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Serum enzymes and some Biochemical Parameters responses of male chinchilla rabbits exposed to crude oil contaminated feed

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Abstract
A study was conducted to investigate the effect of crude oil contaminated feed on serums enzyme and some biochemical parameters of male chinchilla rabbits. The crude oil was incorporated at the graded levels of 0, 4, 8, 12, and 16 ml per kg feed in T_I(control) , T_{II} , T_{III} T_{IV} and T_V respectively. A total of thirty (30) male chinchilla rabbits, aged 12 weeks and weighing 950gms were used. They were randomly assigned into 5 groups (T_I ,T_{II} , T_{III}, T_{IV} and T_V) in a Randomized Complete Design (RCD), six replicates, each of the replicate having one rabbit. At the end of the feeding trial, blood samples were collected from three rabbits from each treatment group for cortisol, total protein, cholesterol and enzymological analysis. Results obtained showed that the inclusion of crude oil had significant ($P< 0.05$) effect on cortisol, total protein, cholesterol and enzymes (Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP).
Key words: Serum enzymes, biochemical parameters and male rabbits.

Introduction

Nigeria is the largest crude oil producer in Africa, the seventh largest in the World and consequently a prominent member of the Organization of Petroleum Exporting Countries (Ezirim, 2008; TELL, 2008). Crude oil exploration / exploitation is known worldwide for its attendant environmental and social concerns at the local level such as loss of indigenous farm lands, destruction of rainforests, contamination of water sources and air pollution, etc (Adeola, 1996; Iruonage, 2008). Nigeria has had it fair share of the negative environmental consequences of a result of oil development since oil was discovered (Ibiba, 2005; Chinonye, 2008). The growth of the country's oil industry, combined with a population explosion and a lack of enforcement of environmental regulations has led to substantial environmental damage, especially in the Niger Delta region (William, 2008). Some of the factors responsible for the rapid depletion of livestock and fisheries resources of the Niger Delta are environmentally related. Various authors (Awosika 1991; Akankali 1998; Egborge 1993 and Ajana, 2003) have cited the following as the major environmental problems affecting livestock and fisheries development in the Niger Delta; oil spillage, gas flaring, industrial pollution, sewage pollution, , erosion and flooding. The devastating consequences of oil spill with its eventual hazards on both aerial and terrestrial environment manifest as an irreversible chain effect on both the biodiversity and human safety (Narayanan, 2007). As this occurs, the oil threatens surface water and a wide range of subsurface marine organisms which are linked in a complex food chain (Katwijik Van *et a*l., 1999). Oil spillage has caused destruction of food resources (Percival and Evans, 1997). Animal species that are not directly in contact with the oil spillage can also be harmed via the food web and predators that consumed contaminated marine preys. Aquatic environments are made up of complex inter-relationship between plant and animal species and any adverse alteration of their physical environment will often lead to the death of one or more species in a food chain, which may also lead to damage for other species further up the chain (Jessup and Leightion, 1996). A variety of pollutants including crude oil and its products are known to induce stress conditions, which impair the health of animals. Ekweozor (1989) reported that frequent spillages of crude oil and its products in creeks and rivers of the Niger Delta have resulted in a marked reduction in the number of both fresh water and marine creatures. Crude oil irrespective of the quantity produces aquatoxicological effects, which is deleterious to aquatic life (Kilnhold, 1980; Afolabi, Adeyemi and Imebore, 1985; Agbogidi, Okonta, and. Dolor, 2005). A number of investigations have been conducted on the direct and indirect effects of crude oil on poultry (Nwokolo *et al*, 1984), goat (Ngodigha *et al*., 1999) and rabbits (Monsi, Kwuinji and Akpan, 1991; Berepubo, Johnson and Sese, 1994; Ovuru, Berepubo and Nodu, 2004). It was reported that there was a decline in feed intake and also severe depression in growth as the level of crude oil contamination increased in the feed

