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Effect of Heavy Metals in feeding Dumpsite forage (Calapo - Calopogonium mucunoides) on Haematological profile of Rabbits (Oryctolagus Cuniculus)

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Abstract

Effect of feeding dumpsite forage (Calopo -*Calopogonium mucunoides*) on the heamatology of rabbits (Oryctolagus cuniculus) was investigated. 24 rabbits: 20 females and 4 males were obtained and distributed randomly into two treatment groups of 10 females and 2 males with each of the groups being fed dumpsite forage and non-dumpsite forage respectively. The forage, specifically Calapo (*Calopogonium mucunoides*) was fed to the two groups' ad-libitum with the non-dumpsite fed group serving as the control. After a period of 20 weeks, soil samples were taken from the sites sent to the laboratory, while the rabbits were sacrificed by anesthesia using chloroform soaked in the control and placed in the desiccators along with the animal one after the other, 5-10 ml of blood obtained were placed into EDTA bottles and transported to the laboratory for haematological and heavy metal analysis. The haematological results revealed a decreased in RBC count, Hemoglobin, PCV, MCH and MCV which may be attributed to the development of anemia in the form of microcytic and hypochromic. Total white blood cell count decreased while the neutrophils and eosinophils values increased in the dump-site treatment. Also, the heavy metal analysis from the dumpsite revealed increase in the content of cadmium, arsenic, lead and mercury above standard permissible limits. In conclusion, feeding of dumpsite forages to rabbits could pose hematoxic and hyperchromasia effect on the rabbits, thus posing health risk in animal and human populations exposed to chemical substances from waste dumpsites.

Keywords: Calapo (*Calopogonium mucunoides*), Rabbits (*Oryctolagus cuniculus*), Haematology, Heavy metals.

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INTRODUCTION

As developing countries of West Africa become industrialized and urbanized, heavy metal pollution is likely to reach disturbing levels. It has been pointed out that Africa's contribution to global lead pollution has increased from just 5% in 1980's to 20% in 1996 (WHO, 1996). In Nigeria, the growing rate of industrialization is gradually leading to contamination deterioration of the environment, and thus industrialization and heavy metal pollution are positively correlated. Excessive levels of heavy metals may occur in the biosphere as a result of normal geological phenomena such as ore formation, weathering of rocks and leaching or degassing (in the case of Hg). Other activities that could contribute to excessive release of these metals into the environment include burning of fossil fuels, smelting, and discharges of industrial, agricultural, and domestic wastes as well as deliberate application of pesticides. Anthropogenic contributions or human activities such as petroleum mining and prospecting as well as oil spillage are also major sources of these metals (Duffus, 1980; Osuji and Onojake, 2004).

In recent times, there has been considerable interest in the level of heavy metallic elements in foods because of their deleterious effect on human health. Apart from those communities exposed to high levels of pollution by industrial effluent or emissions rich in heavy metals, it is evident that, for most individuals, food and diet are the most common source of these potentially toxic elements. These elements in food or drinking water amount to approximately 80% for cadmium, 40% for lead and 8% for mercury (Bennet, 1984). Metal contamination in foods, especially in meat have been broadly investigated (Sharif, et al, 2005). Heavy metal contamination can be transferred to animals through direct exposure, polluted water, crops grown on irrigated sewage, industrial effluents, vehicle emission and dirty slaughter houses (Joseph and Srivastha, 1993; Jukna, et al., 2006).

The proliferation of open and unsafe dumpsites containing multiple disposals of domestic, municipal, industrial and medical waste is common practice in most cities in Nigeria. Dumpsite is an old traditional method of waste disposal similar to landfill method of waste management. Dumpsites are often established in disused quarries, mining or excavated pits away from residential areas, however, studies has shown that all forms of waste disposal have negative consequences on the environment, public health, and local economies (Abdus-Salam and Adekola, 2009). These dumpsites contain build up of heavy metals in soils from anthropogenic source which have been reported to be harmful to crops, animals and human health, their concentrations and transformations to heavy metals in solid municipal waste leads to accumulation in the

food web. These dumpsites serve as feeding grounds for disease breeding animals especially rats, birds and stray animals; thereby contributing greatly to their nourishment and growth (Adewuyi and Opasina, 2010). Leachate from dumpsites are of particular interest as they contain potentially toxic heavy metals. These metals are known to bioaccumulate in soil and have long persistence time through plants or animals (Miranda et al., 2005). The risk associated with the exposure to heavy metals present in food product had aroused widespread concern in human health.

