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Influence of Prolonged Food Deprivation on Ascorbic Acid Levels of the Brain and Liver in *Clarias batrachus* (LINN.)

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Abstract The present paper deals with the effect of starvation on ascorbic acid contents of brain and hepatic tissues in both the sexes of *Clarias batrachus*. The level of ascorbic acid content was found relatively higher in males than in females. The brain showed higher value of ascorbic acid in normal condition (43.24mg/100gm in male and 40.65mg/100gm in female) in comparison to the liver (17.59mg/100gm in male and 13.32mg/100gm in female). During prolonged food deprivation, the gradual decrease in ascorbic acid level has been noticed in both the types of tissues which can be related to enhanced gluconeogenesis. The higher depletion has been observed in liver (73% in male & 78% in female) and lower depletion in the brain (57% in male &48% in female) after 40 days of starvation.

Keywords: *ascorbic acid, brain, Clarias batrachus, liver, starvation*

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1. Introduction

Food, shelter and propagation are the three basic needs of living world and vast majority of population has been suffering from food deprivation since time unknown. According to WHO, hunger is the single greatest threat to the world biota. Food-deprivation influences the biology of animals at various levels, especially the biochemical composition of various organs. In fact, organisms facing the starvation fight it at the cost of their own body reserves till death.

Starvation is experienced in most species of fish during certain periods of every year largely due to environmental conditions and it affects different organs in different ways. Starvation also affects the physiology and other constituents of fish [5,6,14,18]. Prolonged starvation effects on red and white muscles of two fresh water teleost fishes have been studied by Kiran & Talesara [10]. They found a sharp decline in spontaneous activity and metabolic rate during prolonged starvation which was reflected by reduced activities of succinic dehydrogenase and mitochondrial – ATPase in myotomal muscle. Borah & Yadav [3] worked on the biochemical and haematological response to starvation in *H. fossilis*. They reported the decrease in the activity of lactate dehydrogenase in both liver and muscles as a function of starvation. The amount of protein, glucose and glycogen also decreased as the period of starvation increased. Letcher et al. [12] showed the size – dependent effects on starvation and mass loss in yellow perch larvae and juveniles. There is increasing evidence that starvation may be a major cause of mortality in both immature and adult fishes. Fasting also affects metabolic enzymes, RNA/DNA ratio and proteins in fish. It has a great impact on fish growth. Therefore, the present study was planned to determine the starvation effects in an economically important siluroid species.

In the present investigation, the common Indian air-breathing catfish, *Clarias batrachus* were used as experimental animals as they are easily available in local ponds and rivers. These can sustain starvation for prolonged period. The present study is aimed to know the facts and causes of starvation and their consequent impacts on animals. A number of similar studies have been carried out by many workers but most of the works have been confined to mammalian fauna. In Nepal, little works have been done to study the starvation-induced effects in fishes. So, it is pertinent to see and reveal the effect of starvation on fish as there is a unique feature of fish to withstand prolonged starvation through physiological and biochemical changes [15].

In the light of above fact, the present work has been designed to know the level of ascorbic acid content of brain and hepatic tissues of *Clarias batrachus* during prolonged period of starvation of 40 days by estimating ascorbic acid constituents at an interval of every 10 days.

2. Materials & Methods

For the present investigation, healthy live fish were collected from a local fish pond with the help of fishermen. The fish were brought to the laboratory in large earthen

pots covered with mosquito net. They were identified according to Srivastava [23] and were treated with 0.1% $KMnO_4$ solution for five minutes to get rid of any dermal infection. Healthy fish of an average length (18.8 cm) and weight (34.4 g) were transferred one by one with the help of small hand net to a large glass aquarium of about 110 liters capacity measuring about 75cm x 30cm x 45cm in size. They were allowed to acclimatize under laboratory condition for 20 days. During this period, the fish were fed twice daily with commercial fish food to avoid their starvation. Twenty four hours before starting the experiment, the food was stopped to clear off the alimentary canal. The study was carried out from May 2009 to June 2013.

Biochemical estimations were made by taking the samples from each sex of acclimatized and well fed fish and the values obtained were taken as normal value for *C. batrachus.* A control group was kept in tap water. The remaining fish were divided into four batches – A, B, C and D keeping 10 fishes each (5 males and 5 females). The fishes of batch A were kept without food at room temperature for 10 days, that of batch B for 20 days, C for 30 days and D for 40 days.

Starting from 0 day up to 40 days, the fish were dissected at an interval of 10 days. The method adopted for the extraction and estimation of ascorbic acid of the brain and liver was the same as that of Kanungo and Patnaik [9] which is the modification from the Roe [19].