(Monsi, Kwuinji and Akpan, 1991; Berepubo *et al*., 1994; Ovuru, Berepubo and Nodu, 2004). Research results have indicated the adverse effects of crude oil on feed intake and growth performance (Heywood, 1981; Monsi, Kwuinji and Akpan, 1991; Berepubo *et al*; 1994 and Ngodigha *et al*, 2008). Reduction in body weight and size (weight) of some vital organs like the testis, liver, kidney, heart and pancreas in rabbits treated with crude oil contaminated feeds was observed (Berepubo et al, 1994; Ovuru, and Ekweozor , 2003). It was noted that, the reduction in organ size/weight was directly related to the contamination level of the crude oil, which adversely affected the growth performance of the exposed rabbits as against the unexposed treatment (control). Owu *et al*., (2005) reported a significant reduction in red blood cell and haematocrit value in Guinea pigs which were exposed to crude oil. In the findings of Olawale and Onwurah, (2007), they observed that crude oil contaminated diet could pose serious effect on the hormonal system, which may consequently affect the reproduction process in organisms found in a crude oil polluted environment, and change in the endocrine system which may cause changes in reproductive development, growth or behaviour that can affect the animals or human or their offspring (Naz, *et al*., 1999).The aim of this paper was to investigate the effect of crude oil contaminated feed on serums enzyme and some biochemical parameters of male chinchilla 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. 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. A total of (30) male chinchilla rabbits of aged 12 weeks and mean initial weight 950gms were used. They were randomly assigned into 5 groups (T_I ,T_{II} , T_{III}, T_{IV} and T_V) in a Randomized Complete Design (RCD), six replicates, each of the replicate having one rabbit. They were all subjected to standard husbandry routine throughout the study that lasted 12 weeks. The crude oil was incorporated at the graded levels of 0, 4, 8, 12, and 16 ml per kg feed in T_1(control) , T_{II} , $T_{III,}$ T_{IV} and T_V respectively. At the end of the feeding trial, blood samples were collected from three rabbits from each treatment group. Blood samples were collected into well-labeled tubes for serum separation. The samples were taken to University of Port Harcourt Teaching Hospital for analyses. Total protein was determined using the method as described by Peters, Biamonte and Doumas (1982). Cholesterol was determined by the enzymatic endpoint method (Allain et al., 1974). Serum enzymes (SGOT, SGPT, ALP) were determined using spectrophotometric method as described by Rej and Hoder (1983). Data obtained were subjected to Analysis of Variance (ANOVA) (Steel and Torrie, 1980) and treatment means were compared using Duncan's Multiple Range Test (DMRT) as modified by Gomez and Gomez (1985).

Results

Results on cortisol, Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP), Serum Glutamic Pyruvic Transaminase (SGPT), total protein, cortisol and cholesterol are presented in Table 1. Cholesterol levels significantly ($P<0.05$) increased with crude oil contamination. In the control treatment, the value was 1.71 mmol/L ± 0.08. It ranged from 2.05 mmol/L ± 0.70 to 2.14 mmol/L ± 0.34 in the crude oil treated treatment (Table 1). The mean total protein value for treatment I was 39.49 g/L ± 5.19. The values significantly ($P<0.05$) increased with increasing concentration of dietary crude oil contamination and ranged from 41.21 mmol/L ± 1.19 to 53.41 mmol/L ± 3.43 from treatment II (4 ml/kg) to treatment V(16 ml/kg). Significant differences ($P<0.05$) due to treatment effects were observed in cortisol (fig. 1), amylase, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT), Table 1. The mean level of serum glutamic pyruvic transaminase (SGPT) in control rabbit was 25.16 IU/I ± 4.16 but the enzyme levels significantly ($P<0.05$) increased with increasing concentration of crude oil, ranging from 39.42 IU/I ± 5.26 to 60.78 IU/I ± 19.27 in treatment II (4 ml/kg) to IV (12 ml/kg), and then dropped to 57.25 IU/I ± 3.59 at the V (16 ml/kg). The level of alkaline phosphate was 15.29 IU/I ± 3.72 in the control diet fed rabbits. Also these levels significantly ($P<0.05$) increased with increasing concentration level of dietary crude oil contamination and ranged from 17.99 IU/I ± 1.37 to 24.75 IU/I ± 3.73 in treatment II (4 ml/kg) to treatment V (16 ml/kg). The level of serum glutamic oxaloacetic transaminase (SGOT) in treatment I was 20.44 IU/I ± 1.81, but the values significantly ($P<0.05$) increased with increasing concentration of dietary crude oil contamination from 40.21 IU/I ± 2.80 to 72.09 IU/I ± 3.96 in treatment IV, and then dropped to 55.01 IU/I ± 3.05 at treatment V (Table 1).

