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ORIGINAL RESEARCH ARTICLE

Impact of Severe Hypoxia on the Metabolic Alterations in the Fresh Water Catfish *Clarias batrachus*

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ABSTRACT

The present work was aimed to study the effect of hypoxia on some biochemical parameters in muscle, liver and gills tissues; and effect of hypoxia of the air breathing catfish *Clarias batrachus*. The fishes were dissected and excised the required tissues, i.e. muscles, liver and gills and washed with distilled water, prepared a10 % homogenate mixture by grinding with tris buffer (.01 M), and preserved in deep freezer for further biochemical analysis. Pyruvate was estimated by 2, 4-dinitrophenilhydrazine adapted from Lu, protein by Lowery's method, and RNA by Orcinol method Tillmans & Philippi. The supernatant contained the source of all experimental materials (Pyruvate, Protein, and RNA). Highest concentration of protein, pyruvate and RNA measured was found in liver compared to muscle and gills. In gill tissue the observed percentage changes showed decreased level of protein in 2 and 4 hrs. Liver tissue, when compared to normal, the percentage decreased. Similarly, there is a decrease in muscle tissue. RNA evaluation is observed that gill of 2 hrs hypoxia treated fish showed higher than the normal gill and in contrary 4 hrs hypoxia showed percent decreased count. In liver, 2 hrs hypoxia exhibited increase and 4 hrs hypoxia decreased. In muscle 2 hrs and 4 hrs, showed increased count. Pyruvate level of gill from fishes exposed to 2hrs and 4 hrs hypoxia showed increase when compared with normal gills. Liver tissues of 2hrs and 4 hrs hypoxia exhibited percent increase when compared to the normal liver tissues. Muscle tissues showed variation when compared to other two showed increase for 2hrs hypoxia and decrease for 4 hrs hypoxia when compared to the normal muscle tissue.

Key words: Environmental Stresses, Biochemical adaptation, Protein, Pyruvate, RNA content, Air breathing catfish *Clarias batrachus.*

1. INTRODUCTION

Fish is a rich source of animal protein and its culture is an efficient protein food production system from aquatic environment. Air-breathing fish are common in the tropics, and their importance in Asian aquaculture is increasing, as these fishes grow in shallow ponds and tolerate higher water temperature and thereby study to withstand dry summer spell. In contrast to the ideal conditions that normally prevail in the aquatic environment, fishes are sometimes faced with situations where little oxygen is present in the water. In places such as swamps and pools, the absence of wind and water currents prevents the mixing of the oxygen-rich upper layer with the rest of the water column [1].

In other places, an over abundance of aquatic animals or decomposing matter depletes oxygen because of increased consumption. These conditions are not common, and therefore it is not surprising to learn that fishes have evolved special behaviors to cope with low oxygen levels. Biologists, being the curious lot that they are, have sought or created hypoxic (low oxygen) or anoxic conditions and catalogued the reactions of fishes to them , and found that adaptations to low oxygen can be behavioral, physiological or biochemical responses. Fishes that live in frequently hypoxic habitats may have more hemoglobin in their red blood cells, and more of those cells in their blood, and therefore a higher

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blood capacity to take up and transport oxygen. Their body tissues may contain more myoglobin, a molecule that can bind up oxygen and therefore act as an oxygen store [2]. But, their main adaptation to low oxygen is anaerobic metabolism, but is not very efficient and can lead to the accumulation of relatively toxic by-products, such as lactic acid, and therefore when oxygen is present anaerobic metabolism is put aside in favor of its more efficient aerobic counterpart. But when oxygen is rare and metabolic demand is low, as in a cold water fish for example, anaerobic metabolism can contribute to survival for days, weeks, or even months [3].

The Walking catfish *Clarias batrachus* can be found in a variety of habitats, but they are most commonly encountered in muddy or swampy water of high turbidity [4, 5, 6]. The native habitat of walking catfish is tropical Southeast Asia. Intolerance to cold temperature is range limiting [7]. Behavioral avoidance of environmental extremes during cold/dry seasons involves burrowing into pond and river banks to enter a dormancy that is interrupted with the arrival of spring rains. Deep water may also serve as a thermal refugium during cold snaps. *C. batrachus* is euryhaline across its native range, inhabiting fresh and brackish water as well as muddy marshes [8].

