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EFFECT OF HYPOXIA ON CARBOHYDRATE METABOLITES AND HAEMATOLOGICAL INDICES OF SNAKEHEAD FISH, *CHANNA STRIATUS*

ASHOK KUMAR, ARVIND KUMAR SHARMA[1] , *SUSMITA SRIVASTAVA[2] AND ASHOK KUMAR[3]

Department of Zoology,
M. L. K. (P.G.) College,
BALRAMPUR - 271201 (U.P.) INDIA
[1]Department of Zoology,
KS Saket PG College , Ayodhya,
FAIZABAD - 224123 (U.P.) INDIA
[2]Department of Zoology,
Shiv Harsh Kisan PG College,
BASTI-272001 (U.P.) INDIA
[3]Department of Zoology,
BSNV PG College,
LUCKNOW 226001 (U.P.) INDIA
*Corresponding Author
Email : srivastavsusmita@gmail.com

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ABSTRACT

The effect of hypoxia on the level of carbohydrate metabolites glycogen and glucose were studied in brain, muscles and liver of snakehead fish, *Channa striatus*. Hypoxia exposure decreased the glycogen level in brain and muscles of female fishes but increased in male fishes. Hypoxia exposure increased glucose level in brain and muscles of female fishes but decreased in male fishes. The level of glycogen and glucose in liver decreased in both male and female fishes during hypoxia. In the present study, the haematological data of *Channa striatus* revealed that significant increase (P<0.05 and Â0.01) was observed in red blood cell count (RBC) and hemoglobin (Hb) content respectively. White blood cell (WBC) count showed significant (P<0.01) increase when compared to the control. Increase in haematological indices means that fish was exposed to hypoxia and was under stress.

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KEY WORDS : *Carbohydrate metabolites, Haematologyical indices, Hypoxia, Stress*

Introduction

Channa Striatus (family-Channidae) is a striped snakehead fish. It grows up to 1 m in length, though because of fishing, this size is rarely found in the wild. It has a widespread range covering southern China, Pakistan, most of India, southern Nepal, Bangladesh, Sri Lanka and most of Southeast Asia. *Channa striatus* is an important food fish in its entire native range and is of considerable economic importance. Metabolic depression is a key factor in facultative anaerobiosis, anhydrobiosis, hibernation and aestivation. It allows organisms to economize on fuel and energy reserves in the face of environmental stress of unpredictable duration[4]. During exercise fishes requires more energy from the breakdown of fuel reserves to power muscular contraction. The use of different fuel sources

TABLE-1 : Effect of Hypoxia on carbohydrate metabolites of *Channa striatus*

[Data reported as means ± SEM (N=6)]

Metabolites	Tissue	Male		Female	
		Control	Hypoxia	Control	Hypoxia
Glycogen	Brain	10.08±0.78	12.82±0.86***a***(+27.18%)	10.05±0.38	7.92±0.32***a*** (-21.19%)
	Muscles	23.0±0.38	26.0±0.41***b*** (+13.04%)	24.0±0.32	19.3±6.18***b*** (-19.58%)
	Liver	70.30±0.78	63.80±0.18***b*** (-9.24%)	68.30±9.4	64.41±0.32 (-5.69)
Glucose	Brain	1.68±0.31	0.89±0.42***a*** (-47.02%)	1.55±0.34	1.62±0.29 (+4.51%)
	Muscles	3.64±0.44	3.38±0.38 (-7.14%)	3.52±0.41	20.12±0.72***a*** (+47.59%)
	Liver	2.50±0.18	1.70±0.03***a*** (-32.0%)	2.48±0.16	1.68±0.29***a*** (-32.25%)

[*a* P < 0.005,*b* P < 0.001 compared to control (student 't'-test)]

depending on both nutritional status and exercise intensity. Lipids have traditionally been coincided as fuel for long term aerobic swimming[10]. Fishes have low glycogen reserve in liver and muscles in comparison to mammals, but in brain the concentration of glycogen is higher and tends to be rigorously maintained. Besides this, brain glycogen levels are also maintained at their resting values during physical exercise[3]. Use of glycogen reserves of liver and muscles in fishes, during stress condition, has been studied by number of workers but nothing is known about the brain glycogen[9]. In fish, exposure to any stress induce either increase or decrease in haematological levels. Hypoxia is defined as a concentration of dissolved oxygen less than 2 mg/L (2 ppm). the upper limit for hypoxia may be as high as 3-5 mg/L[8]. A number of haematological indices such as haematocrit (the ratio of the volume of red blood cells to the total volume of blood) (Hct), haemoglobin (Hb), red blood cells (RBCs) and so on, are used to assess the functional status of the oxygen carrying capacity of the bloodstream and have been used as an indicator of aquatic environmental stress. Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals. Previous haematological study of pollutants brought knowledge that erythrocytes are the major and reliable indicators of various sources of stress. Haematological studies are useful in assessing the health of fish subjected to changing environmental conditions.