Discussion

Complex animals like the rabbit respond physiologically to the perception of an array of internal and external noxious or stressful stimuli, including predation attempts, harsh environment, habitat change and anthropogenic disturbance, through a rapid cascade of endocrine secretion within the hypothalamic pituitary-adrenal (HPA) axis (Axelrod and Reisine, 1984; Sapolsk, Romero and Munck 2000 . Goldstein, 2003). This response is widely conserved across vertebrates and ultimately involves the secretion of glucocorticoids (GC, cortisol or corticosterone) from the adrenals (Wingfield et al., 1997). Serum cortisol levels are known to increase in the hypothalamic–pituitary–adrenocortical system during a period of stress and may trigger a suite of behavioral and physiological responses, including a rise in locomotion activity and adequate mobilization of energy stores (Wingfield et al., 1997 & 1998). This supports findings in the present study. The cortisol was observed to be increasing with increasing dietary concentration of crude oil in treatment. Tissue damage or necrosis resulting from injury or disease is generally accompanied by increases in the levels of several nonfunctional plasma enzymes bert 2001;.Nicolson,2008). In man and many other mammals, acute inflammation of the pancreas (pancreatitis) causes the release of amylase into circulation (Ganong, 2005). Similar effects were observed in this experiment. As the level of the concentration of the crude oil was increasing, the amylase level was also increasing. The increase in plasma amylase levels in the rabbits may have been as a result of inflammatory reaction of the pancreas. There are many enzymes such as phosphatases, dehydrogenases and transferases that are found in the serum which did not originate from the extracellular fluid. During tissue damage, some of these biomolecules find their way into the serum, probably by leakage through disrupted cell membranes (Ganong, 2005). The increase in serum alkaline phosphatase, glutamaic pyruvic transferase and glutamic oxaloacetic as the concentration of crude oil increased. The mechanisms by which these enzymes are present in high concentration in the blood has been attributed to one in which their membrane pockets are damaged thereby leading to abnormal amounts being released into the blood (Mitoma et al., 1985). This corroborates with the previous evidence found in (Ologunde et al, 2008) who reported that serum alkaline phosphatase levels obtained from blood of rats fed on crude oil contaminated diets were elevated. Cholesterol is a key intermediate in the biosynthesis of related sterols such as bile acids, adrenocortical hormones, androgens and estrogens (Rahmani, Siddiqui, and Athar, 1988). In this study, increase in plasma concentration of cholesterol with increasing concentration of crude oil in the feed was observed. 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. 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. In this study, these parameters increase with increase concentration of crude oil in the feed. 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). In conclusion, it was observed that the inclusion of crude oil had significant ($P< 0.05$) effect on cortisol, total protein, cholesterol and enzymes (Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP).

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Table 1: Effects of treatments on serum enzymes and some biochemical parameters of chinchilla rabbits exposed to varying levels of crude oil contaminated diets.

Parameters	Treatments				
	I	II	III	IV	V
Amylase (Lu/l)	$208.16^{b} \pm 4.75$	$211.20^{b} \pm 10.33$	$225.00^{b} \pm 13.00$	$247.50^{b} \pm 10.11$	$392.00^{a} \pm 42.3$
Alkaline Phosphatase (ALP) (IU/l)	$15.92^{c} \pm 3.72$	$17.99^{bc} \pm 1.37$	$18.98^{b} \pm 0.48$	$23.47^{a} \pm 1.18$	$24.89^{a} \pm 3.37$
Serum glutamic oxaloacetic transaminase (IU/l)	$25.16^{d} \pm 4.16$	$39.42^{b} \pm 5.26$	$56.78^{b} \pm 9.61$	$60.78^{a} \pm 19.27$	$57.25^{b} \pm 3.59$
Serum glutamic pyruvic transaminase (IU/l)	$20.44^{d} \pm 1.81$	$40.21^{d} \pm 2.80$	$48.01^{c} \pm 2.86$	$72.09^{a} \pm 3.96$	$55.01^{b} \pm 3.05$
Cholesterol (mmol/L)	$1.71^{c} \pm 0.08$	$2.05b^{c} \pm 0.07$	$2.24^{a} \pm 0.14$	$2.39^{a} \pm 0.09$	$2.14^{b} \pm 0.34$
Total Protein (g/L)	$39.49^{c} \pm 5.19$	$41.21^{bc} \pm 1.19$	$43.3b^{c} \pm 1.43$	$47.71^{b} \pm 1.44$	$53.41^{a} \pm 3.43$

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

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Fig.1. Effect of treatments on cortisol of male chinchilla rabbits exposed to varying levels of crude oil contaminated diets.