The goals of animal production to produce quality products for increasingly health-conscious consumers and improving the quality of human life tends to be deterred by the presence and bioaccumulation of heavy metals in animal products.

Due to the current trend of industrialization and urbanization, heavy metal pollution is increasing at alarming rates to reach disturbing levels thus leading to the contamination and deterioration of the environment. Apart from those communities exposed to high levels of pollution by industrial affluent or emissions rich in heavy metals, it is evident that most individual foods are contaminated by these potentially toxic elements (Baykov et al., 1996). Within Uyo metropolis where most of the livestock production practices are extensive, these animals graze on the dumpsite areas as they have luxuriant vegetations. Nomadic cattle rearers found within the state also graze cattle on these dumpsites.

Since contamination with heavy metals is a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen and Uruc, 2006), it becomes necessary to study the concentrations of toxic heavy metals in rabbits fed from dumpsites in order to assess the levels of exposure of the consumers to toxic metals and henceforth, maintain an ongoing knowledge on the levels of these metals both in the environment and in meat.

Meat is a very rich and convenient source of nutrients including microelements. The reported cases of heavy metal contamination in meat and other animal products is of great concern for both food safety and human health because of the toxic nature of these metals at relatively minute concentrations. Hence, given the prevalence of these pollutants in the environment, there is a clear need for the sources and effects of their contamination to be known, with the aim of reducing both direct effects on animal health and indirect effects on human health (SCAN, 2003) and the needed improvement in animal production and performance may be achieved by investigating the effects of some of these heavy metals on the physiological responses in rabbits. Ingestion of these contaminants by animals causes deposition of residues in meat especially for animals managed extensively or for most nomadic cattle rearers who make use of these dumpsites as feeding sites, as such higher levels of metals have been found in beef and mutton due to the grazing of cattle on contaminated soils (Baykov et al., 1996). Umesiobi et al. (2000) reported that parameters for assessing the meat quality and physiological performance of these animal based on their feed intake are mainly hematological parameters.

The domestic rabbits are primarily herbivorous and consume most types of grains, forages and hay. Diets, whether home grown or commercially prepared consist of ingredients from plant sources. Since rabbits can utilize a certain amount of forage, they have a place in food production by making use of some non competitive feeds (Herbert et al., 2005). Forage can contribute up to 50% of rabbit diets (Sanni et al., 2005), although there is improvement in performance of rabbit fed concentrate and forage compared to feeding forage or pellets alone (Taiwo et al., 2004). For maximum performance, combination of Centrosema pubescens and concentrates in 50:50 ratios is the most efficient and should be used by rabbit farmers to increase production at a reduced cost (Nworgu et al., 1998).

Heavy metal is a general collective term which applies to the group of metals and metalloids with atomic density greater than 4 g/cm3 or that are 5 times greater than water (Hawkes, 1997). Heavy metals are also called trace element due to their presence in trace (mg kg-1) or in ultra trace $(1\mu g kg-1)$ quantities in the environmental matrices. They are basically recovered from their ores by mineral processing operations (Lenntech, 2004; UNEP/GPA, 2004). Heavy metals such as tin, iron, copper, manganese and vanadium occur naturally in the environment and could serve as plant nutrients depending on their concentrations. Mercury, silver, cadmium, lead, chromium and many others that are indirectly distributed as a result of human activities could be very toxic even at low concentrations. These metals are non-biodegradable and can undergo global ecological circles.

Virtually, all metals can produce toxicity when ingested in sufficient quantities but the heavy metals are especially important because either they are so pervasive or they produce toxicity at such low concentrations. In general, heavy metals produce their toxicity by forming complexes or 'ligands' with organic compounds. These modified biological molecules lose their ability to function properly and results in malfunction or death of the affected cells. The most common groups involved in ligand formation are oxygen, sulfur, and nitrogen. When metals bind to these groups, they may inactivate important enzyme systems or affect protein structure.

Therefore, this study is made to investigate the effect of these heavy metals on hematological of rabbits fed forages from dumpsites with the view to elucidate the risk of contamination to the environment, the living organisms and the secondary consumers being humans, especially of animals that obtain their feeds from these sites.

