Nepalese Journal of Biosciences **5(1): 26-30 (2015)** https://doi.org/10.3126/njbs.v5i1.41725

Prolonged starvation effects on ascorbic acid level in the muscles of a freshwater teleost, *Clarias batrachus*

Nayan Kumar Prasad
Research Laboratory, C. M. Science College, LNMU, Darbhanga.
Department of Zoology, R.R.M. Campus, Janakpur, Tribhuvan University, Nepal.
E-mail: nayankumar.prasad@gmail.com

Abstract

The effect of prolonged food deprivation on ascorbic acid content of muscles has been observed in both the sexes of the fish. The level of ascorbic acid content was found relatively higher in males than in females. In males, the muscles showed higher value of ascorbic acid in normal condition (12.07mg/100gm) in comparison to that of females (9.66mg/100gm). During starvation, the gradual decrease in ascorbic acid level has been noticed in both the sexes of the fish. After 40 days of starvation, the higher depletion was observed in female (about 84%) and lower depletion in the male (about 81%) of *C. batrachus*. This shows substantial decline in aerobic and biosynthetic capacity of the fish during starvation period.

Key words: Ascorbic acid, Clarias batrachus, muscles, starvation.

Introduction

Vertebrates other than fish can withstand starvation only for brief periods. On the other hand, 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 teleosts 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 air-breathing catfish, *Clarias batrachus* were used as experimental animals as they are easily available in local ponds and rivers.

The effects of experimental starvation have been studied on various body and blood constituents of fishes by several investigators in different parts of the world, but in Nepal, such studies on freshwater fishes are scanty. 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; 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 of *C. batrachus*during prolonged period of starvation of 40 days at an interval of every 10 days.

Materials and 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% $KMnO_4$ 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. 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.

On scheduled dates, the fish were dissected out and the muscles were removed from the lateral sides with the help of scalpel and scissor. They were properly cleaned by removing nerves and other attached tissues, and were placed immediately in the ice-cold fish saline, previously prepared (Young, 1933). Before use, the tissues were nicely blotted with filter papers.

The method adopted for the extraction and estimation of ascorbic acid of the muscle was the same as that of Kanungo and Patnaik (1964) which is the modification from the Roe (1954). Weighted quantity of the 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 femalesunder both the normal (12.07mg/100gm in male &9.66mg/100gm in female) and the starved conditions (2.31mg/100gm in male & 1.54mg/100gm in female) (Table 1).

Table 1: Ascorbic acid content of muscles (mg/100gm wet tissue) in *C. batrachus*

Sex	**Days of Starvation**				
	Control	**10**	**20**	**30**	**40**
Male	12.07 ±0.29	8.71 ±0.25	7.92** ±0.26	5.54** ±0.26	2.31** ±0.18
Female	9.66 ±0.52	7.48** ±0.22	4.62** ±0.18	3.62** ±0.18	1.54** ±0.16

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

Starvation of *Clarias* caused a gradual and progressive decrease in the ascorbic acid level of muscles. In the muscles of both the male and female *Clarias*, significant depletion was observed from the beginning of starvation. After 40 days of starvation, the ascorbic acid depletion was

about 81% in male and about 84% in female. The level of ascorbic acid contents during different periods of starvation in the muscles of male and female *Clarias* are given in table 1.

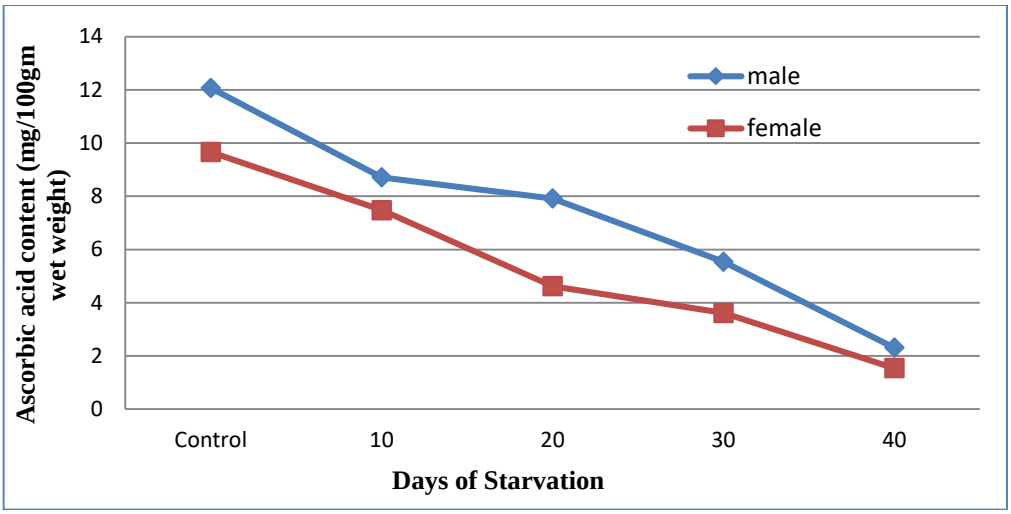

Figure 1. Effect of Starvation on ascorbic acid content of muscles in *C. batrachus*.