Materials and Methods

1.For Carbohydrate metabolites analysis : *Channa striatus* were obtained from the local fish market of Balrampur. These fishes were kept in laboratory condition for one week. During acclimatization period, they were fed on minced goat liver and pelleted food regularly. The female and male of 25.70 ± 4.65 g weight were selected for the experiment. The fishes were grouped into four sets, two sets of males and two sets of females, with six specimens in each set. One set of each sex was treated as control. Whereas other set was used for experiments. The experimental sets of fishes were subjected to force exercise separately. They were forced to swim for more than 120 minutes. Both control and experimental fishes were taken out separately and were then immediately killed by using the concussive blow to the head of each selected fish. The brain, muscles and liver of fishes were dissected out. These tissues were homogenized in 2 ml of 30% KOH for extraction of glycogen. This homogenate was kept in a pre-maintained water bath at 100°C for about 10-20 minutes. The heated homogenate was allowed to cool down at room temperature and 2 ml of 95% ethanol was added, it was again placed in the water bath (premaintained at 100°C) for 10 minutes, after which it was allowed to cool down at room temperature. Subsequently, it was centrifuged for 20 minutes at 3000 rpm. The supernatant was discarded. Precipitate was dissolved in 2 ml distilled water and used as sample. Estimation of glycogen and glucose was made colorimetrically[7].

2. For haematological analysis: The acclimatized healthy fishes were divided into two group in two aquaria. Each group have five fishes. The aquarium I comprising normal control group. The aquaria II are experimental groups of fishes exposed to concentration of dissolved oxygen as 5 mg/L of water, for hypoxic conditions in water respectively. The duration of this experiment is of 20 days. Blood was collected by heart puncture of the fish by heparinised syringe in vials containing ethylenediaminetetra acetic acid (EDTA) as an anticoagulant, which was used to estimate the haematological parameters. The effect of hypoxia was observed by comparing the control and experimental groups. The RBC counts were made by Neubauer haemocytometer. Blood was diluted 1:200 with Hayem's Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as10^6 mm^{-3}, counting was done in the five smaller squares i.e., 1st, 5th, 13th, 21st and 25th. The RBC's on the lower and right sides of a square were added in the total, while those on the upper and left sides were rejected. WBC counts were made by Neubauer haemocytometer .Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. Four large (1 sq mm) corner squares of the haemocytometer were counted under the microscope. The cells touching the boundary lines were not counted. The total number of WBC was calculated in mm^3 x 10^3 .Haemoglobin (Hb) was determined with haemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethaemoglobin method.

Result and Discussion

During the course of present investigation,

the hypoxia condition produced by prolonged summing (more than 120 minute) and their effect on carbohydrate reserves of brain, muscles and liver have been studied and compared with normal (control) fish . Effect of hypoxia exposure on carbohydrate reserve level was differing in each tissue and even in each sex. The glycogen level increased considerable in the muscles and brain of male fish where as decreased in female fish but their level decreased in liver of both sexes.