The species thrives in estuarine waters up to 18 ppt salinity [9]. Several physiological adaptations allow the species to leave the water for extended periods. These include a greatly reduced gas bladder and gills that are structurally stiffened to prevent collapse on land. The gills also exhibit highly vascularized arborescent (tree-like) organs that act as accessory breathing structures aiding respiration on land and in stagnant waters. Considering the behavioral and physiological strategies to cope with hypoxia, the present work reports the metabolic changes observed in the catfish under severe environmental hypoxia, avoiding the air breathing strategy, and considering the role of both responses.

2. MATERIALS AND METHODS

Animal

The catfish (*Clarias batrachus*) were purchased from VEE Care Aquarium. The animals were adult fish but living off their reproductive period. They were kept in laboratory condition, in aerated tanks for two weeks for acclimatization. Fish were fed fish pellets once a day.

Experimental Design

The fish were separated into three groups for exposure to hypoxia condition and anoxia condition. Group I was used as control and kept under normoxia, Groups II and III were subjected to hypoxia, the former for 2 hours and the latter for 4 hours. Hypoxia was induced by disconnection of the aeration system and avoiding the fish to reach the surface by an iron net fitted one-inch below the water surface to prevent bimodal respiration. Anoxia was induced by disconnection of the aeration system and by fitting a glass slab one-inch below the water surface to completely cut off oxygen supply. The animals of Group I, II and III were killed by a blow on the head. The fish were dissected and excised the required tissues, i.e. muscles, liver and gills and washed with distilled water, prepared a10 % homogenate mixture by grinding with tris buffer (0.01 M), and preserved in deep freezer for further biochemical analysis. The supernatant contained the source of all experimental materials (Pyruvate, Protein, and RNA).

Biochemical analysis of tissue sample

Pyruvate was estimated by 2, 4-dinitrophenilhydrazine adapted from Lu [10], protein by Lowery *et al.* [11], and RNA by Tillmans and Philippi [12].

Chemicals

The chemicals were purchased from LOBA, SRL, CDH and Merck, and were of analytical grade.

Statistical Analysis

All the data were collected from the animals for each set of experiment and were statistically analyzed for student “t” test at 0.05% and 0.01% significant level.

3. RESULTS

To observe the biochemical changes in the animal in response to adverse environmental condition, the two different stages of hypoxia condition are compared with normal environmental condition. In hypoxia condition, cat fish can adapt themselves upto around 5 – 6 hours, and can survive for 8 – 9 hours out of water, after which the fishes die. So, the two hypoxia stages I had selected to observe their biochemical changes were 2 hours and 4 hours respectively [13]. Liver is involved in most of the metabolic (biochemical) processes, and due to hypoxia condition protein catabolism takes place in the liver to produce energy for survival. During protein catabolism, amino acids are synthesized; some of which directly enter the Kreb’s cycle and some of them produce carbohydrate through the process of gluconeogenesis [14]. Since, carbohydrate cannot be oxidized due to lack of oxygen; pyruvate accumulation takes place as the glycolytic pathway is continued. To replace the catabolized

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protein new protein has to be synthesized, and for more protein synthesis also more RNA transcription takes place from normal to slight hypoxia [15], but RNA synthesis lowers down at acute hypoxia conditions. In case of LDH activity, it has been observed that it increases from normal to less hypoxia condition, but decreases at acute hypoxia condition, because of the abnormality of the whole function of the body [16]. Likewise the specific activity has also shown high level of increase from normal to 2 hour hypoxia condition, and after that it decreases at 4 hour hypoxia condition. In the present investigation, variation of protein content was recorded in 2 hrs hypoxia and 4 hours hypoxia. In gill tissue, the observed percentage changes showed decreased level of protein in 2 and 4 hrs was 1.40 and 82.24 respectively. Whereas in the liver tissue, when compared to normal, the percentage decreased was 4.36 and 9.40 in 2hrs and 4 hrs respectively. Similarly, there is a decrease in muscle tissue like 48.13 and 55.79 percent for 2 hrs and 4 hrs, hypoxia respectively.

