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Effect of Asphyxiation on the Haemoglobin and Glycogen Content in an African Catfish *Clarias gariepinus*

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ABSTRACT. African catfish *Clarias gariepinus* was exposed to different time of hypoxia. Some of the remarkable changes were observed in stressed fish in comparison to the control one. It was noted that sudden asphyxia produces usual stress reaction, swimming speed is affected below a certain concentration of ambient oxygen. However, significantly decreased haemoglobin and glycogen content were found in the subsequent of hypoxia.

Introduction

In the hypoxic condition, the fish seems in a severe stress and increased oxygen demand which can cause a striking changes in the physiological functioning[1,2]. It is desirable to be able to detect the stressful state before the range of adaptive response is exceeded and a diseased state ensures. Though, if the hypoxic condition of the fish prolonged, the stress-mediated outbreak of some disorder for the aquaculture in the hatchery. In the natural environment, stress caused by various factors, like: temperature, eutrophication, pollution of toxic elements and insecticides have been correlated with epizootic in the wild stock of fish[3]. Various workers in the past have worked on various conditions of stress, like holding[4], anaesthetization[1], and injection[5,6], that are known to be stressful to the fish. The nature of the effect of heavy metals on the fish has been recognised to cause coagulation of mucus on gills resulting in direct damage to gills and asphyxiation[7-10]. But no data are available regarding the effect of asphyxia on the certain blood metabolites and other energy metabolism in African catfish *Clarias gariepinus*, being a hardy fish, have been used in a number of laboratories for experimental purposes. Hence the present investigation confined to evaluate certain physiological changes like glycogen content in liver and muscle and haemoglobin under laboratory conditions with a limit of hypoxia.

Materials and Methods

The fish *Clarias gariepinus* (length 12.6-16.0 cm; weight 11.5-22.2 g) were procured from fish farm (Deerab), located 80 km South of Riyadh (23′30″N., 46′43″E). The fish were transferred in a glass trough (75 gallon) and acclimatized to the laboratory conditions (22.5°C ± 1.0°C, and water hardness 232.58 ± 1.05 mg/L) for 15 days. During the acclimation the fish were fed to satiation with fish pellets (Proximate Analysis: protein, 40%; fat 8.7%; minerals, 10.9% and moisture 6.5%) two times a day. After 15 days of acclimation, ten fish specimen from the glass trough were taken and placed in each of a series of small aquaria (25 L capacity) and were allowed to surface for normal breathing for some time. The same number of fishes were taken as a control and was allowed to surface for normal fresh air in the duration of experiment. The series of aquaria were asphyxiated with Aluminum foil at different interval of time (2, 4, 6 and 8 hours). In each set of experiment the rate of oxygen depletion was determined with the help of the modified Wrinkler's technique or Alsterberg azide modification[11] and after every two hours of asphyxiation, the asphyxiated fish were bleed by tail clipping and the blood was collected in a heparinized tubes for the haemoglobin content. The method of Blaxhal & Daisley[12] was used for the determination of haemoglobin content and expressed in gHb/100 ml of blood. For the determination of glycogen content, the liver and muscle was taken out and extracted according to the method of Ashman & Seed[13] and analysed by the method of[14]. The results of the glycogen content was expressed in terms of µg/g wet weight of tissue. All the assays were performed in duplicate.

Results

It was observed that the fish after asphyxiation showed initial disturbed swimming movement but in a prolonged period of asphyxia, a more or less spiral swimming behaviour was commonly observed. The fish became lethargic and a condition of disorientation was evident. In the aforesaid condition, the opercular beat and surfacing rate of fish increased. The rate of oxygen depletion was minimum in control which was allowed to surface for the entire period in contrast to the groups asphyxiated for different period of time (Table 1).