MATERIALS AND METHODS Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonate V, sodium bicarbornate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, distilled water, hutches, concentrate feed, syringe and hypodermic needles, EDTA treated bottles, latex hand glove, weighing scale, graduated vials, measuring tape, they were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

Experimental Animal And Management

The animals were sourced from the University of Uyo Teaching and Research Farm, Use-Offot, Akwa Ibom, Nigeria. A 2- week experimental period was used to get the animals (rabbits) acclimatized with the experimental procedures. The experiment lasted 20 weeks (June, 2013 to November, 2013). The animals used in this study were 4 bucks and 20 does crossbred rabbits aged 6-7 months. The males weighed between 1350g and 1650g, while the females weighed between 1400g and 1800g.

Four bucks and twenty does respectively were divided into two groups of 12 animals each. When placing the animals into groups care was taken in order to balance the groups such that there were no significant differences between them on the basis of age and weight and the animals were identified individually with the aid of a permanent marker on their ears. The groups were randomly assigned to two (2) treatment diets: dumpsite fed animals and non-dumpsite fed animals.

The experimental animals were housed in a wooden hutch with a wire mesh floor and in-built waste trays. The management techniques employed for all the experimental animals included regular cleaning of the hutch, feeding and watering of the experimental animals on a daily basis. The experimental animals were managed well.

Drinkers and feeders were made of plastics and concrete with narrow but blunt mouth to discourage fed wastage and injuries. Forage (experimental diets) and clean water was also supplied ad libitum. Permission and approval for animal studies were obtained from the college of health sciences animal ethnics committee, University of Uyo.

Experimental Animal Health

The rabbits acquired were treated against internal and external parasites by subcutaneous injection of ivomec (0.2 ml per rabbit) and a broad spectrum antibiotic (Oxytetracyclin L. A.) was also administered at the rate of 0.2 ml per rabbit. Sulphur powder was given occurrences of mange and neomycin was given for diarrhea at the rate of 10g per four (4) liters of drinking water.

Experimental Designing and Feeding Of Experimental Diets

Two treatments being the waste dumpsite fed and the non-dumpsite fed. Forages were obtained from two sites, one being the waste dumpsite within Uyo metropolis and the other being a land, which is the non- waste dumpsite. Forage used was Calapogonium mucunoides due to its palatability to the animals. The forages were supplied daily to the animals and fed adlibitum. Alongside with the forage, the concentrate of pelleted poultry grower's mash meal (20% CP and 2700Kcal/kg) were fed routinely to facilitate the growth of the animals.

Group	Female	Male	Treatment (site)	Duration		
1(NDS)	10	2	Non-dumpsite forage	20 weeks		
2(DS)	10	2	Dumpsite forage	20 weeks		
Table 1 : Showing the experimental designing of the study.						

Soil and Plant Analysis Sample Collection

Soil samples were randomly collected 15 cm depth with the aid of a soil augar from different parts of the dumpsites while control samples were obtained from fallow lands whereby the forage was predominant. For the forage, wholesome and disease free parts were obtained of the plant Calopogonium mucunoides. The soil samples were emptied into well-labelled polythene bags while the plant samples were placed in labeled brown envelops. These were transported to the laboratory for analysis.

Sample Preparation

The soil samples were air-dried at room temperature (Ebong et al., 2008) grinded with the aid of mortar and pestle, then, sieved with a 2 mm mesh to be stored in properly labeled small containers before the analysis. Plant samples were washed with distilled water, placed in labeled envelops and placed in ovens at the temperature of 80 °C until dried. Samples were blended and then stored in air tight containers.

The concentrations of Lead (Pb); Arsenic (As); Cadmium (Cd) and Mercury (Hg) in the digested soil and plant samples were determined using Atomic Absorption Spectrophotometer (Unicam 939/959 model) following the methods described by Ebong et al. (2008).

Sample Analysis

To determine the heavy metals present in the samples, the samples were digested using the Wet Digestion Method to allow the elements in the samples to be liberated (Brady and Weil, 1999).

Procedures for Digestion

1g of <2.00 mm sieved plant sample was placed in a crucible and ashed in a furnace at 500 $^{\circ}$ C for 4 hours. The ash was then dissolved in 5 ml of 20% HCl and filtered using an acid washed filter paper. The volume of the filtrate was increased to 50 ml using distilled water and concentrations were read using Atomic Absorption Spectrophotometer.

