J. of Bio. Res & Biotech. 1, (2), 111-118 (July/December, 2003)

The Effect of Brewery Wastewater on the Total and Differential White Blood Cell Count of the Catfish Clarias Albopunctatus (Lamonte & Nicole)

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Abstract
The total and differential leucocyte count of *Clarias albopunctatus* exposed to Brewery wastewater for 21days were studied. The total leucocyte count increased (leucocytosis) in the wastewater-exposed fish. Compared with the control, there was significant lymphocytosis accompanied by monocytopenia and neutropenia in the treatment groups. These changes are indications of infection following exposure to the Brewery wastewater.

Key words: *Clarias,* wastewater, leucocytes, stress.

Introduction

Ever since the discovery of leucocytes and the elucidation of their functional importance in the mammalian body and human medicine, it has remained an invaluable tool in the diagnosis of diseases. The application of hematological parameters in the assessment of fish health, was emphasized in the review by Blaxhall (1972) This publication and the later review by Ellis (1977) clearly demonstrated the pivotal role which the study of total leucocyte and differential leucocyte counts could play in piscine medicine,

The scope of application of fish haematology has widened to include assessment of the pollutional status of water bodies. This is predicated basically on account of the sensitivity and rapidity of response of blood parameters, particularly the leucocyte, to altered water quality. There are variations in the response of the blood cells to pollutants, chemical irritants and environmental conditions. Increased total leucocyte count was found in fish exposed to heavy metals (Nussey et al 1995; Oluah 2001). Leucocytosis was also reported in *Heteropneustes fossillis* exposed to fertilizer,

sewage and insecticides (Srivastava and Narain, 1982) and in *Labeo* exposed to fertilizer, detergents and metasystox (Van Vuren, 1986). Leucocytosis was also reported in grass carp exposed to danithol (Figar et al, 1995) and in *Anabas testudineus* treated with monocrotphos (Santhakumar et al 1999).

Leucocytopenia however, was reported in fish exposed to chromium (Agrawal et al 1979, Wepener et al 1992) and in Coho salmon exposed to Kraft pulp mill effluent (McLeay, 1975). Neutropenia and thrombocytopenia were reported in *Anguilla anguilla* treated with chloramphenicol and oxytetracycline (Krutzmann, 1977).

In Nigeria there is dearth of information regarding the effect of industrial effluents on the blood parameters of common freshwater fishes. The purpose of this study was to investigate the effect of Brewery wastewater on the total and differential leucocyte counts in the freshwater catfish *Clarias albopunctatus*. The information is necessary not only in biomonitoring but also in providing a firm scientific base for environmental Policy and Regulatory decisions in the country.

Materials and Methods

The fish used in this study were caught using traps from Anambra River at Ogurugu, Uzo-uwani local Government Area of Enugu State, Nigeria. The fish were acclimatized for three weeks in the laboratory before the commencement of the study. The Brewery wastewater was obtained from the outflow from Nigeria Breweries Plc at Nsude, Enugu State in 50l plastic containers. Three different concentrations (10,50 and 100%) of the Brewery wastewater were prepared by dilution method using tap water The Brewery wastewater was filtered using watt man's filter paper before the different concentrations were prepared and used for the study

A total of one hundred and eighty fish (mean weight 25.63±2.08g) divided into four treatment groups of 45 fish each were used for the study. One group was exposed to 100% Brewery wastewater while another was exposed to 50 %Brewery wastewater concentration. The third and fourth groups were exposed to 10 % wastewater and tap water only (control), respectively. Each treatment group was further subdivided into three replicates of 15 fish per replicate. The fish were fed at 3 % body weight at 8.00h daily. The water in each aquarium was changed every 24h and aerated continuously. At specified intervals (2, 7, 14, 21, days), two fish from each replicate were removed using dip hand net and killed to determine the total and differential white blood cell counts of the fish. The blood was obtained by the severance of the caudal peduncle.

The leucocyte count was made using improved neubauer haemocytometer after diluting the blood 1:100 with Shaw's solution {Shaw, 1930). To prepare the blood for the differential counts, a drop of blood was placed on a fat-free slide. The blood smear was prepared using a second slide (spreader) held at 30^0 against the drop of blood. The spreader was drawn against the drop of blood to produce a thin film.

This was allowed to dry at room temperature and thereafter fixed in methanol for 3 minutes. This was allowed to dry and stained with Giemsa Romanowsky stain. The blood and the stain were allowed to react for about 40 minutes after which the stain was decanted and the slide rinsed with distilled water. The stained blood smear was viewed under oil immersion using binocular microscope. Statistical analysis was done using Analysis of variances (A NOVA) followed by FLSD and a significance level was taken as $P<0.05$. The total alkalinity, dissolved oxygen and free carbon

dioxide were determined by titrimetric method while the elemental analyses were done using atomic absorption spectrophotometer. The chloride and the nitrate were determined colorimetrically (Table1).