Weighted quantity of the liver, muscles, gonads and brain were taken separately and homogenized with 5 ml of 6% (w/v) trichloro-acetic acid (TCA) in pre-cooled tissue homogenizer, containing acid washed chemically pure sand. The homogenates were centrifuged at 3000 rpm in a centrifuge. The supernatant was decanted and the process was repeated twice with the residue with 5 ml of 6% TCA. A few drops of bromine water were added to the supernatant for the oxidation of ascorbic acid. The solution was stirred and filtered. Excess of bromine was removed from the filtrate by bubbling air. Samples (2ml) of the filtrate were taken in duplicate for the determination of ascorbic acid, by 2-4 dinitro-phenyl-hydrazine method of Roe [19]. The extinction of the colour developed after the addition of hydrazine-thiourea reagent and concentrated sulphate acid was read in colorimeter at 520 mμ. The concentration of ascorbic acid in each sample of liver and brain homogenates was determined from the standard.

3. Results

In the present study, the level of ascorbic acid was found relatively higher in males than in females under normal conditions. The brain had higher ascorbic acid content in comparison to that of liver. The minimum value of ascorbic acid concentration was observed in the liver of female *Clarias* among the solid tissues investigated.In the liver of male, significant depletion was observed only after 10 days of starvation whereas in female significant depletion was marked after 20 days of starvation. In brain, non-significant depletion was observed up to 20 days of starvation but thereafter the depletion was sharp and significant. The ascorbic acid contents in brain and liver of male and female *Clarias batrachus* are given in Table 1 and the Figure 1 & Figure 2, respectively.

Table 1. Ascorbic acid content of brain and liver(mg/100gm wet tissue) of C. batrachus

Name of Organ	Sex	Control	Periods of Starvation			
			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 male and female ± SE; ** Significant.

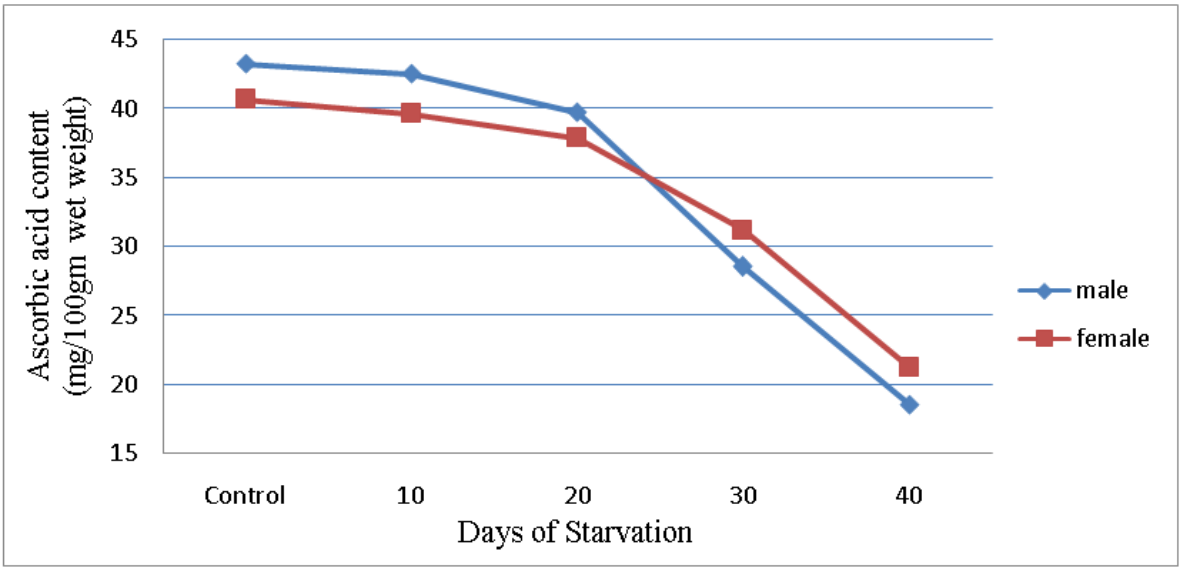

Figure 1. Effect of Starvation on ascorbic acid content of brain in *C.batrachus*