Glycogen content of fish tested for different durations of asphyxiation are presented graphically in Fig. 1. The trend of declination in quantity of glycogen content and haemoglobin are seems to be inversely proportional to the period of asphyxiation. Percentage of depletion in glycogen content and oxygen depletion are given in Table 1. It is clear that the increase of asphyxiation time, resulted in a significant drop of glycogen level in liver and muscles and haemoglobin content (Table 1).

Discussion

Exposure of *Clarias gariepinus* to different hypoxia brought about a general hyperexitability because if the hypoxic condition of the fish prolonged, the stress mediated

TABLE Haemoglobin, oxygen consumption and percentage depletion of glycogen content in the muscle and liver due to asphyxia in African catfish *Clarias gariepinus*.

Time of asphyxiation	Haemoglobin g Hb / 100 ml	Dissolved oxygen	Oxygen depletion	Percentage of depletion in glycogen	
				Muscle	Liver
Control	8.58	8.46 ± 0.05	–	–	–
2 hours	7.25	6.93 ± 0.65	1.53	9.05	7.32
4 hours	6.50	5.53 ± 0.16	2.93	15.60	30.27
6 hours	6.0	5.20 ± 0.16	3.26	23.96	40.64
8 hours	5.20	4.46 ± 0.14	4.0	28.64	48.86

Haemoglobin:

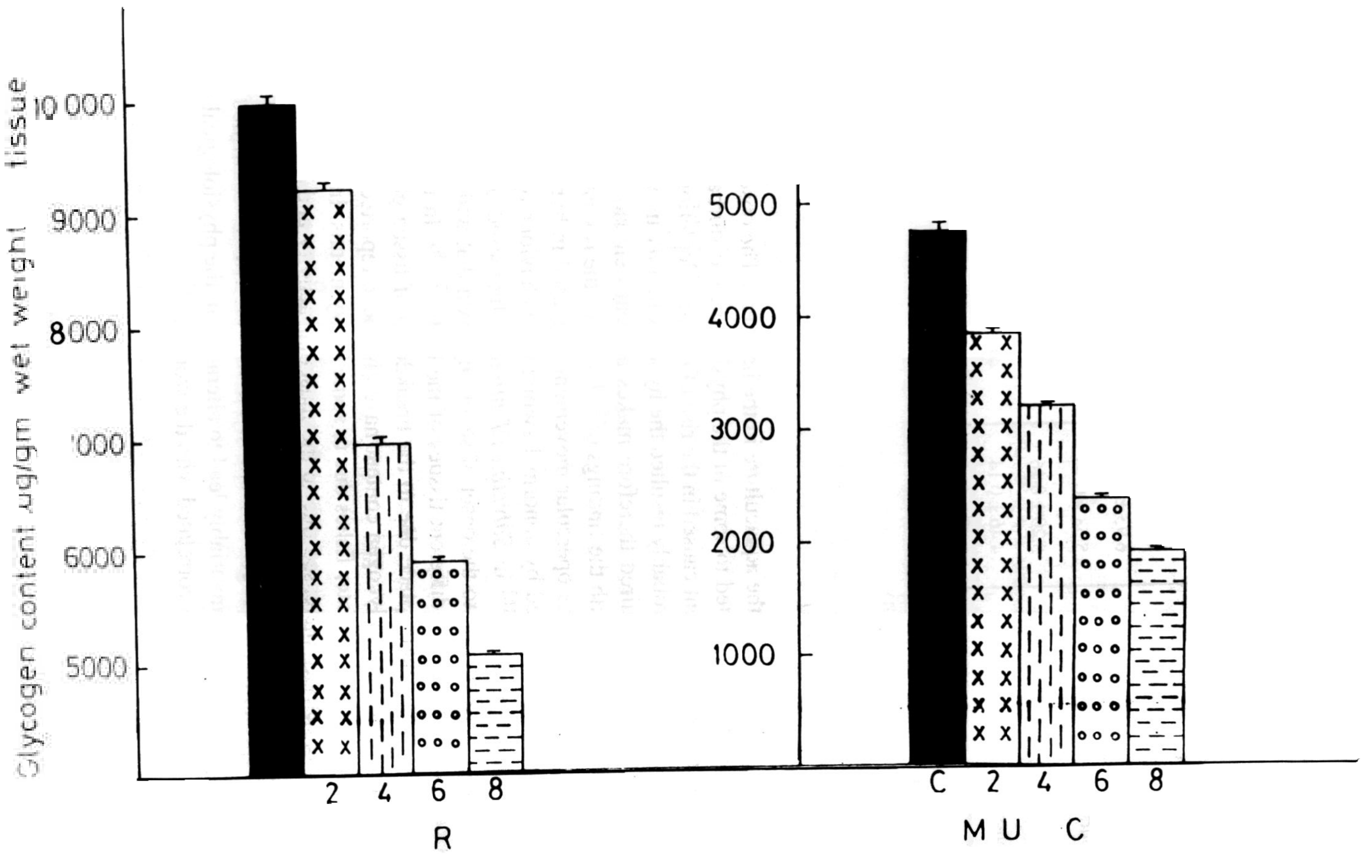
Control vs 2 hr ($P < 0.05$)
Control vs 4, 6, 8 hr ($P < 0.01$)

