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THE EFFECTS OF CRUDE OIL CONTAMINATED FORAGE ON ORGAN DEVELOPMENT OF FEMALE RABBITS

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Abstract

Oil spill is a major challenge of the petroleum sector. Oil spill on land affects rodents and other burrowers. A study was carried out to assess the impacts of graded oil contaminated forage on the organ development of female rabbits in a completely randomized experimental design. Forty semi-adult female mixed breed rabbits aged 16 – 17 weeks weighing 1.30 - 1.42kg were used for this study. The forty rabbits were therefore randomly allocated to the five dietary groups including the control (A), which was fed with plain feed containing no crude oil. Four other groups contained 0.05 %, 0.10%, 0.15% and 0.20% of crude oil contaminated forage representing treatments B, C, D and E respectively. After 24 weeks, results showed that the organs of the female rabbits including ovary size and weight, heart, liver and kidney weights decreased as the percentage of crude oil in the contaminated forage increased. It can therefore be concluded that the crude oil contaminated forage decreased the performance of female rabbits.

Key words:
Oil spill, performance, organ development

1.0 Introduction

Crude oil exploration and production is the most important economic activity in Nigeria. Crude oil accounts for 80% of Nigeria export, 95% of foreign exchange earnings, and 20% of her GDP (Nwilo and Badejo, 2005). The country is ranked as the 12th largest producers and 7th largest exporter of crude oil in the world (Ohimain, 2013). Oil and gas exploration has contributed immensely to the development of the Nigerian state and economic empowerment of multinational oil corporations, but to the neglect of the host communities, who bear the brunt of oil exploration. Host communities suffer for the direct environmental impacts of oil exploration such as oil spills, routine gas flaring and disposal of oily waste water and produced water. The environmental impacts of decades of oil exploration in the Niger Delta has manifested as contaminated surface and ground water, vegetation and soil pollution, depletions of fisheries and air pollution (Moffat and Linden, 1995; UNEP, 2011).

Oil spill is one of the major cause of concern for the Nigerian government, operating oil companies and host communities. When oil spill occurs it affects virtually all facets of the environment including air, surface and ground water, sediment and soil, vegetation, fisheries and wildlife. And because humans are linked to their environment, oil spill could directly or indirectly threatens the nutrition, economic activities and health of the host communities. For instance, in Ogoni land, UNEP (2011) reported that oil spills affected surface and ground water, sediment, soil, vegetation and fisheries and other natural resources that the people are dependent upon for their survival. In the past, oil spill was mostly caused by routine production activities of multinationals such as equipment failure, and pipeline corrosion. Now, crude oil spill incidents and quantity spilled are increasing mainly due to illegal bunkering and artisanal refining (Ogbeibu and Iyobosa, 2013; Ogodo and Esemuede, 2013). Ogbeibu and Iyobosa (2013) estimated that about 10% Nigerian oil is lost to oil vandal annually.

The effects of oil spill on the environment have been well documented, especially on surface and ground water (UNEP, 2011), soil and vegetation (Ogodo and Esemuede, 2013), fisheries and aquatic mammals (Ogbeibu and Iyobosa, 2013). But few literature document the effects of oil spill on land mammals, such as Yahaya (2001), Berepubo et al. (1994), Johnson (1994), Ngodigha et al. (1998), and Nodu and Timibitei (2011). In this study, we worked on the effect of crude oil contaminated forage on organ development of female rabbits.

2.0 Materials and methods

2.1 Animals procurement

Forty semi-adult mixed breed female rabbits aged 16 – 17 weeks were used for this study. The rabbits initially weighed between 1.30 - 1.42kg. The rabbits were procured from the farm of Rivers State Agricultural Development Programme (ADP) farm (livestock unit), Rumuodomaya in ObioAkpor Local government area

2.2 Pre-experimental management of animals

The pre-experimental period lasted two weeks during these period all the animals used in this study were subjected to the same management conditions including housing, feeding and medications (i.e antibiotics, coccidiostat and vitamins).

Housing

During the pre-conditioning period, all the rabbits were properly housed in hutches. These single tier hutches had three compartments per hutch. The rabbits were kept two per compartment. These single tier conventional hutches were made of wire mesh and wooden frame. The compartments were large enough to accommodate about four rabbits each. All the hutches were housed in a single room (shed) under the same environmental condition. This made it convenient for the experiment. All the hutches and animals were properly identified on individual basis using identification tags.