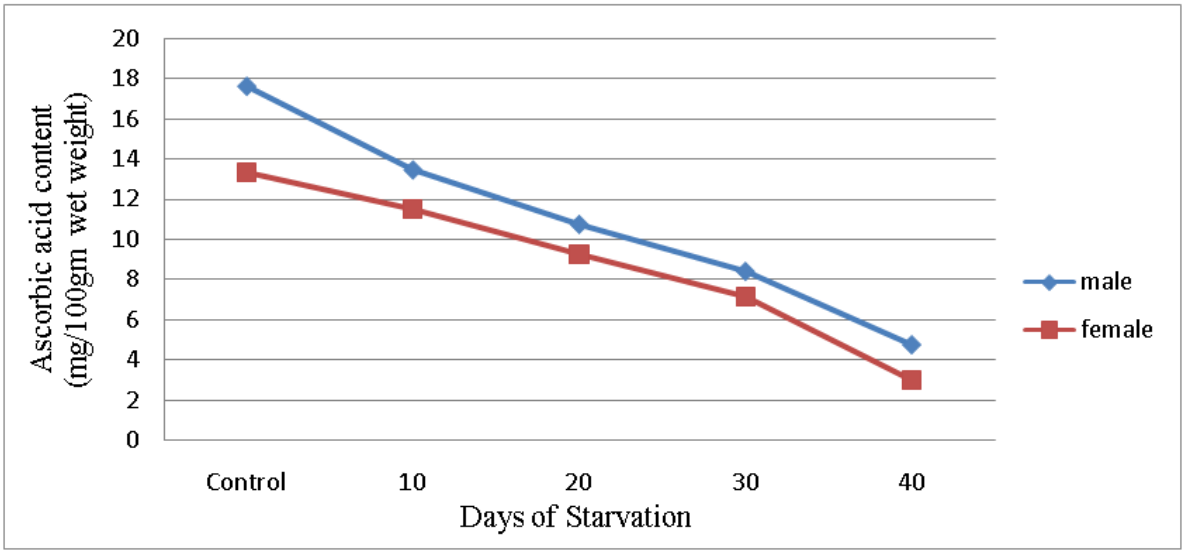

Figure 2. Effect of Starvation on ascorbic acid content of liver in *C. batrachus*

4. Discussion

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. It is one form of vitamin C. Little is known about metabolic reactions which require ascorbic acid, but it is said to be involved in oxidation-reduction system as catalyst [1]. It is considered as a fatigue retardant and anti-oxidant [16]. Ascorbic acid is necessary for the production of connective tissue and the healing of wounds [21]. The concentration of ascorbic acid has been noticed to rise in summer and fall in winter [8,20]. It is essential for the proper functioning of the formative cells of various tissues and maintenance of normal state of the intarcellular substance like muco-protein and collagen in bone, cartilage, teeth, skin and connective tissues [17].

Ascorbic acid is synthesized by almost all vertebrates from hexose's including glucose. The only species known to depend on dietary sources for ascorbic acid are human beings, monkey and guinea pig [17]. Fish, like other animals, contain ascorbic acid in their tissues and synthesize it for themselves. The values of ascorbic acid observed in liver and brain of *Clarias batrachus* are in conformity with Leblond *et al.* [11] and Bal and Kalyani [2]. The concentration of ascorbic acid in the liver of *Clarias batrachus* declines after 10 days of starvation and continues to decline further with the progressive days of starvation (Table 1). There is much more ascorbic acid in the brain (Table 1) than in hepatic tissues.

It is known that during starvation animals live on component tissues of their own body for energy purposes [25]. The animal depends on the dietary source of hexoses for ascorbic acid synthesis [4]. Due to rapid consumption of carbohydrates during starvation, the animal fails to get a sufficient amount of haxoses. Hence, the ascorbic acid synthesis deceases during starvation as indicated by the decreased ascorbic acid concentration in various organs and tissues of the body. It is significant to mention that the decreased concentration of ascorbic acid during starvation follows the same pattern as that of glycogen.

Glycogen depletion in brain is comparatively slower than the glycogen depletion in liver and similar the ascorbic acid concentration of brain is less depleted than that of liver due to starvation. This indicates the dependence of ascorbic acid synthesis on hexoses. The present observations are in conformity with Sinha [22] and Dvorak [7].

5. Conclusion

The fresh water teleost, *Clarias batrachus* can sustain starvation for prolonged period. Food deprivation influences the biology of the body at various levels, especially the biochemical composition of various organs. The ascorbic acid content was higher in males than in females in all the tissues investigated under normal conditions. It was high in the brain and low in the liver. However, after 40 days of starvation, the higher depletion was observed in liver (73% in male & 78% in female) and lower depletion in the brain (57% in male & 48% in female).

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