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Immunological Changes in Two Sizes of *Tilapia guineensis* Exposed to Carbofuran

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Abstract: *The acute effects of Carbofuran a pesticide on immune profiles in juvenile and adult sizes of Tilapia guineensis was carried out. The fish was exposed to five concentrations of 0.000 (control) 0.025, 0.050, 0.075 and 0.100 mgL-1 of the chemical in triplicates for a period of 15 days. During the exposure, six water quality parameters which include temperature, pH, salinity, dissolved oxygen, nitrite and ammonia were evaluated. At the end of the experimental period samples of blood were collected from the fish and were analyzed for immune profiles using standard laboratory methods. The results obtained indicated that the values of ammonia and nitrite increased with increasing concentrations of the chemical. While the values of dissolved oxygen reduced. However, temperature, pH and salinity were within the same range. The chemical caused a significant distortions in the immune profiles of T.guineensis, with an increase in the number of leucocytes (leucocytosis) , a normal reaction of the fish body, against attacks of foreign substances, such as carbofuran, which can alter the normal physiological function in fish. Significant increases ($P<0.05$) in the number of lymphocytes (lymphocytosis) and eosinophils (eosinophilia) combined with significant decreases in monocytes (monocytopenia) and neutrophils (neutropenia), are indicative of changes (infections) that set in after exposure to this chemical.*

Keywords: *Immunology, Toxicity; Lymphocytes; Tilapia, Environmental pollution.*

1. INTRODUCTION

The release of chemicals into the aquatic environment results in some changes, which may threaten functional attributes, the integrity and existence of aquatic organisms, especially fish (Chindah and Hart, 2000). Recently, haematological parameters have become promising biomarkers in measuring the effects of chemical pollutant in fish. Blood samples can regularly be obtained from test organisms, thus allowing the use of non- destructive approach in effect assessment (Akinrotimi *et al.*, 2010). Typically, haematological parameters are non-specific in their responses towards chemical stressors. Nevertheless, they may provide important information in assessment studies, by providing an indication as to the general physiology and health status of the organism under investigation (Beyer, 1996). Much emphasis has recently been placed on the development of biological markers that can predict exposure to, and effects from, environmental pollutants. As monitors for exposure, biomarkers have the advantage of quantifying only biologically-available pollutants. As measures of effects, biomarkers can integrate the effects of multiple stressors and can assist in elucidating particular mechanisms associated with those effects.

Fish are the oldest and most diverse of the vertebrate groups and consist of more than 20 000 different species (FAO, 1997). Their immune system is quite varied and appears to be associated with fish phylogeny (Ellis et al., 2003). Although even the most advanced teleost species do not posses bone marrow or lymph nodes, fish contain functionally equivalent hematopoietic tissues primarily in areas of the kidney, spleen, and thymus. In addition, fish also have circulating white blood cells that are functionally and morphologically similar to mammalian lymphocytes, granulocytes, and monocytes (Cain *et al.*, 2000; Bowden, 2008). Conversely, the innate immune system is something common to all multicellular organisms. A number of other mammalian equivalent factors have been found in fish

serum: complement (Kadar et al., 2005), lysozyme (Oakes et al, 2004), interferon (Svobodova *et al.*, 1996), C-reactive protein (Nestman *et al*., 1980), hemolysins (Lowry et al. 1951), and hemagglutinins (Meister, 1983).

Teleosts are immunologically competent animals if properly maintained (Hrubec *et al.*, 200). Nonspecific responses in fishes involve phagocytosis and inflammation Innate or non-specific immunity comprehends defence mechanisms that protect an organism against infection without depending upon prior exposure to any particular microorganism. Many components of the innate immune system appear to be evolutionary conserved (Sole *et al.*, 1996; Oakes *et al.*, 2003). In mammalian systems, variations in the integrity of disease resistance and the immune response are very sensitive indicators of toxic insult due, at least in part, to the complex nature of the immune system (Andreson, 1992). Thus, sensitivity of immune system mechanisms to a particular contaminant might be similar among different species. For fish populations, a link between environmental contamination and disease has long being discussed. Understanding the impact of contaminants on fish immunity is of economic relevance for fisheries as well as aquaculture. Therefore, this study assessed the immunological changes in two sizes of *Tilapia guineensis* exposed to carbofuran