Result and Discussion

The water quality parameters of the Brewery wastewater are shown in Table 1. Tables 2 to.5 show the effect of the Brewery wastewater on the total and differential leucocyte counts of *C. albopunctatus*. At each phase of the study, there was significant difference ($P < 0.05$) in the total leucocyte counts of the fish in the treatment groups. Compared with the control, there was significant leucocytosis in the treatment groups ($P < 0.05$) and it persisted throughout the exposure period.

The increased total leucocyte count in *C. albopunctatus* exposed to Brewery waste water agreed with the result of Nussey et al (1995) on *Oreochromis mossambicus* exposed to copper. It is also consistent with the effect of cadmium on *C. gariepinus* (Oluah, 2001). Similarly, leucocytosis was observed in *Heteropneustes* exposed to fertilizer, sewage and insecticides (Srivastava and Narain, 1982) and in *Labeo* exposed to fertilizer, detergents and metasystox (Van Vuren, 1986). Leucocytosis was also reported in *Anguilla anguilla* and *Aphanus dispar* exposed to lead and mercury (Hilmy et al 1980; Santos and Hall, 1990). Leucocytosis was also reported in grass Carp exposed to danithol(Figar et al,1995),in *Anabas testudineus* treated with monocrotophos(Santhakumar et al,1999). On the contrary, Kraft pulp mill effluent induced leucocytopenia in Coho salmon (McLeay, 1975).

The leucocyte subpopulations identified in the *C. albopunctatus* were of five types namely: lymphocyte, monocyte, neutrophil, eosinophil and basophil. The lymphocytes with no granular cytoplasm and round nucleus rich in chromatin were the most abundant in the peripheral blood of *C. albopunctatus* exposed to Brewery wastewater. Both small and large lymphocytes were identified but were all considered as " lymphocytes" in this study.

The lymphocytes increased significantly ($P<0.05$) with increasing Brewery wastewater concentration and duration of the study. In *Aphanius dispar*, Hilmy et al (1980) noted increased lymphocyte production when exposed to mercury. Lymphocytosis was also found in *Ictalurus punctatus* subjected to stress (Scott and Rogers, 1981).

The high proportion of lymphocytes in *C albopunctatus* exposed to Brewery wastewater also agreed with the report of Ezzat et al (1974) and Nussey et al (1995) on the effect of heavy metals on *Oreochromis and Tilapia zilli*. Srivastava and Narain (1982) reported that lymphocyte constitute the dominant differential leucocyte in *Heteropeustes fossillis* treated with some pollutants. However, Iwama et al (1976) reported lymphocytopenia in rainbow trout subjected to stress.

Compared with the control, the monocyte decreased significantly ($P <0.5$) in the treatment groups ($P<0.05$). The monocyte decreased with increasing wastewater concentration and the duration of exposure except at day 2 and 7 in the group exposed to 10 and 50% Brewery wastewater, respectively. This observation agreed with the effect of copper on *O. mossambicus* (Nussey et al 1995) and sewage, urea, nuvacron, endrin and dimecron on *H. fossillis* (Srivastava and Narain, 1982).

The neutrophils were the most abundant granular differential leucocytes in the blood of fish in the treatment groups. The neutrophil subpopulation varied in the treatment groups ($P<0.05$). The

neutrophil decreased with increasing Brewery wastewater concentration and duration of exposure. The eosinophil, the second largest granulocyte in the fish in the treatment groups, decreased also with increased wastewater concentrations after two days of exposure. The basophil was not identified in the treatment groups after 2 days. This is consistent with the earlier work on *Tilapia zilli* (Ezzat et al, 1974) where basophil was thought to be rare. Since monocyte was believed to represent part of the phagocytic system in fish (Ellis et al, 1977; Srivastava and Narain, 1982), the monocytopenic condition in the fish exposed to 100 % Brewery wastewater, could be indication of impaired phagocytosis. This could have contributed to the total mortality of the fish in this group before the end of the study (Table5).

