synthesis of hexoses, such as glucose. According to Prosser (1984), the only species that rely on food sources for ascorbic acid are some mammals like humans, monkeys, and guinea pigs. Fish, similar to other organisms, possess ascorbic acid in their tissues and have the ability to produce it internally. The ascorbic acid levels in the brain and liver of *Clarias batrachus* are similar to the findings published by Leblond et al. (1938), and Bal and Kalyani (1960).

Madhuban and Kaviraj (2009) suggest that fish may utilize ascorbic acid depletion as a defensive mechanism to cope with stress, hence increasing the need for ascorbic acid in stressful circumstances. After ten days of fasting, the amount of ascorbic acid decreases in the liver of *Clarias batrachus* and keeps going down with each additional day of starvation, as shown in Table 1. The brain contains a considerably higher amount of ascorbic acid (Table 1) compared to hepatic tissues.

During periods of starvation, animals utilize their own bodily tissues as a source of energy (Wright, 1976). The animal produces ascorbic acid by converting hexoses found in its meal. As the carbohydrates are consumed rapidly during the periods of starvation, the animal does not obtain a sufficient amount of hexoses. During fasting, there is a decrease in the production of ascorbic acid, which is evident from the reduced concentrations of ascorbic acid in the muscular tissues of the body (Prasad, 2016). It is important to mention that the levels of ascorbic acid decrease during fasting in a similar manner to the decrease in glycogen levels (Prasad, 2014).

The rate of glycogen depletion in the brain is relatively slow as compared to the liver, and hunger causes a lesser fall in the brain's ascorbic acid content as compared to the liver. This illustrates the dependency on hexoses for the synthesis of ascorbic acid. The current findings are similar to the findings of Sinha (1966) and Dvorak (1974).

Table-I: *The level of ascorbic acid (mg/100gm wet tissue) in the brain and liver of C. batrachus*

Organs	Gender	Control	Periods of Food Deprivation			
			10 days	20 days	30 days	40 days
Brain	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
Liver	Male	17.59 ± 0.27	13.44** ± 0.25	10.74** ± 0.14	8.41** ± 0.22	4.77** ± 0.13
	Female	13.32 ± 0.27	11.51 ± 0.40	9.22** ± 0.27	7.13** ± 0.23	2.98** ± 0.20

Values are the mean of eight samples of both the male and female fish
± SE; ** Significant

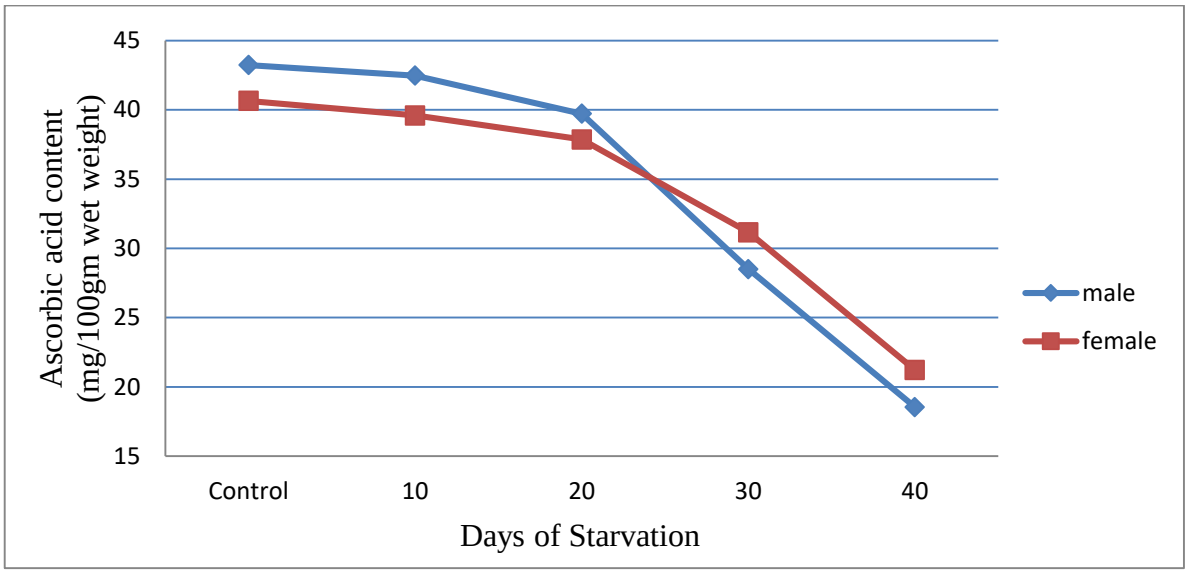

Figure 1. Effect of food deprivation on ascorbic acid level of brain in *C. batrachus*.

