Voyager: Vol. VIII, No. 2, Dec. 2017, ISSN :(p) 0976-7436 (e) 2455-054x Impact Factor 3.8919 *(ICRJIFR)*
UGC Approved Journal No. 63640

Effect of Different Nitrite Concentrations on Haemoglobin Percentage of *Heteropneustes Fossilis*

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Reference to this paper should be made as follows:

Renu Verma & Kapil Kumar,
" Effect of Different Nitrite Concentrations on Haemoglobin Percentage of *Heteropneustes Fossilis*",
Voyager: Vol. VIII, No. 2, Dec2017, pp.109- 113

Abstract

Nitrates themselves are relatively non-toxic, however, when swallowed they are converted to nitrites that can react with haemoglobin in the blood forming methemoglobin, which can not bind oxygen. So, this study was to assess the effect of different nitrite concentrations in the fish Heteropneustes fossilis and knowing the haemoglobin values at different intervals of nitrite exposure. Evaluation of haemoglobin concluded that with increasing concentration of sodium nitrite and also with increasing exposure period, the haemoglobin value decreases. At the end of experimental period, it was lowest i.e., 5.60g/dl. This decrease signifies that the fish's ability to get sufficient oxygen to the tissues is restricted considerably and that results in decreased physical activity.

Key words: *Heteropneustes fossilis, Haemoglobin, non-toxic.*

Introduction

In the environment nitrates are naturally present in soil water and food. Microorganisms can convert nitrate to nitrite. However, human activities have increased environmental nitrate/nitrite concentrations with agriculture being the major source. With rain water this nitrate/nitrite flush out into the water bodies and affect the live stock. In fish nitrite is actively taken up by the gills and accumulates in plasma, gills, liver, brain and muscles (Bath and Eddy, 1980). Nitrite subsequently enters tissues, cells and within the red blood cells, it oxidises haemoglobin to methaemoglobin, which is unable to transport oxygen (Cameron, 1971). Earlier it has been studies that during heavy rainfall and winter feeding this condition boost up. During the winters, this witness, ammonia tends to accumulate in ponds because the water is too cold for these bacteria to function. In extreme cases, this ammonia can contribute to winter kill problems. As water temperature begins to warm in the spring, the bacteria that turn ammonia into nitrate "wake up" long before those that convert nitrite into nitrate. As a result until pond temp is fairly warm, nitrate levels may increase to a point where brown blood becomes a problem (LSU Ag. Center, Com. 2009).

Materials and Methods

The fish, *Heteropneustes fossilis* (Bloch) is selected as an experimental animal and was obtained from local fish market. During the course of investigation, the collected fish was brought to the laboratory in plastic containers with proper handling and then washed in $KMnO_4$ solution and normal saline for 5 minutes each, in order to remove superficial infections. There fishes were held in laboratory conditions for 15 days in order to recover from stress. During this period they were fed on commercially prepared fish feed. The fish of 15±1 cms length and 35±0.5 gm weight was selected for the experiment. To avoid effect of seasonal environmental changes on fish, the physico-chemical characteristics of the water during the experiment were:

- Ø Temperature 23°C – 28°C
- Ø pH 7.0 – 7.5
- Ø Hardness 106mg/L as calcium carbonate.
- Ø CL – 38.6 mg/L

The fishes were divided into 10 groups, each containing 6 fishes. First group was the control group and the rest groups were the experimental group. In experimental group the sodium nitrate concentrations ranged from 50mg/L to 250mg/L with the increment of 25mg/L was added. Nitrite and fresh water controls were included in triplicate. The blood samples were taken from candal vein of the anaesthetized fish.

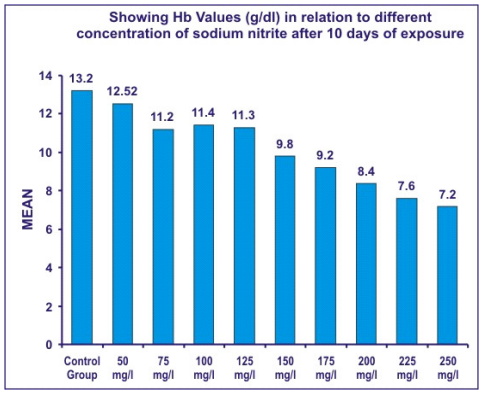
Haemoglobin of the blood sample evaluated by collier method.

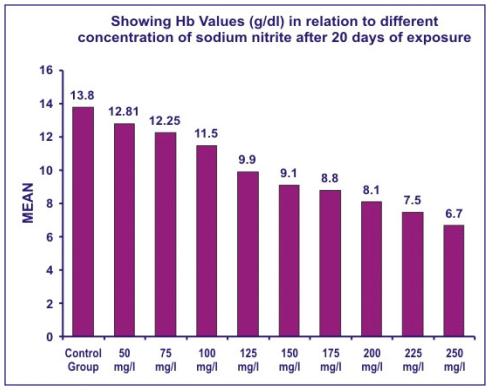
Student 't' test was employed to determine whether there was any significance difference ($p<0.05$) between measured control and experimental group.