Similarly, the sustained leucocytosis in the treatment groups indicated tissue damage since leucocyte mobilization was stimulated by some products of tissue damage (Britton, 1969), which elicits allergy. Although evidence from some earlier works (Ellis 1977; Krutzmann, 1977) showed that lympocytosis is accompanied by neutropenia, our result indicated that lympocytosis runs parallel with increased neutrophil number. The reason for the total morality observed in the group exposed to 100% Brewery wastewater is not known with certainty. The result showed that as from the 7th day of the study, the total leucocyte count remained relatively lower than in other treatment groups. It is likely that the severity of the stress and tissue damage consequent upon exposure to the wastewater, may have overwhelmed the leucopoietic centers, there by impairing the immuno-competence and hence, response to inflammation and infection in this group resulting in death. Due to the inextricable involvement of leucocytes in the immuno-defence systems of fish, the determination of leucocyte cell types could profitably be used as early warning signal of pollution and disease outbreak.

Table 1: The physico-chemical characteristics of the Brewery wastewater

Parameters	
pH	8.2
Total alkalinity	42,8mg/L
Dissolved oxygen	4.8mg/L
Free carbon dioxide	129.1mg/L
Magnesium	49.9mg/L
Calcium	8.447mg/L
Iron	6.41mg/L
Copper	0.05mg/L
Zinc	1.39mg/L
Cadmium	0.01mg/l
Sodium	69.80mg/L
Lead	0.3mg/L
Potassium	66.15mg/L
Phosphate	0.195mg/L
Chloride	4.97mg/L
Nitrate	0.042mg/L

Table 2: Mean differential. and total leucocyte counts of *C. albopunctatus* exposed to Brewery wastewater for 2 days

	Concentrations of Brewery Waste water%			
Leucocyte sub-	Control	10	50	100 **population**
Agranulocyte				
Lymphocyte	62.9±2.60	72.0± 2.56	80.64±3.28	82.36±1.98
Monocyte.	6.20±0.78	9.60±0.07	5.02±1.22	4.0±0.64
Granulocyte				
Neutrophil	22..41±1.6	15.68±1.16	10.20±1.02	11.54±1.26
Eosinophil	6.42 ± 1.38	5.58±1.10	4.0±1.37	2.8±1.11
Basophil	2.0±0.26	1.10±0.06	1.0±0.04	—
Total leucocyte Count ($10^4/mm^3$)	46.3±2.88	154.5±2.04	178.5±3.10	194.5±.02

Table 3: Mean Differential and total leucocyte of *C. albopunctatus* exposed to Brewery wastewater for 7 days.

Brewery Wastewater Concentration (%)				
Leucocyte sub-population (%)	Control	10	50	100
Agranulocyte				
Lymphocyte	63.0±2.60	78.4±1.98	74.5±2.46	83.2±2.48
Lymphocyte	5.8±0.062	5.8±0.062	7.0±0.08	2.0±0.61
Granulocytes (%)				
Neutrophil	22.7±1.14	12.60±1.08	14.0±1.80	10.0±1.22
Eosinophil	6.50±1.46	5.0±0.92	5.5±0.54	3.0±0.66
Basophil	2.1±0.62	-------	-------	-------
TotalLeucocyte ($10^4/mm^3$)	45.9±1.20	154.5±2.81	162.00±1.96	70.5±1.72

Table 4: Mean Differential and total leucocyte counts of *C. albopunctatus* exposed Brewery wastewater for 14 days

	Concentration of Brewery wastewater (%)			
Leucocyte sub-population %	Control	10	50	100
Agranulocyte (%)				
Lymphocyte	61.2±1.88	78.1±2.06	78.12±2.10	90.4±2.79
Monocytes	5.90±0.81	4.0±0.62	4.8±0.70	4.8±0.70
Granulocyte (%)				
Neutrophil	22.6±1.08	14.0±1.12	12.0±1.10	5.0±0.82
Eosinophi	6.6±1.46	4.0±0.68	4.0±0.54	3.0±0.31
Basophil	1.0±0.01	-----	-----	-----
Total Leucocyte ($10^4/mm^3$)	46.1±1.46	86.0±1.76	86.0±1.76	86.0±1.76

Table 5 : Mean Differential and total leucocyte counts of *C. albopunctatus* exposed to Brewery wastewater for 21 days.

	Brewery waster concentration (%)			
Leucocyte sub-population %	Control	10	50	100
Agranulocytes				
Lymphocytes	62.0±1.26	82.1±1.86	82.4±266	------
Monocytes	6.0±0.08	6.0±0.04	5.0±0.11	------
Granulocytes				
Neutrophil	22.6±1.10	9.0±1.69	9.5±1.08	------
Eosinophi	6.0±1.60	3.0±0.14	3.5±0.54	------
Basophil	1.0±0.01	------	------	------
Total Leucocyte ($10^4/mm^3$)	46.0±1.35	54.5±1.20	85.0±1.68	------

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