

Studies on the Changes in Ascorbic Acid Level of Gonads in *Clarias batrachus* (Linn.) due to Prolonged Food Deprivation

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Abstract

The present paper deals with the influence of starvation on fish which has great ability to adapt in starvation stress. In the present investigation, the effect of prolonged food deprivation on ascorbic acid contents of gonadal tissues has been observed in both the sexes of *Clarias batrachus*. The level of ascorbic acid content was found relatively higher in males than in females. The testes showed higher value of ascorbic acid in normal condition (13.26mg/100gm) in comparison to that of ovaries (8.41mg/100gm). During prolonged food deprivation, the gradual decrease in ascorbic acid level has been noticed in both the types of gonads which can be related to enhanced gluconeogenesis. After 40 days of starvation, the higher depletion was observed in female gonad (about 53%) and lower depletion in the male gonad (about 51% only) of *C. batrachus*.

Key words: ascorbic acid, *Clarias batrachus*, gonads, starvation.

Introduction:

Higher vertebrates are found to withstand starvation only for brief periods. In contrast, fishes can withstand prolonged periods of food deprivation in their natural environments during various stress conditions (Hinch *et al.*, 2005 & Miller *et al.*, 2009). Many species of bony fishes demonstrate the ability to withstand prolonged periods of starvation, ranging from few months to up to few years, (Larsson & Lewander, 1973). 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.

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 (Mustafa, 1983). Starvation affects the physiological status and biochemical constituents of fish (Rajyasree & Naidu, 1989; Mukhopadhyaya *et al.*, 1991; and Tripathi & Verma, 2003).

In the light of above fact, the present work has been designed to know the level of ascorbic acid content of muscles 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.

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 Shrestha (1981) and Srivastava (2006). The fish were treated with 0.1% KMnO₄ 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. 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 muscles and gonads was the same as that of Kanungo and Patnaik (1964) which is the modification from the Roe (1954).

Weighted quantity of the gonads, muscles, gonads and muscles 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 (1954). 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 gonadal homogenates was determined from the standard.

Results:

In the present study, the level of ascorbic acid was found relatively higher in males than in females under both normal and starved conditions. Starvation of *Clarias* caused a gradual and progressive decrease in the ascorbic acid level of gonads. In the gonads of both the male and female, significant depletion was observed only after 20 days of starvation. After 40 days of starvation, the ascorbic acid depletion was about 51% in testes and about 53% in ovaries. The level of ascorbic acid contents in the gonads of male and female *Clarias* are given in Table-I and the Figure-1.

Table-I

Ascorbic acid content of muscles and gonads (mg/100gm wet tissue) of C. batrachus

Sex	Control	Periods of Starvation			
		10 days	20 days	30 days	40 days
Male	13.26 ± 0.17	12.03 ± 0.24	11.49 ± 0.25	8.91** ± 0.25	6.43** ± 0.31
Female	8.41 ± 0.16	8.05 ± 0.24	7.59 ± 0.25	5.53** ± 0.25	3.96** ± 0.12

Values are the mean of eight samples of both male and female ± SE

** Significant

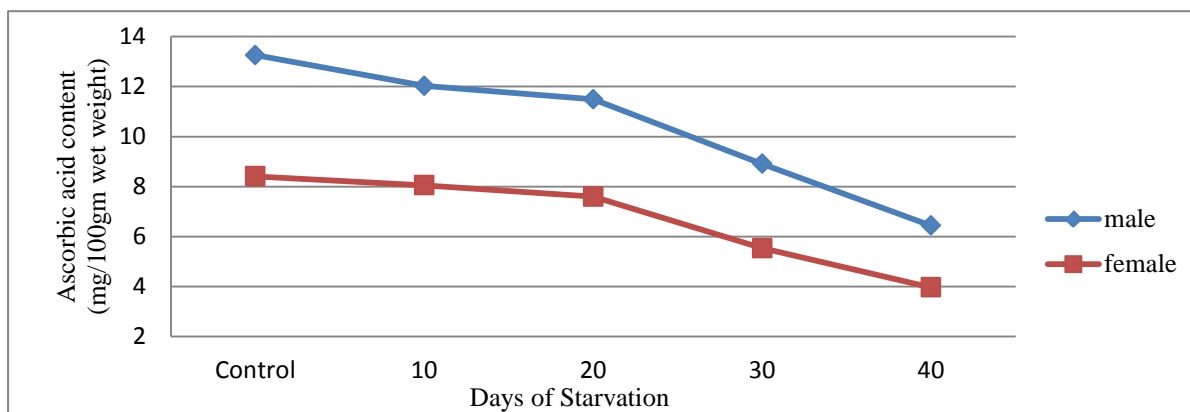


Figure 1. Effect of Starvation on ascorbic acid content of gonads in *C. batrachus*.

Discussion:

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. It is one form of vitamin C. A little is known about metabolic reactions which require ascorbic acid, but it is said to be involved in oxidation-reduction system as catalyst (Bal & Kalyani, 1960a). It is considered as a fatigue retardant and anti-oxidant (Paratheswararao, 1967). Ascorbic acid is necessary for the production of connective tissue and the healing of wounds (Siddiqui, 1967). It has been reported that the concentration of ascorbic acid rises in summer and falls in winter (Fomin & Romanjuk, 1937 and Shanta & Motelica, 1962). It is essential for the proper functioning of the formative cells of various tissues and maintenance of normal state of the intercellular substance like mucoprotein and collagen in bone, cartilage, teeth, skin and connective tissues (Prosser, 1984).

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 (Prosser, 1984). Fish, like other animals, contain ascorbic acid in their tissues and synthesize it for themselves. The values of ascorbic acid observed in liver and muscles of *Clarias batrachus* are in conformity with Leblond *et al.* (1938) and Bal & Kalyani (1960b). The concentration of ascorbic acid in the gonads of *Clarias batrachus* declines after 10 days of starvation and continues to decline further with the progressive days of starvation (Table-I).

During starvation animals live on component tissues of their own body for energy purposes (Wright, 1976). The animal depends on the dietary source of hexoses for ascorbic acid synthesis (Briggs, 1962). Due to rapid consumption of carbohydrates during starvation, the animal fails to get a sufficient amount of hexoses. Hence, the ascorbic acid synthesis decreases 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. The present observations are in conformity with Sinha (1966) and Dvorak (1974).

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 under both the normal as well as starved conditions. However, after 40 days of starvation, the higher depletion was observed in female gonads i.e., about 53% and lower depletion in the male gonads i.e., about 51% only.

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