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). The concentration of ascorbic acid has been noticed to rise in summer and fall in winter (Fomin & Romanjuk, 1937; Shanta&Motelica, 1962). It is essential for the proper functioning of the formative cells of various tissues and maintenance of normal state of the intarcellular 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 and Kalyani (1960b). The concentration of ascorbic acid in the muscles of *Clarias batrachus* declines after 10 days of starvation and continues to decline further with the progressive days of starvation (Table 1).

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 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. 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 as all the control fish survived during the entire experimental 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 muscles i.e., about 84% and lower depletion in the male muscles i.e., about 81% only.This shows substantial decline in aerobic and biosynthetic capacity of the fish during starvation period. It has a great impact on fish growth.

Acknowledgement

The author renders his heartfelt gratitude to Dr. R. P. Sinha and, Dr. A. K. Verma, Professors, Department of Zoology, C. M. Science College, LNMU, Darbhanga, India, for his generous and constant guidance as well as for providing lab facilities for this research work.

References

Bal, P. A. & Kalyani, M., 1960 a. Studies on the ascorbic acid content of fish tissues. *J. Anim. Morph. Physiol*., 7: 162-166.

Bal, P. A. & Kalyani, M., 1960 b. Studies on the ascorbic acid content of fish tissues. *J. Zool. Soc. India*., 12: 216 – 219.

Briggs, M.H., 1962. Some aspects of the metabolism of ascorbic acid in insects.*Comp. Biochem. Physiol.*,5:241–252.

Dvorak, N., 1974. Effect of corticotrophin, starvation and glucose on ascorbic acid levels in the blood plasma of piglets.*Nutr.Catabolism*.,16: 215-222.

Fomin, S.V. & Romanjuk, N.M., 1937. The ascorbic acid contents of the tissues of various fish. *Ukr.Biokhem.Zh.*,10: 365-378.

Hinch, S.G., Cooke, S.J., Haeley, M.C. & Farrel, A.P., 2005. Behavioral physiology of fish migrations; salmon as model approach.*Fish Physiol*., 24: 239-295.

Kanungo, M.S. & Patnaik, B.K., 1964. Ascorbic acid and ageing in rat. *Biochem. J.*, 90: 637.

Larsson, A. & Lewander, K., 1973. Metabolic effects of starvation in the eel, *Anguilla anguilla* L. *Comp. Biochem. Physiol.*, 44 A: 367-374.

Leblond, C.P., Giroud, A., Ratsimamange, R. & Gero, E., 1938. The normal level of ascorbic acid.*Bull. Soc. Chim. Biol*., 20: 1079-1087.

Miller, K.M., Schulzem, A.D., Ginther, N. Lis, Patterson, D.A., Farrell, A.P. & Hinch, S.G., 2009. Salmom spawning migration; metabolic shifts and environmental triggers.*Comp. Biochem. Physiol*., 4D: 75-89.

Mukhopadhyaya, P.K., Mohanty, S.N., Das, K.M., Sarkar, S. & Batra, B.C., 1991. Growth and changes in carcass composition in young of *Labeorohita* and *Cirrhinusmrigala* during feeding and starvation. In: *Fish Nutrition Research in Asia*. Proc. of the 4th Asian Fish, *Spec. Publ. Asian Fish. Soc.*, 5: 87-91.

Mustafa, S., 1983.Changes in Biochemical composition in Starving Catfish, *Heteropneustes fossilis*.*Japanese Journal of Ichthyology*,29: 4.

Paratheswararao, V., 1967. Some mechanisms underlying thermal acclimation in a fresh water fish, *Etroalus maculates* (Teleosti). *Comp. Biochem. Physio*.,21: 619-625.

Prosser, C.L., 1984. *Comparative Animal Physiology*.3rd Edition.Satish Book Enterprise Booksellers and Publishers, Agra.

Rajyasree, M. & Naidu, K.R.P., 1989. Starvation induced changes in biochemical aspects of hepatic tissue of fish, *Labeorohita*. *Indian J. Fish.*,36: 339-341.

Roe, J.H., 1954.Chemical determination of ascorbic and dehydroascorbic and diketogulonic acid.In *Methods of Biochemical Analysis*, vol. i (ed. D. Glick.) New York: Interscience Publishers.

Shanta, N. & Motelica, I., 1962. Influence of starvation on muscles and gonads cholesterol levels of the catfish *Heteropneustes fossilis. Proc. Indian Academy of Science*.,881: 205-208.

Shrestha, J., 1981. *Fishes of Nepal* (1st Ed.) Curriculum Develop. Centre, Tribhuvan University, Kathmandu, Nepal.

Siddiqui, M.A., 1967. Seasonal variation in the ascorbic acid content and calcium content in different tissues of *Ophiocephalus punctatus* (Bloch).*I. J. Exp. Biol*., 5: 54-55.

Sinha, R.C., 1966. Effect of starvation on the concentration of ascorbic acid in the pedipalp muscle of scorpion, *Palamnasusbengalensis*.*Experiments*, 22: 221.

Srivastava, G.J., 2006. *Fishes of UP and Bihar*.11th ed. VishwavidyalayaPrakashan, Varanasi, India.

Tripathi, G. & Verma, P., 2003. Starvation-Induced Impairment of Metabolism in a Freshwater Catfish.*Z. Naturforsch*., 58c: 446-451.

Wright, S., 1976.*Applied physiology*, Oxford Univ. Press, London. 12th edition, 204-466.