For the soil sample, 1g was weighed into digestion flask, 20 ml of nitric acid, 10 ml of Perchloric acid and a drop of Concentrated Sulphuric Acid was added and heated in a sandbath. The mixture was allowed to stand for 30 minutes. The digestion chamber in the laboratory was used and the sample digested until the colour turned white. This was allowed to cool, thereafter, the digested samples were filtered and the filtrate was made up to 50 ml solution with addition of distilled water. The digested solutions were stored in labeled sample bottles.

Determination of Heavy Metals

The heavy metals concentrations in the digested samples were determined using the Unicam 939 model Atomic Absorption Spectrophotometer.

The principle of the Atomic Absorption Spectrophotometer involves absorption of light at a wavelength specific to that element by free atoms of the element. The Atomic Absorption Spectrophotometer was calibrated with standard solutions for each element then the sample solution was aspirated into the equipment and the reading was shown on the Atomic Absorption Spectrophotometer.

Heamatological Analysis Blood collection

The blood collection was done at the end of the 20th week of the feeding trial. Bleeding was done between 9.00 am and 10.30 am on the day of collection. Blood was taken from their jugular vein. The rabbit was first removed from the cage by holding it securely on the scruff, held by the hind and fore limbs and was laid on the slaughter slab. The animals were anaesthetized using chloroform, thereafter with the aid of a sharp knife the jugular vein was severed. The blood was then collected immediately into a set of sterile plastic bottles containing EDTA for haematological test.

Haemoglobin concentration

This was determined using the haemoglobin cyanide method formerly called cyanmethaemoglobin method.

The haemoglobin in the blood was oxidized to haemoglobincyanide by the action of potassium ferrocyanide. The haemoglobin concentration was finally determined as described by Schalm et al. (1975).

Erythrocyte count

A solution of formal citrate was prepared by mixing 10 ml of formalin with one litre of trisodium citrate solution. 31.3 g per litre is used as diluent for visual red blood cell count. The blood was taken into a positive displacement pipette and 40 ml of diluent prepared to give a final dilution of 1 in 201. The diluent sample was mixed and loaded into the counting chamber. The erythrocyte was counted using a haemocytometer method as described by Schalm et al. (1975).

Packed cell volume (PCV)

The sample was mixed very well. The Wintrobemicrohaematocrit tube was filled with blood by capillary action up to 2/3. The samples were spun for 5 minutes at 10,000 rpm and the PCV was read as a percentage using the designed scale reader Boron et al. (2005).

Leucocytes count

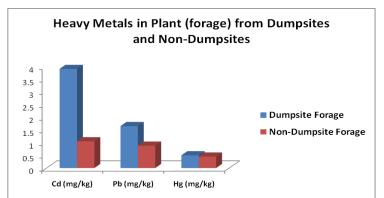
The leukocytes count was obtained using a haemocytometer with Natt and Hendricks diluent to obtain a 1:200 blood dilutions. The number of leucocytes was thereafter estimated in accordance with method of Schalm et al. (1975) as a % (relative number of each type of white blood cell in relationship to the total white blood cell count) or as an absolute value.

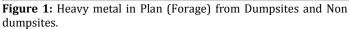
Red Blood indices

The Mean Cells Haemoglobin (MCH), Mean Cell Volume (MCV) and Mean Cell Haemoglobin Concentration (MCHC) were determined from RBC, PCV and haemoglobin (Hb). These haematological indices were calculated using the appropriate formulae as described by Jain (1986).

MCH = <u>Haemoglobin in g/1000 ml of blood</u>	pg/cell	
Red blood cell count in millions /ml		
MCV = <u>Volume of packed cells/1000 ml of blood</u>	fl or \rm{mm}^3	
Red blood cell count in millions /ml		
MCHC = <u>Haemoglobin in g/100 ml of blood</u>	X 100	g/dl or %
Volume of packed cells/1000 ml of blood		

Results Soil and Plant Analysis





Sample Identity	Cd (mg/kg)	Pb (mg/kg)	Hg(mg/kg)			
Dumpsite forage (DF)	3.90	1.65	0.50			
Dumpsite soil (DS)	5.70	8.30	0.55			
Non-dumpsite for (NDF)	age 1.05	0.88	0.45			
Non-dumpsite soil (N	OS) 4.20	3.20	0.35			
Table 2. Heavy motel content of soil and plants						

