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HAEMATOLOGICAL AND SERUM PROFILE OF GROWING RABBITS FED VARYING LEVELS OF CRUDE OIL CONTAMINATED FEED

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ABSTRACT
Thirty (30) 6-7 weeks old Chinchilla rabbits weighing between 430 – 460 were used in a Complete Randomized Design to evaluate the effect of crude oil contaminated feed on the haematological and serum profile of growing rabbits. Graded levels 0 ml, 4 ml,8 ml, 12 ml and 16 ml crude oil were incorporated in 1kg of feed in treatment T_I, T_{II}, T_{III}, T_{IV} and T_V respectively with 6 replicates per treatment, one animal per replicate in a study that lasted for 12 weeks. Concentrate feed with iso-nitrogenous and iso-caloric were formulated for the study. After 12 weeks of rearing, four rabbits were randomly selected from each treatment for blood samples. Result obtained showed increased serum concentration which suggest decreased glomerular filtration rate. The results from this study also demonstrated that crude oil toxicity caused anaemia.

KEYWORDS: Haematology, serum, growing rabbits, crude oil and contaminated feed.

INTRODUCTION:

Investigation have revealed that prolonged ingestion of oil (either crude or refined) polluted plant material, seeds and water by livestock and poultry do affect tissues and organs and consequently influence growth and performance (George and Sese, 2012). The haematological and serum profile reflects the physiological responsiveness of the animal to its internal and external environment (Esonu *et al*., 2001) and are important indicators of health status in animals and have been an indispensable tool in the diagnosis, treatment and prognosis of many disease. This study investigates the effect of crude oil on the haematology and serum profile of growing rabbits.

MATERIALS AND METHODS

The study was conducted at the Rabbit Unit of the Teaching and Research Farm, Rivers State University of Science and Technology, Port Harcourt, Rivers State, Niger Delta region, Nigeria and lies within longitude 4° 35 " and latitude 7° 00" and 7° 53" E. A total of thirty (30) 6-7 week old growing Chinchilla rabbits weighing 430 – 460 g were used in a study that lasted for 84 days. The rabbits were properly identified with ear tag and were housed singly in clean single-tier metal hutches (50 x 30 x 30 cm). The rabbits were subjected to two weeks of acclimatization prior to the beginning of the experiment. The crude oil used in this study was the Bonny light grade obtained from the Bonny Terminal in Bonny Local Government Area of Rivers State, Nigeria. Concentrate feed with iso-nitrogenous and iso-calorie were formulated and the simulation of feed contamination was achieved by incorporation of the crude oil in the formulated feed by mixing and homogenization with electrical blender (Sonic Japan, SB-1515) for 5 minute to ensure imbibitions. The rabbits were divided into five treatment groups of six rabbits each. Each treatment group was further subdivided into 6 replicates of a rabbit each in a completely randomized design. The rabbits were assigned to 0 ml, 4 ml, 8ml, 12 ml and 16 ml level of crude oil per Kg of feed. All routine management practice and vaccination were maintained. Feed and water were offered ad libitum. At the end of the study, four (4) rabbits per treatment were randomly selected and bled by severing the jugular vein. Blood samples were collected with Ethylene diamine tetra-acetic acid (EDTA) bottles and non-anticoagulant bottles for haematological and blood Chemistry analysis. The haematological parameter and blood chemistry were determine as described by Dacie et al., (1991). The blood parameters measured were Red blood cell (RBC), Packed Cell Volume (PCV), haemoglobin (Hb), White Blood Cell (WBC), lymphocytes, Neutrophils and monocytes . The mean corpuscular Volume (MCV), and Mean corpuscular Haemoglobin (MCH) and Mean Corpscular Haemoglobin Concentration (MCHC) where calculated from the method described by Schalm et al., (1975). The total protein was obtained from the blood serum and all the data collected were subjected to Analysis of Variance (ANOVA) according to Steel and Torrie and means were separated where necessary using Duncan new Multiple Range Test (DNMRT) of Obi (1990).