Likewise, the RNA level was also observed in 2 hrs and 4 hrs hypoxia which was compared with normal tissues. It was observed that gill of 2 hrs hypoxia treated fish showed 121 percent higher than the normal gill and in contrary 4 hrs hypoxia showed 14.28 percent decreased count. The same criteria has been observed in liver, 2 hrs hypoxia exhibited 164 percent increase and 4 hrs hypoxia decreased by 1.70 percent. But in muscle unlike gills and liver both 2 hrs and 4 hrs, showed increased count, the observation was 196.75 and 72.99 percent for 2hrs and 4 hrs respectively.

It was noticed that pyruvate level of gill from fishes exposed to 2hrs and 4 hrs hypoxia showed 86.6 and 16.66 percent increase when compared with normal gills. Liver tissues of 2hrs and 4 hrs hypoxia exhibited 50.63 and 29.11 percent increase when compared to the normal liver tissues. Muscle tissues showed variation when compared to other two; the percentage increase was 16.66 for 2 hrs hypoxia and decrease of 16.66 percent for 4 hrs hypoxia when compared to the normal muscle tissue.

The liver contained the highest amount of protein and gills the lowest among the tissues examined. Muscle contained highest amount of protein in normal and when exposed to hypoxia the protein content decreased gradually. Highest protein content in liver was found in normal and lowest in 4 hour hypoxia condition. In gills protein content decreased gradually from normal to hypoxia condition (**Fig 1**). Highest amount of pyruvate was found in liver and lowest in muscle. In muscle, lowest amount of pyruvate was present in 4 hrs hypoxia and highest in 2 hour hypoxia condition. In liver, it was somewhat different from the muscle in that the lowest amount of pyruvate was found in normal condition, and highest in 2 hour hypoxia condition. In case of gills normal condition contained lowest amount of pyruvate and the highest in 2 hour hypoxia condition (**Fig 2**). Liver contained the highest amount of RNA and gills the lowest. In normal condition muscle contained lowest amount of RNA whereas 2 hour hypoxia condition showed highest amount. Liver showed somewhat different results from muscle. Highest amount of RNA was found in 2 hour hypoxia condition and lowest in 4 hour hypoxia condition. Gills also contained the highest amount of RNA in 2 hour hypoxia condition and decreased to 4 hour hypoxia condition (**Fig 3**).

Fig 1: Variation of Protein content (mg/g wet weight) in muscle, liver and gill tissue in normal and hypoxia condition fishes

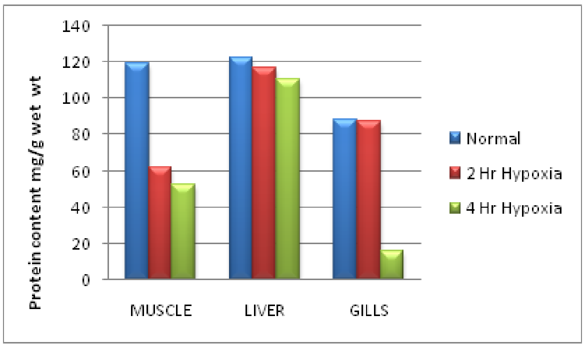


Fig 2: Variation of Pyruvate content (mg/g wet weight) in muscle, liver and gill tissue in normal and hypoxia condition fishes