2. MATERIALS AND METHOD

2.1. Experimental Site and Fish

The experiment was conducted at Fish Disease Laboratory, African Regional Aquaculture Center, Buguma, Port Harcourt, Nigeria.Fish samples were caught from recruitment ponds at low tide. The fish were transported to the laboratory in a plastic container and thereafter acclimatized for 7 days at water temperature of 28 ^{o}C.

2.2. Experimental Chemical

Carbofuran is a systemic, broad spectrum N-methyl carbamate insecticide and nematicide. It was purchased off shelf in solid form, 1 g was weighed and dissolved in 1 L of water to obtain the stock solution (1 gL^{-1}). Various concentrations were measured into bioassay tanks for range finding and definitive tests.

2.3. Experimental Procedure

Two life stages of the fish comprising of juvenile of mean weight of 65.33 ± 2.12 g and adult size of mean weight 105.38 ± 2.79g used in the study were randomly divided into five groups of 10 fish each. Each group was further randomized into three replicates experiments of 10 fish per replicate. The fish in group 1 and 2 were exposed to 0.025 and 0.005 mg/L of Cabofuran, respectively. While the fish in groups 3 and 4 were exposed to 0.075 and 0.100 mg/l of Cabofuran, respectively. The fish in the fifth group which served as the control was exposed to dechlorinated water only. The experiment lasted for 15 days in a static bioassay system. The water and the pesticide were changed every 24 hour to maintain constant concentration and avoid the accumulation of wastes and food remnants. The feeding regime and blood collection methods used were done according to the methods described by Gabriel et al. 2012.

2.4. Evaluation of Immune Systems of Fish

The fishes were taken out individually using a small hand net and placed belly upward on a table. Blood samples of about 5 mL was collected from the caudal peduncle with the aid of a 2 mL plastic syringe, 2 mL of the blood was dispensed into Ethylene Diamine Tetra-acetic Acid (EDTA) anticoagulant for haematological studies .Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after appropriately diluted. Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Hrubec *et al*., 2000). The leucocytes count was made using improved Neubauer haemocytometer after diluting the blood 1:100 with Shaw's solution (Shaw, 1930).

2.5. Evaluation of Water Quality Parameters

Water quality parameters in the experimental tanks during the study were evaluated: Water temperature was measured with mercury in glass thermometers, pH with pH meter (Model 3013,

Jenway, China), and Salinity was determined with hand held refractometer (Atago products, Model H191, Japan). The values of dissolved oxygen, nitrite and ammonia were evaluated using the method described by APHA (1998).

2.6. Data Analysis

The results were analyzed using two way analysis of variance (ANOVA) followed by F-LSD post hoc test. The significance level was taken as $P < 0.05$.

3. RESULTS

The results of physico-chemical parameters in the experimental tanks during the exposure period are presented in Table 1. The values of temperature, pH and salinity were within the same range in all concentrations of cabofuran. While ammonia, and nitrite increased significantly. However, the dissolved oxygen reduced with increasing concentrations of the chemical. The effects of the chemical on the total leucocyte and differential white blood cell counts of the juvenile and adult sizes of *T. guineensis* are shown in Tables 2 and 3 respectively. The result showed that the total leucocyte in the treatment groups were significantly higher ($P < 0.05$) than the control. Also the leucocyte counts in the treatment groups were significantly different ($P < 0.05$). The results also showed that the lymphocytes, neutrophils, and monocytes, s were the recognizable types of white blood cells found in the peripheral blood of *T. guineensis* . These were classified as granulocytes or agranulocytes, depending on the presence or absence of granules in their cytoplasm. The lymphocytes are the most dominant leucocyte type in the blood of *T. guineensis* . In the control, the leucocytes were significantly higher ($P<0.05$) when compared with the treated groups. Lymphocytosis occurred with increased duration of exposure. The monocytes, which are round cells with oval nuclei with clumped chromatin, are the second agranulocytes in the blood. When compared with the control, there was significant decrease ($P<0.05$) in the monocytes in the exposed fish. Monocytopenia occurred with increasing duration of exposure. The neutrophil, were the granulocytes in the blood of *T. guineensis*. The neutrophils were significantly reduced in the treated groups ($P < 0.05$) when compared with the control. The neutrophil was the dominant granulocyte in the fish *T. guineensis*. Neutropenia was most pronounced in the fish exposed to 0.10mg/l of the chemical.