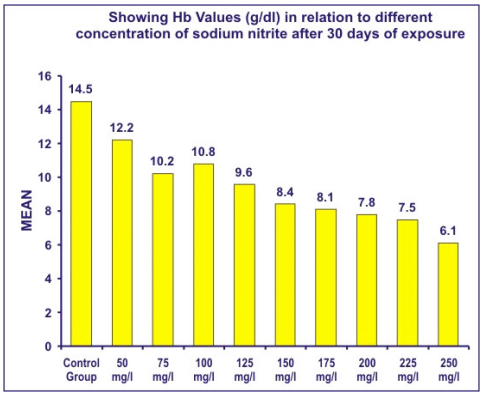
Showing Hb (g/dl) values in relation to different concentration of sodium nitrite and time; values are mean ± S.D.;

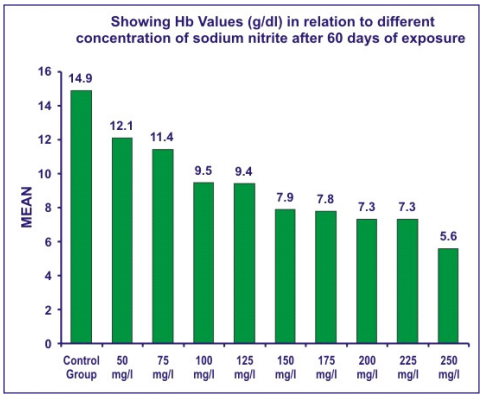
Time in days	Control Group	Experimental group treated with different concentration of Sodium nitrite								
		50 mg/l	75 mg/l	100 mg/l	125 mg/l	150 mg/l	175 mg/l	200 mg/l	225 mg/l	250 mg/l
10 days	13.20± 0.8	12.52± 0.5*	11.20± 0.4*	11.40± 0.5*	11.30± 0.7*	9.80± 0.4*	9.20± 0.8*	8.40± 0.5*	7.60± 0.6*	7.20± 0.8*
20 days	13.80± 0.5	12.81± 0.7*	12.25± 0.3*	11.50± 0.7*	9.90± 0.8*	9.10± 0.5*	8.80± 0.4*	8.10± 0.8*	7.50± 0.4*	6.70± 0.6*
30 days	14.50± 0.8	12.20± 0.4*	10.50± 0.5*	10.80± 0.4*	9.60± 0.5*	8.40± 0.3*	8.10± 0.3*	7.80± 0.5*	7.50± 0.3*	6.10± 0.2*
60 days	14.90± 0.6	12.10± 0.6*	11.40± 0.5*	9.50± 0.8*	9.40± 0.4*	7.90± 0.5*	7.80± 0.6*	7.30± 0.8*	7.30± 0.2*	5.60± 0.5*

* Significant at 0.05 level









Result

Active and aggressive behaviour in early stages of toxicity in *Heteropneustes fossilis* (Bloch) confirms the earlier findings of Singh and Reddy (1989).

In the later periods of toxication, the

fish became sluggish, in active, dull and in emaciated state. This correlate with the findings of Bowser PR, Falls WW (1983) and Jenson FB (2003). This was also observed during present investigation that fish subjected to nitrite toxicity were darker in colour, blood and gills were brownish as compared to the controlled fish. This correlates with the findings of Kroupova H, Machova, Svobodova Z. (2005).

Discussion

It was concluded that with increasing concentration of sodium nitrite and also with increasing exposure period, the haemoglobin values decrease. At the end of the experimental period, it was lowest i.e., 5.60 g/dl. Similar results were obtained by Pratap *et. al.* (2004a and b). This fact is also supported by the findings of Kiese (1971). He explained the fact that from blood plasma, nitrite diffuses into red blood cells where it oxidises iron of haemoglobin (Hb). Haemoglobin that is changed in this way is called methemoglobin or ferrihaemoglobin, which lacks the capacity of binding the oxygen reversibly (Bodansky, 1951). The methemoglobin reduces the total oxygen carrying capacity of the blood (Camerson 1971). It gives whole blood a brown colour. & Johal, 2000).

The brown coloured blood was observed in Nile-tilapia (*Oreochromis niloticus*) as soon as methemoglobin levels reached about 20% of total haemoglobin (Svobodova *et. al.* 2005a). According to Blaxhall and Daisley (1973) the determination of haemoglobin concentration can be a good indicator of anemic condition in fish. Cyriac *et. al.* (1989) considered decreases in haemoglobin concentration as a contribution to haemodilution. Haemodilution is a mechanism that reduces the concentration of pollutants in the circulatory system.

The decrease in haemoglobin concentration signifies that the fish's ability to provide sufficient oxygen to the tissues is restricted considerably and results in decreased physical activity (Grobler, 1988; Wepener 1990; Nussey, 1994). According to Reddy and Bashnibidden (1989) this significant decrease in haemoglobin concentration of fishes under toxic stress must be due to either an increase in the rate at which the haemoglobin is destroyed or due to decreased rate of haemoglobin synthesis. Other reason for the progressive reduction in the haemoglobin content might be attributed to depression exhaustion of haemopoietic potential of the fish (Sawhney

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