G.J.A.S.R

Global Journal of Animal Scientific Research

Journal homepage: www.gjasr.com

Print ISSN:2345-4377 *Online ISSN:2345-4385*

Original Article

White Blood Cells Tolerance Levels of Adult Rabbits Exposed To Crude Oil Ingestion in Niger Delta Region Nigeria

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ARTICLE INFO

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How to Cite this Article:
Yahaya, M.A., Ajuogu, P.K., & Ekine, A.O. (2015). White Blood Cells Tolerance Levels of Adult Rabbits Exposed To Crude Oil Ingestion in Niger Delta Region Nigeria. *Global Journal of Animal Scientific Research*, 3(2), 469-472.

Article History:
Received: 26 February 2014
Accepted: 1 April 2015

ABSTRACT

A study was conducted to determine the white cells threshold levels of rabbits exposed to crude oil ingestion in the oil producing region of Niger Delta of Nigeria. a total of thirty four (34) rabbits consisting of two bucks kept separately away from the thirty two (32) does randomly allotted to four treatment groups of A, B, C and D with the following (Bonny light) percentage crude oil inclusion rate per kg of feed mash of 0.0%, 0.01%, 0.02% and 0.03% were used in this study respectively. Each treatment was replicated into two replicates of two does. Results obtained revealed significant difference ($p<0.05$) in the percentage values of lymphocytes, ersinophils and giant cells- recorded amongst the treated groups. The percentage values of basophils showed that, group B recorded lower values than the root group. It was therefore concluded that, rabbits can tolerate exposure to crude oil ingestion up to 0.03% in formulated feed. The degree of stress imposed can be monitored by studying the leucocytes. Such indices can form useful parameters for laboratory application in clinical examination.

Keywords: White Blood Cells, Tolerance, Rabbits, Crude Oil, Niger Delta.

INTRODUCTION

This study is motivated by the many crude oil pollution episodes covering wide expanse of Farm lands, swamps, rivers and creeks in the Niger Delta Area of Nigeria, moreso in consideration of the destruction of the abundant natural economic resources of the area, some of which include marine life, wildlife very fertile soil, virgin forest reserves, wild fruits and Foods. Conscious and Foresighted individuals have complained and cried in their publications in journals, magazines and newspapers, both in Nigeria and other countries of the world.

Crude oil spillage on crops, soil, marine life, wildlife and poultry have been specifically reported by Bakare (1967), Albers and Gray (1982), (Amadi 1990), and Amadi *et al.*, (1992). Deleterious

pathological effects which include Fatty degeneration of the liver, nephrosis, splenomegaly, adrenocortical hypoplasia, pancreatic atrophy, signs of intestinal irritation and lipid pneumonia were reported by Hartung and Hunt in 1966, while Miller *et al.,* (1978) recorded depressed growth, impaired avoidance behaviour and pathological changes in the kidney. Berepubo and Sese (1994) have also observed sub fertility in experimental rabbits fed with graded levels of crude oil.

Crude oil has been found to change the blood chemistry, cause antibody depression and lymphatic involution, via the release of ACTH and glucocorticoids in animals (Brown, 1961; Thomson and Lippman, 1974; Siegel, 1980; Sudakor, 1992). Ngodigha *et al.,* (1999) observed that lymphocytes decreased as crude oil contamination increased and that the production of ACTH and glucocorticoids increased in the experimental goats. The PCV, neutrophil, basophil and cosinophil counts of goats fed highest level of contaminated forage were lower.

The study was thus designed to investigate the response of white blood cells in rabbits fed tolerable crude oil concentrations.

MATERIALS AND METHODS

Thirty four New Zealand rabbits aged 7-8 months including two males and 32 does females procured from Agricultural Development Programme (ADP) Rivers State were used for this experiment. They were housed in 3-tier wire mesh/iron frames set of cages kept in open-sided big halls in the RSUST Teaching and Research Farm. Each set of cages comprised 9 hutches. While the two males were kept in separte single hutches, distant from view of the females the 32 females were randomly assigned to four groups, A, B, C & d. eight rabbits were allotted per group but in separate hutches. While they were being preconditioned for two weeks in their cages, they were fed chicken grower mash and forage. Exactly after two weeks, groups B, C & D were fed graded levels of crude oil (Bonny Light) in mash of concentrations of 0.1g/kg mash (0.01%), 0.2g/kg (0.02%) and 0.3g/kg (0.03%) respectively, whereas A served as control. Before crude oil was mixed with feed, it was exposed for 24 hours in a shallow tray to evaporate in order to ensure its stability in feed (White 1975). After 12 weeks of dietary crude oil ingestion blood slide smears were made in duplicates from all the rabbits in all the groups. This was done using a hypodermic needle to puncture the car vein after cleaning the site thoroughly with methylated spirit. The blood oozing from the punctures were used to make duplicate slide smears from every rabbit. When the smears dried they were fixed in methanol and stained with Leishman solution for 3-5 minutes, rinsed with water, allowed to drip dry and subsequently observed under 100 x (immersion oil) light microscope. Differential white blood cell count was conducted for lymphocytes, monocytes, eosinophils, basophils and neutrophils. The average of the seven in each group was taken as the relevant reading. The data were analyzed using student's 't' test for comparison of means according to Bailey (1981), using the formula:

$$t = \sqrt{\frac{N_1\ SD^2{}_1 + N_2\ SD^2{}_2}{N_1 + N_2 - 2}} \quad \left(\frac{1}{N_1} + \frac{1}{N_2}\right)$$

RESULTS

Results indicate (Table 1) that lymphocytes were significantly higher ($p<0.05$) in the test rabbits than in the control while the neutrophlis differed significantly ($P<0.05$) lower than the control but not among themselves. For the monocytes, groups A C and D exhibited normal monocyte count (2-4%) whereas B count of 0.4% as significantly ($P<0.05$) lower than normal. Eosmophil read as

Follows 1 .50, 0.40, 0.25 and 0.33 respectively for groups A B C &D. The values of B C & D differed significantly (P<0.05) lower than A. The values of basophils were A-2.25, B-0.65, C-2.83 and D-1.33, showing that group B differed significantly (P<0.05) lower than others. The giant cells were significantly higher (P<0.05) in the test groups B C & D than in the control A. The readings so recorded for giant cells are 0.25, 1.83, 0.90 and 1.25 for A B C and D respectively.