Table1. *Physico-chemical parameters of water in Experimental tanks (Meant ± SD)*

Parameters	Concentrations of Carbofuran (mg/L)				
	0.000	**0.025**	**0.050**	**0.075**	**0.100**
Temperature (^{0}C)	28.08 ± 1.55^a	28.82 ± 1.97^a	28.99 ± 1.88^a	28.99 ± 1.92^a	28.01 ± 1.93^a
pH	6.59 ± 1.66^a	6.59 ± 1.07^a	6.53 ± 1.47^a	6.59 ± 1.99^a	6.71 ± 1.93^a
Ammonia (mg/L)	0.21 ± 0.01^a	0.35 ± 0.02^{ab}	0.49 ± 0.02^b	0.49 ± 0.02^b	0.65 ± 0.23^c
DO (mg/l)	6.65 ± 0.02^a	6.44 ± 0.22^a	5.47 ± 0.22^{ab}	4.1 ± 0.31^b	3.01 ± 0.11^b
Nitrite (mg/l)	0.03 ± 0.01^a	0.09 ± 0.01^b	0.09 ± 0.01^b	0.09 ± 0.01^c	0.13 ± 0.01^c
Salinity (ppt)	14.55 ± 1.21^a	14.77 ± 2.04^a	14.59 ± 1.88^a	14.49 ± 2.88^a	14.09 ± 3.77^a

Means within the row with different superscripts are significantly different ($p<0.05$).

Table2. *Immune Profiles in Juveniles of T. guineensis Exposed to Different Concentrations of Cabofuran (mean ± SD)*

Parameters	Concentrations OF Carbofuran (mg/L)				
	0.00	**0.025**	**0.050**	**0.075**	**0.100**
WBC (cellsx10^9)	15.44 ± 1.89^a	17.83 ± 1.69^a	23.99 ± 3.01^b	27.99 ± 3.55^b	33.66 ± 2.44^c
Leucorit (%)	13.88 ± 1.87^c	9.07 ± 0.66^b	8.33 ± 0.82^b	7.02 ± 0.99^b	4.01 ± 0.11^a
Thrombocytes (%)	141.01±9.70^c	110.81±3.88^c	102.67±9.29^b	90.09 ± 9.02^a	88.99±9.07^a
Neutrophils (%)	23.39 ± 2.12^a	19.22 ± 1.17^b	16.61 ± 1.19^b	13.08 ± 1.21^a	10.21 ± 2.61^a
Lymphocytes (%)	60.61 ± 1.01^c	66.36 ± 1.09^c	73.61 ± 1.17^{ab}	80.03 ± 1.99^a	84.89 ± 1.99^a
Monocytes (%)	16.00 ± 1.66^a	14.42 ± 1.12^a	10.78 ± 1.88^{ab}	8.09 ± 1.77^c	5.68 ± 1.07^c

Means within the row with different superscripts are significantly different ($p<0.05$).