RESULTS & DISCUSSION

The results of the haematological indices and serum profiles are presented in Table 2. Haematological analyses, which include Packed Cell Volume (PCV), erythrocyte counts, total leukocyte counts and differential leukocyte counts, provide information about the haematopoietic system and immunological responses (Udensi *et al*., 2007). These blood tests can serve as diagnostic adjuncts in the development of a presumptive of definitive diagnosis (Campbell, 1995). Significant dose dependent reduction in the Packed Cell Volume, haemoglobin and erythrocytes of rabbits that consumed graded level of crude oil was observed in this study. The observed

reduction in the concentration of PCV, haemoglobin and erythrocytes suggest an anaemic condition in the crude oil treated rabbits. The significant reduction ($P < 0.05$) may be attributed to the cytotoxic effects and suppression of the erythropoiesis caused by constituents of the crude oil (Sunmonu *et al.*, 2008; Keller *et al*., 1998; Ovuru *et al*., 2003 & 2004; Owu *et al*., 2005 and Ngodigha 2009).

TABLE 1: The composition of the experimental diets and proximate analysis

Ingredient	Dietary Treatment I (control) 0/ml	II 4ml	III 8ml	IV 12ml	V 16ml
Maize	11.15	11.15	11.15	11.15	11.15
Palm kernel cake	35.00	35.00	35.00	35.00	35.00
Groundnut	15.00	15.00	15.00	15.00	15.00
Wheat Bran	35.00	35.00	35.00	35.00	35.00
*Vit/ Min premix	0.25	0.25	0.25	0.25	0.25
Bone Meal	3.00	3.00	3.00	3.00	3.00
Dl Methionine	0.10	0.10	0.10	0.10	0.10
Salt	0.40	0.40	0.40	0.40	0.40
Lysine	0.10	0.10	0.10	0.10	0.10
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Chemical analysis (on dry matter basis)					
Dry matter	89.41				
Crude protein	21.62				
Crude fibre	16.02				
Ash	14.43				
Ether extract	4.72				
Nitrogen free ext.	43.21				

*Vitamin A 8000000 I.U, vitamin D_3 1600000 I.U, vitamin E 5000 I.U, vitamin K 2000 mgr, Thiamine 1500 mgr ,Riboflavin B_2 4000 mgr, Pyridoxine B_6 1500 mgr,Niacin 15000 mgr vitamin B_{12} 10 mgr, Pantothenic Acid 5000 mgr, Folic Acid 500 mgr, Biotin 20 mgr, Choline chloride 200 gr, Antioxidant 125 gr, Manganese 80 gr, Zinc 50 gr, Iron 20 gr, Copper 5 gr, Iodine 1.2 gr, Selenium, 200 mgr Cobalt 200 mgr.

TABLE 2: Effects of treatments in haematological and Serum profile of growing rabbits exposed to varying levels of crude oil contaminated diets.