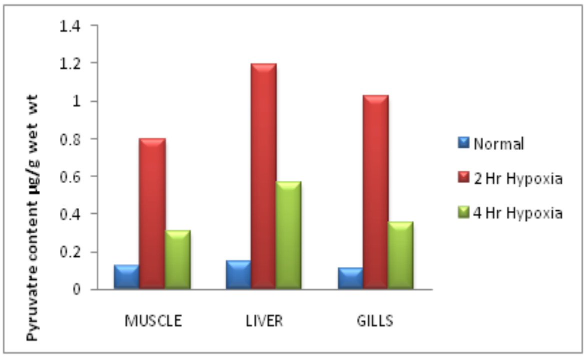
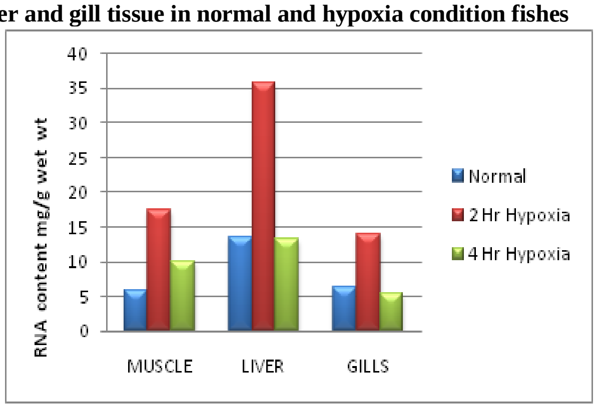


Fig 3: Variation of RNA content (mg/g wet weight) in muscle, liver and gill tissue in normal and hypoxia condition fishes



4. DISCUSSION

For normal growth and functioning a good environmental condition is necessary for all living organisms. If the environmental conditions are changed it has an effect on the organism, but all organisms to some extent have to adapt themselves to the unwanted environmental conditions. However, prolonged environmental changes might cause death of the organism.

Among the vertebrates, almost all species are capable of adapting to the various changes in environmental conditions for their survival. But, there is a limit of adaptation beyond which the animal cannot survive. Among the fishes, cat fish (*Clarias batrachus*) is an example of such an animal. They are air breathing fish having the ability to withstand the changes in environmental conditions. Several physiological adaptations allow the species to leave the water for extended periods. These include a greatly reduced gas bladder and gills that are structurally stiffened to prevent collapse on land. The gills also exhibit highly vascularized arborescent (tree-like) organs that act as accessory breathing structures aiding respiration on land and in stagnant waters. Hypoxia is one of the adverse condition due to which the changes occurs not only in their anatomical and morphological levels but also in their biochemical, haematological, and physiological levels.

Several species besides *Clarias batrachus* have been reported as responsive to environmental oxygen concentration, particularly in terms of blood parameter variations [17]. Blood responses, prior to many others, are considered fundamental to internal adjustments to cope with a number of stressors like hypoxia, anoxia condition. Among that, increase of hematocrit is usually observed as the result of erythrocyte swelling, decrease of plasma volume, increase of red blood cell number or a combination of such factors [18]. *C.batrachus* presented the usual blood response to hypoxia, i.e., increase in haematocrit (PCV). Haemoglobin concentration also increased, along with increase in RBC count, which may be due to enhanced erythropoiesis because of environmental stress. Blood glucose level increased in 2 hour hypoxia, may be due to its mobilization from liver to meet the increase in energy demand, but decreased in 4 hour hypoxia. Protein showed a different trend than glucose, in 2 hour hypoxia condition there were no changes in protein level compared to normal condition, but decreased in 4 hour hypoxia condition suggesting stress induced proteolysis.

Among the three tissues examined under hypoxia condition, liver showed more changes in all the biochemical parameters in hypoxia condition than in normal condition, as liver is involved in most of the metabolic (biochemical) processes. Due to hypoxia condition protein catabolism takes place in the liver to produce energy for survival. During protein catabolism, amino acids are synthesized; some of which directly enter the Kreb's cycle and some of them produce carbohydrate through the process of gluconeogenesis. Since, carbohydrate cannot be oxidized due to lack of oxygen , pyruvate accumulation takes place as the glycolytic pathway is continued To replace the catabolized protein new protein has to be synthesized , and for more protein synthesis more RNA transcription takes place from normal to slight hypoxia , but RNA synthesis lowers down at acute hypoxia conditions

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