Table3. *Immune Profiles in Adults of T. guineensis Exposed to Different Concentrations of Cabofuran (mean ± SD)*

Parameters	Concentrations OF Carbofuran (mg/L)				
	0.00	**0.025**	**0.050**	**0.075**	**0.100**
WBC (cellsx10^9)	17.09 ± 1.99^a	21.99 ± 7.03^a	27.99 ± 8.55^b	37.88 ± 6.99^c	42.88 ± 6.99^c
Leucorit (%)	15.77 ± 4.33^c	8.99 ± 0.87^b	7.61 ± 0.95^b	6.62 ± 0.99^b	5.03 ± 0.55^a
Thrombocytes (%)	170.88±9.99	146.77±9.99	122.99±2.06	110.61 ± 1.03	103.6± 9.88
Neutrophils (%)	25.00 ± 2.76^c	21.73 ± 1.86^b	18.61 ± 1.19^b	15.88 ± 1.99^a	13.01 ± 2.99^a
Lymphocytes (%)	58.61 ± 1.96^a	65.37 ± 1.87^b	72.01 ± 1.99^b	$75.33 \pm 1.89b^c$	79.89 ± 9.99^d
Monocytes (%)	18.39 ± 1.17^c	13.43 ± 1.99^b	10.08 ± 1.49^b	9.01 ± 1.62^b	7.08 ± 3.77^a

Means within the row with different superscripts are significantly different ($p<0.05$).

4. Discussion

In this study the values of White blood cell (WBC) increased tremendously with increasing concentrations of the chemical. This is in line with the reports of Akinrotimi et al. (2012) in *Clarias gariepinus* exposed to cypermethrin in the laboratory. It should be noted that WBC is an important cell in the immune system, because of their main defensive function. The increase in number of WBCs may play an important role in immunological defense systems during exposure to toxicants like carbofuran and appears to be associated with increased circulatory levels of granulocytes, which are known to respond for phagocytosis of foreign body in the system of the fish (Akinrotimi *et al*., 2013). The WBCs respond immediately to the change in medium due to xenobiotic transformation. During toxic exposure period of the fish to carbofuran, the WBC counts were enhanced. It indicates that fish can develop a defensive mechanism to overcome the toxic stress.

The cell population numbers may be increased and termed *cytosis* or *philia*; reductions of cell numbers are termed *penia*. Our result show increased in lymphocyte in contrast to decrease of monocyte and neutrophils during the chemical exposure, so *Lymphocytocysis* versus *Monocphilia* and *Neutrophilia* occurred in current results. This results agrees with the findings of Akinrotimi et al.(2009) in *Tilapia guineensis* exposed to acute stress.. A stress-induced leukocyte response refers to a combination of changes observed in animals receiving corticosteroids or producing increased endogenous corticosteroids because of some stressful condition. It generally consists of neutrophilia, lymphopenia and sometimes monocytosis depending on the animal species. The neutrophilia develops as a consequence of increased release of segmented cells from the kidny storage pool, decreased margination of cells, decreased movement of cells into tissues and increased stability of lysosomal membranes (Adhikari *et al.*, 2004). Lymphocytosis results from steroid-induced lysis and cell redistribution. Monocytosis, when it occurs, is thought to result from mobilization of marginated cells. It is interesting that the stress-induced leukocyte response is a relatively infrequent finding in toxicology studies even though the study design or the test material often creates physical conditions that appear to be quite stressful (Adhikari *et al.*, 2004).

It is believed that neutrophils and monocytes have phagocytic activity which might explain their increased percentage during infectious situations. George and Akinrotimi (2017) also found an increase of neutrophil counts in channel catfish exposed to high doses of atrazine in the laboratory. However, Gabriel *et al.(*2011),find a reduction in some immunological parameters (leukocyte and lymphocyte counts) and the increase in neutrophil and monocyte percentages were demonstrated in exposed fish. It is known that toxicants can induce abnormal responses in the immune system, including leukocyte count, a marker of cellular defense (Nte and Akinrotimi, 2018). The increase in neutrophil and monocyte percentages which represents the activity of the first and second lines of defense against the cellular damage, has been reported after chemical exposure exposure (Akinrotimi *et al*., 2007).

5. Conclusions

Fish have well developed immune systems, with full representation of all known fundamental components of innate and adaptive immunity, although with some specializations and unique features. The key characteristics of the piscine immune system, as exemplified by *Tilapia guineensis* exposed to carbofuran. Significant future contributions to immunology from research using piscine models can be anticipated. With standardization, information on the changes in the white blood cell types in fish could be applied as a diagnostic tool in evaluation and prediction of pollution in aquatic environment

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