Haematological Parameters	**Treatments**				
	I	**II**	**III**	**IV**	**V**
Hb (g/dl)	$10.45a \pm 0.72$	$9.61^{ab} \pm 0.76$	$8.88^{abc} \pm 0.50$	$8.58^{bc} \pm 0.28$	$7.6^{c} \pm 0.40$
PCV (%)	$34.05^{a} \pm 2.10$	$32.78ab \pm 2.50$	$30.55^{abc} \pm 1.50$	$28.10^{bc} \pm 0.87$	$26.94^{c} \pm 1.30$
RBC (X 10^{12}/l)	$4.18^{a} \pm 0.21$	$4.00a \pm 0.27$	$3.83^{ab} \pm 0.18$	$3.29^{b} \pm 0.11$	$3.28^{b} \pm 0.71$
Platelets (X 10^{9}/1)	$181.25^{a} \pm 8.18$	$168.00^{ab} \pm 6.12$	$151.75^{bc} \pm 6.25$	$146.00^{bc} \pm 7.84$	$142.75^{c} \pm 9.92$
WBC (X 10^{9}/l)	$6.75^{a} \pm 0.38$	$6.23^{a} \pm 0.31$	$4.6^{b} \pm 0.32$	$4.57^{b} \pm 0.82$	$3.95^{b} \pm 0.32$
Neutrophils (%)	$45.00^{b} \pm 7.21$	$46.83^{b} \pm 3.51$	$53.53^{c} \pm 2.59$	$56.73^{a} \pm 3.82$	$55.37^{a} \pm 3.62$
Lymphocytes (%)	$55.34^{a} \pm 5.01$	$53.43^{a} \pm 2.59$	$49.18^{a} \pm 6.06$	$41.33^{b} \pm 5.96$	$36.82^{b} \pm 2.31$
Monocytes (%)	$1.04^{a} \pm 0.02$	$0.09^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$	$0.00^{b} \pm 0.00$
Eosinophils (%)	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.04^{b} \pm 0.46$	$1.82^{b} \pm 0.04$	$2.09^{a} \pm 2.88$
Metabolites					
Urea (mmol/L)	$5.42^{b} \pm 1.13$	$5.98^{b} \pm 0.36$	$6.32^{ab} \pm 0.19$	$7.21^{a} \pm 0.52$	$7.02^{a} \pm 1.08$
Creatinine (mmol/L)	$135.61^{ab} \pm 11.67$	$153.41^{a} \pm 5.11$	$161.23^{a} \pm 6.65$	$179.41^{bc} \pm 12.59$	$189.71^{a} \pm 1.08$
Glucose (mmol/L)	$5.42^{b} \pm 0.27$	$5.53^{b} \pm 0.12$	$6.81^{a} \pm 0.57$	$6.97^{a} \pm 0.40$	$6.81^{a} \pm 1.11$
Total Protein (g/L)	$39.49^{c} \pm 5.19$	$41.21^{bc} \pm 1.19$	$43.3b^{c} \pm 1.43$	$47.71^{b} \pm 1.44$	$53.41^{a} \pm 3.43$
Albumin (g/L)	$24.00^{b} \pm 1.08$	$24.21^{b} \pm 2.32$	$25.03^{b} \pm 0.82$	$25.28^{b} \pm 1.44$	$30.02^{a} \pm 1.78$
Total Bilirubin (mmol/L)	$0.00^{c} \pm 0.00$	$3.78^{b} \pm 0.49$	$4.42^{b} \pm 2.35$	$4.89^{b} \pm 0.87$	$5.02^{a} \pm 0.28$
Conjugated Bilirubin (µmol/L)	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$0.01^{c} \pm 0.00$	$0.39^{b} \pm 0.25$	$0.46^{a} \pm 0.29$
Cholesterol (mmol/L)	$1.71^{c} \pm 0.08$	$2.05b^{c} \pm 0.07$	$2.24^{a} \pm 0.14$	$2.39^{a} \pm 0.09$	$2.14^{b} \pm 0.34$

a,b,c, within column means ± SEM with different superscript (s) differ significantly ($P<0.05$)

The present study also demonstrated a significant increase in the total leucocyte count in rabbits that consumed the low level of crude oil (0ml & 4 ml / kg of feed). Subsequently, the total leukocyte counts were significantly reduced from 8ml – 16 ml concentration of crude oil (treatment III-V). This may be an indication that an initial proliferation response by the immune cells was followed by immunosuppression. The immune system is synonymous with circulating leukocytes, all of which derive from a single precursor, the pluripotential haemopoietic stem cell (Scott and Gordon, 1995). It has been shown that immunodeficiency often follows contact with toxicants (Rocke and Samuel, 1991). Experimentation with petroleum ingestion in rats (Eyong *et al.*, 2004), rabbits (Ovuru *et al.*, 2003, 2004), goat (Ngodigha, 2009) as well as evidence from seabird

rehabilitation centres (Leighton, 1986; McOrist *et al*, 1992).