Table 1: Mean count of leucocytes at 12th week of exposure to dietary crude oil Diets

Type of WBC	A (%)	B (%)	C (%)	D (%)
Lymphocytes	60.75[a]	89.90[b](47.98↑)	89.4[b](47.16↑)	86.80[b](42.89↑)
Neutrophils	33.75[a]	7.40[b](78.07↓)	7.50[b](77.78↓)	9.50[b](71.85↓)
Monocytes	3.00[a]	0.40[b](86.67↓)	2.08[a](30.67↓)	1.75[a](41.67↓)
Eosinophils	1.500[a]	0.40[b](73.33↓)	0.250[b](83.33↓)	0.33[b](48.89↓)
Basophils	2.25[a]	0.65[b](71.11↓)	2.83[a](25.78↑)	1.33[a](48.89↓)
Giant cells	0.250	1.830(632↑)	0.90(260↑)	1.2500(400↑)

Mean with the same letter along the same row are not significantly different (P>0.05) A =0.0g/kg mash; B = 0.1g/kg mash; C = 0.2g/kg mash; D = 0.3g/kg mash

↑ = percent increase

↓ = percent decrease

DISCUSSION

Livestock and poultry subjected to spillages have suffered various degrees of toxicity (Lolomori, 1983). Rabbits specifically fed crude oil contaminated feed at various levels suffered high mortality (Berepubo *et al*., 1994) when subjected to 0.05%, 0.1% and 0.15% graded levels of experimental contamination. This present study has shown that rabbits survived on contamination levels of 0.01%, 0.02% and 0.03%. This notwithstanding the white blood cell reading show the response of leucocytes to the stress imposed in the body of the rabbits. In their study, Berepubo *et al.,* 1994 reported atrophy of female reproductive organs, which should be associated with subfertility or infertility hematological.

Although literature in formal ion on the effects of crude oil in rabbits is scanty, effects of ingestion of a similar chemical like fluoride have been studied by Hiraq (1972). Also in 1975, Matsumura investigated the effects of chlorinated hydrocarbon, cyclodiene insecticides and telodrin on the hematology of rabbits. Both Hiraq and Matsumura observed increase in the number of lymphocytes and decrease in other white cells.

In this study also the lymphocytes have significantly (P<0.05) increased in number in the test rabbits. This observation also agrees with White (1975) who reported that fluoride administered to rabbits caused increase in the number of lymphocytes as well as the structure and function of lymphoid tissues. The increase in lymphocytes is usually a natural reaction of the body to stress, especially involving antigens. The lymphocytes being antibody producers defend the body against stress or debilitation imposed by foreign agents (antigens).

The neutrophil count of test groups BC & D depressed significantly lower than control group A. Usually when there is stress in the body the glucocorticoid level rises, Hiraq (1972) and Saita (1974). When the effect persists it causes depression of lymphoid organs and tissues which consequently fail in their production of neutrophils. This trend leads to lowered resistance of the animal to disease infection. These animals under test however, did not die since they were not exposed to infection coupled with the high level of nutrition provided by the mash. The observed cosinopenia is attributed to depression suffered by the lymphoid tissues as explained previously. Carola *et al.,* (1990) explained that the functions of eosmophils are to modulate allergic

inflammatory reactions and to destroy antibody-antigen complexes. Consequently there could have been failure in this function since the high level of lymphocytes would correlate to high level of antibody-antigen complex which was rather over whelming for the significantly low level of cosinophils observed in the experiment.

The normal basophil percentage values for some domestic animals are 0.2-0.5 (bovine), 0.3-1.4 (swine), 0.3-0.5 (caprine), 0.03 -0.7 (canine) and 0.01-I (feline). The values 0.65 and 1.33 obtained for the test groups B and D respectively fall within range of the above listed animals whereas the values 2.25 and 2.83 for A and C respectively are very much higher than the recorded range. The inconsistency in the values between the groups is apparently due to experimental errors in reading the basophils. This discrepancy thus obliterates meaningful interpretation of the effect upon basophils in this experiment. Carola *et al*., (1990) however confessed that the exact function of bsophils is not known but they may be playing some role in leucocytosis caused by anaphylaxis or circulatory shock. The monocyte values were normal for all the groups except B, which is also attributed to errors in reading. Otherwise there is no cause why the reading should solitarily differ even when the two extremes A and B agree.

The increase in giant cells in the test groups B, C and D was indicative of fortified defence by the body. They are on the increase during overwhelming invasions. Their presence in the blood could be an index of assessing the severity of stress or infection in the body.

CONCLUSION

Rabbits Call tolerates crude oil ingestion up to 0.03% in feed. The degree of stress so imposed can be monitored by reading the leucocytes or the giant cells in the blood. Such readings can form useful indices for laboratory application in clinical diagnosis.

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