Feeding

All the rabbits during the two weeks of pre-conditioning were given the same feed and water. Feeders and drinkers in the hutches which were made of heavy clay to prevent over turning and spillage of feed and water by the rabbits. The animals were fed *ad libitum* with feed made of green forage prepared from mixture of commonly available grasses and legumes. The forage was supplemented with concentrate feed to boost their nutrition and enhance their sustenance (Aduku and Olukosi, 1990; Fielding, 1991). The green forage fed to these rabbits composed of *Panicum maximum* (Guinea grass), *Brachiar amutica* (Para grass), *Centrocema pubescens* (Centro), *Puereria phaseolides* (Pecero), *Calapogonium muconoides* (Calapo) and *Aspiria africana* (Bush marigold). The concentrate feed supplement was the grower's mash as recommended by Ibeawuchi and Fajuyitan (1986). Clean and cool water was provided *ad libitum* on daily basis. The feeding and drinking troughs were cleaned and washed on daily basis and also disinfected as often as necessary.

Medication

During pre-conditioning, all the animals were administered prophylactic coccidiostat (25% embazin) as well as broad spectrum antibiotics like terramycin soluble power. Some vitamin drugs like biovite were also administered to the rabbits. These drugs are to protect the animals from some common rabbit diseases that would have attacked the animals and interfered with the study during the experimental period.

2.3 Crude oil source and means of contamination

The crude petroleum used in this study is the Bonny light grade obtained from Nigeria Agip Oil Company (NAOC) at Obrikom flow station in Ogba-Egbema-Ndoni local government area of Rivers state. Prior to actual commencement of the study and contamination of the forages, the crude oil was exposed for 24hours in shallow pans to allow evaporation of the light fraction in order to ensure a stable product (White, 1975). This was to simulate

naturally occurring conditions following oil spillages in the oil bearing communities.

2.4 Experimental design

The completely Randomized Design (CRD) experimental method was used for this study. The use of the CRD experimental design was based on the fact that the subjects (animals) in this study were homogenous. The forty female rabbits used for this study were randomly divided into five groups (Table 1) of eight animals per group representing one control group and four treatment groups (A, B, C, D and E). Group A served as the control group. The forty rabbits were therefore randomly allocated to the five dietary groups including the control (A). Each treatment group comprised of four replicates with two animals per replicate giving a total of eight animals per treatment group. The duration of the experiment was 24 weeks.

Table 1: Graded oil composition in the forage

Treatment No	% oil composition of forage	Mass composition
Treatment A	Plain feed with contamination (0.0% crude oil)	-
Treatment B	0.05% crude oil contamination	0.5g crude oil/kg of forage
Treatment C	0.10% crude oil contamination	1.0g crude oil/kg of forage
Treatment D	0.15% crude oil contamination	1.5g crude oil/kg of forage
Treatment E	0.20% crude oil contamination	2.0g crude oil/kg of forage

2.5 Diet mixture and levels of contaminated crude oil inclusion

The use of these levels of crude oil contamination in this study was informed by the results of a study reported by Berepubo et al. (1994) providing these dose of crude oil not to be too lethal compared to the pilot study of Johnson (1994) that reported a high mortality of 25 out of 32 rabbits (78.13%) used in the experiment was recorded within one week of administration of the crude oil at the different level of 1%, 2%, 3% contamination. It therefore suggest that the above levels of crude oil contamination/ingestion by animals are highly lethal. Hence, lower concentration were used in this study (Table 1).

On commencement of the experiment, all the animals from the various treatment groups were then fed forage contaminated with the graded levels of crude petroleum. The contamination of the forage was done by incorporation of the measured amounts of crude oil in the forage by simple mixing and homogenization for at least two hours to ensure imbibition. Rabbits in treatment, A which serve as the control group, were not however fed the crude oil contaminated forage. They were fed crude oil free forage. In addition to forage, all the rabbits in the various group (A – E) were fed of commercial growers mash (Ibeawuchi and Fajuyitan, 1986). The supplementation of concentrate feed was to provide basic required nutrients in order to avoid malnutrition and its attendant effects. The concentrate was not however given to the rabbits until a substantial proportion of the test feed have been eaten by rabbits in the treatment groups (B – E).

2.6 Determination of organ weight

Two rabbits from each of the dietary groups (including the control group) were sacrificed for the post mortem examination. The organs including ovary, heart, liver and kidney were weighed.

2.7Statistical Analysis

Data obtained from this study were subjected to analysis of variance (ANOVA) according to the procedures of Steel and Torrie (1981), where significant differences existed, the treatment means were compared using Duncan Multiple Range Test (DMRT) (Duncan, 1955).

Results

Some organs (ovary, heart, liver and kidney) that were examined are presented in Table 2. Ovarian weight decreased as the level of oil contamination of the forage increased. Treatment E had the lowest mean weight of 0.51±0.01g, while treatments B, C and D had mean weights of 0.64±0.02, 0.60±0.02 and 0.58±0.02g respectively and were not significantly different (P>0.05) from each other. Ovaries in the control group had the highest mean weight of 0.87±0.02g and were significantly different (P<0.001) from those of all other groups.