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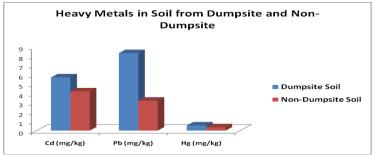


Figure 2: Heavy metal in Plan (Forage) from Dumpsites and Non dumpsites. **Hematological Analysis**

Blood pH

The blood pH concentrations were of mean values 7.35 for the dumpsite treatment and 7.25 for the nondumpsite treatment (Fig 3)

Red Blood Cells

The erythrocyte counts were of mean values 5.30 × 10^6 /mm³ for the dumpsite treatment and 6.95 × 10^6 /mm³ for the non-dumpsite treatment. (Fig 3)

Packed Cell Volume (PCV)

The Packed cell volumes were between 33.50% for the dumpsite and 49.50 % for the non-dumpsite group. The rabbits on control diet (NDS) had the highest PCV mean value while the dumpsite group had the low mean value. (Fig 3)

Haemoglobin concentration

Haemoglobin concentrations obtained were between 10.5 g/dl for the dumpsite and 15.0 g/dl for the nondumpsite group. The rabbits on control diet (NDS) had the highest haemoglobin mean value while the dumpsite group (DS) had the low mean value. (Fig 3). **White blood cells count and differential white**

blood cells count The leucocyte counts were found within the range of 8.0×10^{9} /l and the non-dumpsite group had mean values of 10.5×10⁹/l. The leucocyte count of the control group was higher than that of the dumpsite group on test diets. The eosinophil mean values were 7.0×10^9 %/l and 1.50×10^9 %/l for the DS and NDS treatment groups respectively. The basophils mean values were $0.2 \times 10^9 \%/l$ for dumpsite group (DS) and 0.1×10^9 %/l for non-dumpsite groups (NDS). The neutrophils mean values were found within the range of 63.5× 109 %/l and 40.00 × 109 %/l for DS and NDS respectively. The lymphocytes mean counts were found within $72.00 \times 10^9 \%/l$ and $45.40 \times 10^9 \%/l$ for DS and NDS groups respectively. The monocytes mean value also indicated 3.5× 109 %/l and 1.5× 109 %/l for DS and NDS groups respectively. The result of this study indicates that the mean lymphocyte value decreased in the DS group while the differential white blood cell counts increased in the DS treatments as compared to the NDS groups. (Fig 3)

Haematological indices ((MCV), (MCH) and (MCHC))

Haematological constant mean values were found for the DS and NDS treatment groups respectively as 46.5mm³ and 62.0 mm³,17.5 pg/cell and 21.5 pg/cell and 21.5% and 31.5 % for MCV, MCH and MCHC respectively. These haematological indices tended to decline in the dumpsite treated group as against the increase in the non-dumpsite groups. (Fig 3)

HEMATOLOGICAL PARAMETERS	DS	NDS
Blood pH	7.35	7.25
Red Blood Cells, or Erythrocyte(10 ⁶ / mm ³)	5.3	6.95
Packed Cell Volume (%)	33.5	49.5
Mean Corpuscular Volume (mm ³)	46.5	62
Hemoglobin (g/dl)	10.5	15
Mean Corpuscular Hemoglobin (pg/cell)	17.5	21.5
Mean Corpuscular Hemoglobin		
Concentration (%)	21.5	31.5
White Blood Cells (10 ⁹ /l)	8	10.5
Basophils (10º %/l)	0.2	0.1
Eosinophils (10º %/l)	7	1.5
Lymphocytes (10º %/l)	72	45.5
Monocytes (10 ⁹ %/l)	3.5	1.5
Neutrophils (%)	63.5	40
Band neutrophils (109%/l)	0.15	0
Adult neutrophils (10 ⁹ /l)	3.5	1.5
	P	

Table 3. Hematological profile of Rabbit from Dumped (DS) andNon-dumped (NDS) sites.

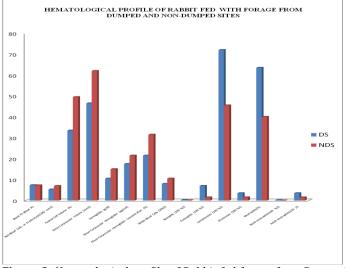


Figure 3: Hematological profile of Rabbit fed forage from Dumped (DS) and Non-dumped (NDS) sites.