In this study, the number of circulating monocytes and lymphocytes was significantly ($P<0.05$) reduced with increasing level of crude oil concentration. The primary function of B lymphocytes is to produce antibodies, and circulatory monocytes which are precursors of tissue macrophases (Junqueira *et al.*, 1998). The proliferations of monocytes and lymphocytes following crude oil consumption may be related to the response of the immune system to the presence of the toxicant. The number of circulating neutrophils and eosinophils was significantly increased in rabbits that received the crude oil contaminated diets. Neutrophils and eosinophils form part of the granulocytes that make up leukocytes in animals. Neutrophils are most active in phagocytosis, while eosinophils are less active. Both cells participate in the destruction of pathogens through phagocytic uptake, intracellular and extracellalar enzymatic degreadation of toxic substances (Gordon *et al.*, 1995). A decline in the number of these cells may depress the ability of the treated animals to phatocytize bacterial pathogens and thereby increase their susceptibility to infection (Eppley, 1992). In this study the platelets values decreased with increasing concentration of crude oil concentration. The platelets are nucleated cells and function as the first line of defense against bleeding. Since the toxic components in the crude oil change the blood chemistry and also induce anemia by causing bone marrow hypoplasia (Briggs *et al.*, 1996; Scott *et al*, 1995), the crude oil in the diet may have interfered with the production of platelets in the animals, which is observed in the reduction of the platelets number. This observation is in consistence with that of Ovuru *et al*, 2005, Sudakov, 1992; Snyder, 1987.The concentrations of the total proteins, bilirubin and albumin in the serum may indicate the state of the liver and the type of damage (Yakubu *et al*., 2005). Similarly, the serum concentrations of electrolytes, urea and creatinine could give an insight into the effect of a compound on the tubular and on the glomerular part of the kidney (Abolaji *et al*., 2007). This study demonstrated that urea and creatinine increased with increasing concentration of crude oil in the feed (Table 2). A rise in serum level of these metabolites suggest the inability of the kidney to excrete these product, which further suggest a decrease in glomerular filtration rate (Robert 2001). A common manifestation of nephritic damage is acute renal failure characterized by decline in glomerular filtration rate (Wasan *et al*., 2001), which may have been induced by the hydrocarbon fractions present in the feed. This affirmation is supported by Counts *et al*., (1995) who reported that chemically induced nephrotocity by halogenated hydrocarbons injure the proximal tubule monolayer, resulting in gaps in epithelial lining, leading to back leak of filtrate and diminishing glomerular filtration rate.Bilirubin is the major breakdown product that results from the destruction of old red blood cells. It is removed from the blood by the liver; hence it is a good indicator of the function of the liver (Sunmonu and Oloyede, 2007). This study also demonstrated that toxic metabolites (Total bilirubin and conjugated bilirubin) increased with increasing with increasing concentration of crude oil in the feed. The observed increase in bilirubin concentration (Table 2) confirms the fact that there may be evidence of liver dysfunction, which may be as a result of the effect of the toxic components of crude oil in the formulated diets. This assertion is supported by the work of Ovuru *et al*, (2004) who reported an increase in total serum bilirubin concentration in semi adult rabbits exposed to crude oil contaminated diet and attributed this to a metabolic disturbance in the liver arising from defective conjugation and/ or excretion of bilirubin. Cholesterol is a key intermediate in the biosynthesis of related sterols such as bile acids, adrenocortical hormones, androgens and estrogens (Rahmani *et al.*, 1988). In this study, increase in plasma concentration of cholesterol with increasing concentration of crude oil in the feed was observed (Table 2). This increase in cholesterol may be an indication of renal retention disease resulting in diminished removal lipoprotein from the plasma, thus causing the concentration of cholesterol to increase markedly. Previous studies in which cholesterol was measured in blood, Oruwari *et al*., (1998) reported that palm oil, a saturated fatty acid was implicated in increased level of cholesterol in roosters and rabbits. Their findings confirm with the observation in this study. Blood glucose is a sensitive indicator of environmental stress (Ganong, 2005). In this study, increase in blood glucose with increasing concentration of crude oil in the feed was observed (Table 1). It is well documented that an increase in glucose concentration is a common finding in animals affected by the stress of disease (Turgut., *et al*., 2000). Kaneko *et al*. (1997) also reported that the increase in blood glucose concentration was in response to hypocalcaemia because of interference with the secretion of insulin from the pancreas. An adequate amount of calcium ions in extracellular fluids is required for insulin secretion in response to glucose and other secretagogues for insulin. This may explain the increase in glucose concentrations observed in this study and it may be associated with the diabetes mellitus (Sahal *et al*., 1994).Total proteins, which include globulins, fibrinogens and albumins, are important in the control of water balance in animals. Serum total protein levels is a rough measure of protein status but reflects major functional changes in kidney and liver functions (Agrawal and Johri 1990).They have nutritive, transporting, protective, buffering and energy functions (Cheesborough, 1992). In this study, these parameters increase with increase concentration of crude oil in the feed (Table 1). A rise in plasma total protein and albumin may be an indication of haemoconcentration, presence of abnormal globulins or some form of liver and kidney dysfunction (Ganong, 2005).

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