The treatments significantly (P<0.01) influenced the heart weight of the rabbits with the highest mean heart weight of 8.02±0.18g observed in the control animals. The lowest heart weight was recorded in animals fed treatment E (4.75±0.35g). The other treatment groups showed mean weights of 6.48±0.16, 5.95±0.09 and 5.51±0.48g for treatment B, C and D respectively. Heat generally decreases as the oil contamination of the forage increased.

The liver weight of the rabbits in this study showed significant differences (P<0.05) among the different treatment groups. The control group had the highest liver weight (39.21±0.89, followed by treatment B with 31.25±1.25g. Others are treatment C (29.50±0.50), treatment D (27.65±0.35) and the least weight of 26.39±0.17g was observed for animals in treatment E. Generally, liver weight decreases as the oil contamination of the forage increased.

Results obtained on the kidney weights of the experimental rabbits show a decrease in weight with increasing levels of crude oil contamination in the feed. The results revealed that the control rabbits had a mean weight of 10.24±0.38g, while the least weight was observed in treatment E with 6.03±0.03g. Treatments B, C and D recorded mean kidney weights of 8.06±0.06, 7.20±0.20 and 6.94±0.08g respectively. The results indicate that these values were significantly different (P<0.01) from each other.

Table 2: Effect of graded levels crude oil contamination on organ weight (g) of female rabbits

Organ	Treatment				
	A	B	C	D	E
	Control	0.05	0.10	0.15	0.20
Ovary (g)	0.87 ± 0.02^{a}	0.62 ± 0.02^{b}	0.60 ± 0.02^{b}	0.58 ± 0.82^{b}	0.51 ± 0.01^{c}
Ovary size (cm)	0.030	0.024	0.018	0.018	0.004
Heart (g)	8.02 ± 0.18^{a}	6.48 ± 0.16^{b}	5.95 ± 0.09^{b}	5.51 ± 0.48^{bc}	4.75 ± 0.35^{c}
Liver (g)	39.21 ± 0.8	31.25 ± 1.25^{b}	29.50 ± 0.50^{bc}	27.65 ± 0.35^{d}	27.39 ± 0.17^{e}
Kidney (g)	10.24 ± 0.38^{a}	8.06 ± 0.06^{b}	7.20 ± 0.20^{c}	6.94 ± 0.08^{c}	6.03 ± 0.03^{d}

Within row mean ±SEM with different superscripts are significantly different at P<0.05

Discussion

Results of the effects of dietary crude oil (contaminated forage) on organ weights showed significant differences (P<0.001) between the control and contaminated groups in ovary, heart, liver and kidney weights. It was observed that there was a general reduction in organ weights/development with increasing levels of crude oil ingested. This may have probably affected the normal functioning of the organs. The control group had higher organs weights, while the least organs weights were recorded among

rabbits fed the highest percentage of contaminated forage. This observation is in consonance with the reports of Berepubo et al. (1994) and Ngodigha et al. (1998) who noted similar reduction in organ weights in rabbits and West African Dwarf goats respectively following crude oil ingestion by these animals. Organ weight analysis has been reported to be very important in general toxicity studies in animals (Heywood, 1981). The importance of these organs in the effective functioning of the animals systems is already an established fact. Therefore any impairment would imply inefficiency in their functions. For instance, the heart is the organ which pumps and supplies blood to the other organs and systems of the body. A normal heart (in size and function) supplies blood containing the necessary nutrients to all parts of the body including a developing foetus. However, the reduced sizes of heart (abnormal size) in the rabbits placed on contaminated diets were caused by the stressor factor (crude oil). The implication of this observation is a possible cardiovascular disorder resulting in the malfunctioning of the heart among these rabbits. These may be related to the consequential release of stress-related adreno-corticular trophic hormone (ACTH) and glucocorticoids, especially when the stressor is approaching an intolerable level (Ganong, 1975). Although this was not monitored in the rabbits, it could possibly be implicated because when the animals were initially introduced to the crude oil contaminated forage (stress factor), they reacted by showing initial weakness and later developed some level of tolerance and continued the ingestion of the contaminated feeds. These culminated in the reduction in the size of the heart with a consequent effect on its blood pumping function and distribution of nutrients.