Glycogen:

Control vs 2, 4 hr ($P < 0.01$)
Control vs 6, 8 hr ($P < 0.001$)

outbreak of some disorder for the aquaculture in the hatchery. The observed behavioural changes obtained are attributed to some of the physiological disturbances which may increase the opercular movement caused in the rise of oxygen, depletion in the asphyxic condition. This consumption probably resulted the hyperexitability in which a considerable amount of energy is required therefore makes a great demand of oxygen. This hypothesis can be correlated with the findings of[8-9]. One of the researchers[15] pointed out that in air breathing fish, the opercular movement increased in hypoxic conditions. Loss of equilibrium as indicated by abnormal swimming behaviour by intoxication is also available in the work of[16] in *Salvelinus fontinalis*. Increased opercular beat in stressed fish may be attributed to the onset of severe hypoxia that will trigger on some of the biochemical changes in different tissues of the body[17, 18]. In the stressed fish, the anaerobic glycolysis takes place due to the breakdown of tissue glycogen. Similar reduction in the stored tissue glycogen content have also been reported by other workers[8-10,19,20] in different freshwater fishes due to some heavy metals. In hypoxic condition the swimming activity increased due to the muscular activity and more energy is required by the fish. This energy can be taken from different organs of the body and may cause the depletion of glycogen content in the tissues. This is confirmed by the fact that all stressed conditions invariably lead to alteration in the physiological mechanism. These observations can be correlated with the work of[21].

Apart from this the haematopoitic tissues also affected from these such changes[22]. The destruction of these tissues is likely to result in decreased blood cell production and a subsequent reduction of red blood cells (RBC) which may have reduced the haemoglobin content. Such reduction can also be obtained by the process of haemodilution through a net uptake of water[10].



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تأثير الاختناق الهوائي على المحتوى الهيموجلوبيني والنشا الحيواني لسمك القرموط الأفريقي *Clarias gariepinus*

علي بن سليمان العقل
قسم علم الحيوان ، كلية العـــلوم ، جامعة الملك سعود
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المستخلص . عرضَ سمك القرموط الأفريقي *Clarias gariepinus* للاختناق الأوكسجيني لفترات متباينة من الزمن .

وتبين من التجربة أن نقص الأوكسجين قد تسبب في حدوث تأثير كبير على الأسماك المعاملة مقارنة بالأسماك التي لم تتعرض للاختناق حيث تمثل هذا التأثير في السباحة السريعة باتجاهات مختلفة وكذلك في النقص الحاد في الهيموجلوبين مقترنًا بالانخفاض الملحوظ في النشاء الحيواني (الجلايكوجين) في كل من العضلات والكبد .