The level of glycogen in brain and muscles of male fish increased with decreasing the level of glucose during stress condition reveals that glucose is converted into glycogen and enabling a glycogen sparing effect during intense exposure (Table-1). Workers[9] also reported that glycogen level increases in brain of male fish during exercise. During exercise fishes use lipid as source of energy fuel and accumulate glycogen in muscles[10]. Thus due to aerobic use of lipids (fatly acids) glycogen sparing occurs in males during intense exercise. Possibly due to this reason, increase in glycogen takes place in males than depletion.The level of glycogen decreases with decreasing the level of glucose in female fishes reveals that during stress conditions female fish utilize glycogen as a source of energy and due to break down of glycogen into glucose, the level of glucose increases in brain and muscles. This glucose provides energy during stress. The theory of metabolic depression applies well to fish in hypoxic or anoxic states. A drop in the contribution of oxidative phosphorylation to energy production and an enhancement of the anaerobic contribution are well documented but are often accompanied by an overall suppression of net ATP turnover that helps to conserve fuel use when inefficient fermentive pathways are the primary source of ATP. During forced exercise, hypoxia develop, due to which drop in oxidative phosphorylation takes place and for energy demands enhancement of anaerobic contribution resulted[1,2,4,9]. This anaerobiosis finally leads to suppression of net turn over of ATP. Therefore demand for more and more glucose arises in the body which acts as cause for increase in glycogenolysis and decrease in glycogen. But this explanation holds good for female fishes only, where depletion of glycogen in brain, muscles and liver has been recorded.In case of male, elevation has been noticed during course of study in muscles and brain. In our opinion, during stress condition male use other alternative fuel to fulfill their energy demand rather to break glycogen into glucose. Lipid acts as a source of energy during stress conditions[5,10]. Thus due to aerobic use of lipid (fatty acid) glycogen sparing occurs in male during hypoxia created by intense exercise help in increasing the glycogen level in brain and muscles of males than depletion.Thus, observations suggest that conventional theories concerning, fuel utilization and regulation during hypoxia may not completely accurate as in male snakehead fishes depend on lipid to fulfill their energy requirement.

On the other hand the haematological analysis reveal that all the three variable ie. RBC,WBC and Heamoglobin have significant changes due to hypoxic stress (Table-2). The erythrocyte count of healthy controls showed a mean value of 5.37×10^6 $mm^{-3.}$ The fishes that were exposed to stress in form of hypoxia showed mean value of RBC as 7.52×10^6 mm^{-3}. The values mentioned above showed a significant ($P<0.05$) increase when compared to the control.

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 4.56 $mm^3 \times 10^3$. The fishes of experimental groups exposed to concentration of dissolved oxygen as 5 mg/L of water, for hypoxic conditions in water for 20 days showed mean value of WBC as 8.23 $mm^3 \times 10^{-3}$. The values mentioned above showed a significant increase ($p<0.01$) when compared to the control.

The control fishes showed mean value of 13.6 g/dL for haemoglobin. The fishes of experimental group showed mean value of haemoglobin as 17.75 g/dL. The values for treatments showed a significant ($p<0.01$) increase when compared to the control.

In general, hematocrit is the number of red blood cells (RBC) in circulation and is highly variable among fish species. Active fish, like the blue marlin, tend to have higher hematocrits[6], whereas less active fish, such as the starry flounder exhibit lower hematocrits[11]. Hematocrit may be increased in response to both short-term (acute) or long-term (chronic) hypoxia exposure and results in an increase in the total amount of oxygen the blood can carry, also known as the oxygen carrying capacity of the blood[12]. Acute changes in hematocrit are the result of circulating stress hormones activating receptors on the spleen that cause the release of RBCs into circulation[6]. In the present study we conclude that during chronic

TABLE- 2 : Total count of RBC's, WBC's, and Haemoglobin in the control and exposed to stress due to hypoxia in *Channa striatus*

Variable	Control (mean±SEM)	Stress (mean±SEM)
Number of RBC (10^6 mm^{-3})	5.37± 0.034*	7.52 ±0.329*
Number of WBC (10^3 mm^{-3})	4.56 ± 0.183**	8.23±1.655**
Haemoglobin(g/dL)	13.06 ± 0.265**	17.75±0.713**

Values are given as Mean ± SEM (n = 6). **Significantly different at (P<0.01); *Significantly different at (P<0.05).

hypoxia exposure, the mechanism used to increase hematocrit is independent of the spleen and results from hormonal stimulation of the kidney by erythropoetin (EPO).Increasing hematocrit in response to erythropoietin is observed after approximately one week and is therefore likely under genetic control of hypoxia inducible factor hypoxia inducible factor (HIF). While increasing hematocrit means that the blood can carry a larger total amount of oxygen, a possible advantage during hypoxia, increasing the number of RBCs in the blood can also lead to certain disadvantages. First, a higher hematocrit results in more viscous blood increasing the amount of energy the cardiac system requires to pump the blood through the system and secondly depending on the transit time of the blood across the branchial arch and the diffusion rate of oxygen, an increased hematocrit may result in less efficient transfer of oxygen from the environment to the blood.

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