Similar reduction in size was also observed in the two other important organs –liver and kidney. These two organs work to eliminate toxic compounds or substances from the body. The reduced size of the liver observed among crude oil treated rabbits in treatment B – E was also as a result of the stressful effects of the treatment factor. The atrophy of the liver will definitely affect its function (detoxification) as it tries to eliminate the harmful effects of the crude oil from the body. The poor functioning capacity of the liver as a result of its atrophy will therefore lead to infiltration and accumulation of hydrocarbon molecules in the hepatic cells. To this, Selye (1974) and Baklavajyan (1983) had observed a decrease in the glycogenic and detoxifying functions of the liver as toxic metabolites accumulate in the blood systems. This leads to weakening of the body defensive mechanisms, which is detrimental to in animal production. This observation is in conformity with the works of Yahaya (2001), Selye (1974) and Rathiff et al. (1970).

The kidney, which is another important organ in the elimination of waste or toxic matters from the body, was also adversely affected in this study. The kidneys of the control were superior in size and weight to those of the other treatment groups. The reduction in size of this organ (kidney) among the crude oil treated rabbits is attributed to the crude oil stressor effect which may also not be unconnected with the presumed inadequate blood and nutrient supply from the already atrophied heart. This would negatively affect the kidneys resulting in kidney dysfunction and invariably leading to accumulation of toxins in the body. Some of the effects may include weakening of the body defense mechanisms like in the case of liver dysfunction resulting in the animals easy succumbing to pathogens, mutagens or carcinogens. This observation lends credence to the work of Sawicki (1977), Carter (1983), Berepubo et al. (1994). The significant ($P<0.001$) decrease in weights of these organs examined is not however surprising since it is consistent with the overall decline in body weight gain observed in the test rabbits (Table 2). This decline in weight as it affects both organs and overall body weight gain in the test animals therefore makes it possible to relate this phenomenon to the toxic effects of the ingested contaminated feeds. However, because of paucity of literature in this regard, it is therefore not very clear whether the decrease in the organ weight as the animals attempt to adapt to the foreign toxic compound (hydrocarbon toxicity) in the system or a real pathological response to the toxic compounds present in the crude oil (Berepubo et al., 1994). Information in this regard is yet to be elucidated and calls for further investigations.

Of much interest however in the present study is the similar reduction in ovarian weights of the rabbits placed on contaminated diets compared with those in the control group. It is observed in this study that the

ovaries of the tested rabbits suffered significant reduction in size and weight when compared with those reported by Berepubo et al. (1994) and Yahaya (2001) who fed rabbits with crude oil contaminated feed (forage) and observed a reduction in the size and weight of their reproductive organs. Nwokolo et al. (1984) has earlier reported similar reduction in the reproductive organs of poultry fed crude oil contaminated feed. The decrease in size and atrophy of the follicles among the tested rabbits (B - E) observed in the present study could be associated with subfertility. Similar situation of subfertility resulting from size reduction in reproductive organs has been reported in previous studies on marine animals (Ekweozor and Snowden, 1987), and in bovine (Plyaschenko and Sidorov, 1987).

It has been noted that rabbits in the treatment groups (B – E) in this study showed poor reproductive characteristics beginning with late puberty attainment to poor oestrous behavior, imbalance secretion of reproductive hormones, poor receptivity of males (most of them engaging the males in fighting), and no conception among them. These negative reproductive characteristics are evidences or subfertility substantiated by the decrease ovarian size and weight, which could be otherwise regarded as underdevelopment of the ovaries. Similarly, atrophy of the ovaries may have affected hormonal production and caused the non-acceptance of males by most rabbits in the treatment groups. All these negative effects could be inferred, were caused by the stress factor exerted on rabbits fed the crude oil contaminated forage feed. It thus agrees with an earlier report by Robert (1956) who observed that atrophy of ovaries and underdevelopment of varies in an abnormality which is ordinarily bilateral, found primarily in poorly fed and managed animals, identified as a stress-causing agent. Animals respond sensitivity to stress factors as observed in the present study which agrees with the work of Plyaschenko and Sidorov (1987) who noted that crude oil is an environmental stressor which delays sexual maturity, increases duration of reproductive cycles, causes relative quiescence of the ovaries and lowers conception rate in animals. Rabbits in the tested groups of this study could be categorized as being poorly fed as they were subjected to feed contaminated with crude oil. They are therefore bound to suffer the fate of poisoning and nutrient deficiency.

Conclusion

oil spill is a major challenge of the petroleum industry. Spills often occur during routine production activities. On land spills have rodents and their forage (vegetation). This study investigated the impacts of crude oil contaminated forage on female rabbits. The results showed that the organs of the female rabbits including ovary size and weight, heart, liver and kidney weights decreased as the percentage of crude oil in the contaminated forage increased. It can therefore be concluded that the crude oil contaminated forage decreased the performance of female rabbits.

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