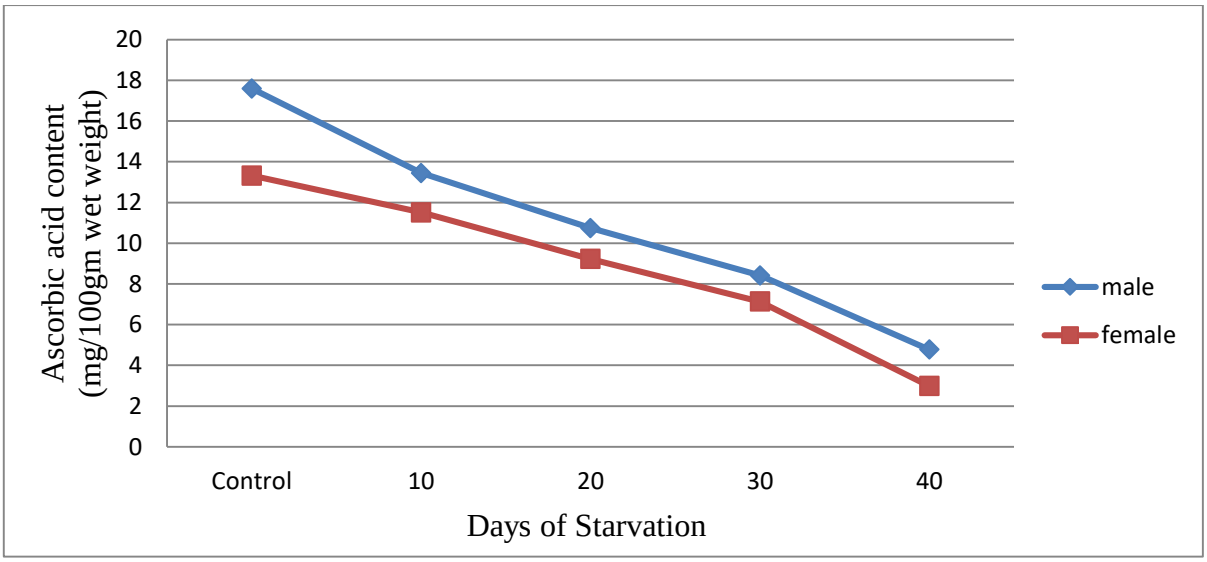

Figure 2. Effect of food deprivation on the ascorbic acid level of the liver in *C. batrachus*.

5. Conclusion

Clarias batrachus, a type of freshwater fish, can endure long periods without food. Food deprivation exerts many impacts on the physiological processes of the body, namely altering the biochemical makeup of several organs. In all tissues examined under normal conditions, males exhibited higher amounts of ascorbic acid compared to females. The concentration was elevated in the brain but diminished in the liver. Following a 40-day period of famine, the liver showed the most significant depletion, with a reduction of 73% in males and 78% in females. Conversely, the brain exhibited the lowest depletion, with reductions of 57% in males and 48% in females.

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References:

1. Azad, I. S., Dayal, J. S., Poornima, M., & Ali, S. A. (2007). Supra dietary levels of vitamins C and E enhance antibody production and immune memory in juvenile milkfish, *Chanos chanos* to formalin-killed *Vibrio vulnificus*. *Fish Shellfish Immunology*, 23, 154-163.
2. Bal, P. A., & Kalyani, M. (1960a). Studies on the ascorbic acid content of fish tissues. *Journal of Animal Morphology and Physiology*, 7, 162-166.
3. Borah, S., & Yadav, R. N. S. (1996). Biochemical and haematological response to starvation in an air-breathing freshwater teleost, *Heteropneustes fossilis* (Bloch). *Indian Journal of Fish*, 43, 307-310.
4. Chin, P., & Shin, Y. K. (1992). Effect of starvation on the total metabolism of *Crangon affinis*. *Bulletin of the Korean Fishery Society*, 25, 371-382.
5. Deng, H., Wang, J., & Zhang, X. (1993). The changes in biochemical constituents in serum liver and muscular tissue of German mirror carp under different periods of starvation. *Journal of Dalian Fishery College*, 8, 57-61.
6. Dvorak, N. (1974). Effect of corticotrophin, starvation, and glucose on ascorbic acid levels in the blood plasma of piglets. *Nutrition and Metabolism*, 16, 215-222.
7. Fomin, S. V., & Romanjuk, N. M. (1937). The ascorbic acid contents of the tissues of various fish. *Ukrainian Biochemical Journal*, 10, 365-378.
8. Kanungo, M. S., & Patnaik, B. K. (1964). Ascorbic acid and ageing in rat. *Biochemical Journal*, 90, 637.
9. Kiran, S., & Talesara, C. L. (1985). Prolonged starvation effects on myotomal muscle of two freshwater teleost fishes, murrel (*Channa punctatus*) and catfish (*Clarias batrachus*). Indian Journal of Experimental Biology, 23, 17-22.
10. Leblond, C. P., Giroud, A., Ratsimamange, R., & Gero, E. (1938). The normal level of ascorbic acid. *Bulletin of the Society of Biological Chemistry*, 20, 1079-1087.
11. Madhuban, B., & Kaviraj, A. (2009). Toxicity of the pyrethroid pesticide fenvalerate to freshwater catfish *Clarias gariepinus*: Lethality, biochemical effects and role of dietary ascorbic acid. *Journal of Environmental Science and Health*, 44(B), 578-583.
12. Misra, C. K., Das, B. K., Mukherjee, S. C., & Pradhan, J. (2007). Effects of dietary vitamin C on immunity, growth and survival of Indian major carp *Labeo rohita*, fingerlings. *Aquaculture Nutrition*, 13, 35–44.
13. Mukhopadhyaya, P. K., Mohanty, S. N., Das, K. M., Sarkar, S., & Batra, B. C. (1991). Growth and changes in carcass composition in young *Labeo rohita* and *Cirrhinus mrigala* during feeding and starvation. In Fish Nutrition Research in Asia (pp. 87-91). *Proceedings of the 4th Asian Fish*. Special Publication of the Asian Fisheries Society, 5.
14. Mustafa, S. (1983). Changes in biochemical composition in starving catfish, *Heteropneustes fossilis*. *Japanese Journal of Ichthyology*, 29(4), 416-420.
15. Paratheswararao, V. (1967). Some mechanisms underlying thermal acclimation in a freshwater fish, *Etroalus maculates* (Teleost). *Comparative Biochemistry and Physiology*, 21, 619-625.
16. Prasad, N. K. (2014). Effects of Prolonged Starvation on Glycogen Content of Muscles in *Clarias batrachus. Himalayan Biodiversity*, Pokhara, 2(1), 52-55.
17. Prasad, N. K. (2016). Prolonged starvation effects on ascorbic acid level in the muscles of a freshwater teleost, *Clarias batrachus*. *Gun Sagar.* 3(2), 65-68.
18. Prosser, C. L. (1984). *Comparative Animal Physiology* (3rd ed.). Satish Book Enterprise Booksellers and Publishers.
19. Rajyasree, M., & Naidu, K. R. P. (1989). Starvation-induced changes in biochemical aspects of hepatic tissue of fish, *Labeo rohita. Indian Journal of Fisheries*, 36, 339-341.
20. Roe, J. H. (1954). Chemical determination of ascorbic and dehydroascorbic and diketogulonic acid. In D. Glick (Ed.), *Methods of Biochemical Analysis* (Vol. 1). New York: Interscience Publishers.
21. Shanta, N., & Motelica, I. (1962). Influence of starvation on brain and liver cholesterol levels of the catfish *Heteropneustes fossilis*. *Proceedings of the Indian Academy of Sciences*, Section B, 881(1 part 3), 205-208.
22. Siddiqui, M. A. (1967). Seasonal variation in the ascorbic acid content and calcium content in different tissues of *Ophiocephalus punctatus* (Bloch). *Indian Journal of Experimental Biology*, 5, 54-55.
23. Sinha, R. C. (1966). Effect of starvation on the concentration of ascorbic acid in the pedipalp muscle of scorpion, *Palamnaeus bengalensis. Experiments*, 22(4), 221-222.
24. Srivastava, G. J. (2006). *Fishes of UP and Bihar* (11th ed.). Vishwavidyalaya Prakashan.
25. Wright, S. (1976). *Applied Physiology* (12th ed.). Oxford University Press.