DISCUSSION

In this study, the mean concentration of heavy metals analyzed for both in refuse dumpsite and non-refuse dumpsites (control sites) soils and forages are indicated in Figure 4.1a and 4.1b. Results obtained revealed higher concentration of heavy metal from refuse dumpsites soils as compared to their control soil site samples. This result is in accordance with studies by Amusan et al (2005). This could also be attributed to the availability of metal containing wastes at dumpsites which are eventually leached into the underlying soils. This enhanced level of Cadmium, Mercury, and Lead could be attributed to the dumping of PVC plastics, nickel-cadmium batteries, motor oil, Household and industrial garbage and disposal sludge on the dumpsites (Jarup, 2003, Ebong et al., 2008). The enhanced level of Pb, in this study agrees with the findings of Osuji and Onojake, (2004) who reported enhanced levels of Ni, Cu and Pb in the soils in Niger Delta. This may result in enhanced absorption by plants, leading to possible bio-accumulation of such plants and the animals which feed on them thus the high concentration of these metals in dumpsite plant samples as indicated in Table 4.1.

Haematological indices are considered to be pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of animals exposed to pollutants (Anonymous, 1995). Some haematological indices such as Hematocrit, Haemoglobin, Total erythrocyte count and Total leucocyte count are used to assess the functional status of the oxygen carrying capacity and immunological condition of the bloodstream and have been used as indicators of heavy metal pollution (Davis, 1998).

From Table 4.2, it can be deduced that the packed cell volume decreased in the DS group below recommended mean of 34.0% as reported by Mitruka

and Rawnsley (1977) for clinically healthy rabbits while the NDS was high with mean value of 49.5%. The red blood cells counts recorded in this study were 5.3×10⁶/mm³ for DS which was lower and 6.95×10^6 /mm³ for NDS which was within the recommended mean of 5.6 ×106/mm³ reported (Mitruka and Rawnsley, 1977; Hewitt et al., 1989) for healthy rabbits. This indicates that the DS forage had deleterious effects on erythropoietic tissues of rabbits. The mean cell haemoglobin (MCH) was 17.5 pg/cell for DS thus lower and 21.5 pg/cell for NDS which was within the normal range of 19.2-29.5 pg/ cell (Hewitt et al., 1989; Mitruka and Rawnsley 1977). The mean cell haemoglobin concentration (MCHC) falls within the normal range of 31.1 - 37.0 g/l as earlier reported (Hewitt et al., 1989; Mitruka and Rawnsley, 1977) for healthy rabbits. The MCHC values have been shown to be the most accurate and absolute values that indicate anaemic condition in animals (Esonu et al., 2006) and for DS group this was lower with mean values of 21.5% while in NDS with mean value of 31.5%, it fell within recommended range. This suggests that the DS group rabbits had microcytic and hypochromic red cells, meaning that the feeding of dumpsite forages affected iron utilization by the rabbits, hence these animals did suffer from reduced haemoglobin and mean corpuscular volumes (Esonu et al., 2006).

For the white blood cells differentials, the neutrophils values for DS were higher with mean value 63.5% as against NDS that was 40% thus falling within the normal range of 35 - 43.2 % (Hewitt et al., 1989; Kronfield and Mediway, 1975; Mitruka and Rawnsley 1977) recommended for clinically healthy rabbits. The are concerned with day to day neutrophils immunological defense against pathogens. This implies that the ingestion of the dumpsite forage had triggered the production of this blood component. The lymphocytes value are given at a standard range of (53.5 - 65.8%) (Hewitt et al., 1989; Mitruka and Rawnsley 1977) recommended for clinically healthy rabbits. Compared to this value, the DS group gave a high mean value of 72 $\times 10^{90}$ /l as against the NDS group with 45.5×10^{90} /l. The eosinophils have the normal range (1.0- 2.5%) (Hewitt et al., 1989; Mitruka and Rawnsley 1977) and for the NDS group with mean value 1.5×10^{9} %/l, they fell within range as against the DS group with a higher value of 7.0×10^{90} /l. The high presence of eosinophils in the DS group in this study is an indication that the animals did suffer allergic reactions due to the toxic metals and parasitic infections (Robert et al., 2003).

From this investigation, it is obvious that exposure of rabbits to heavy metals ingestion caused a significant decrease in erythrocyte values. Consequently, haemoglobin (Hb) and packed cell volume (PCV)

decreased with intake of contaminated dumpsite forages (Table 4.2). The observed reduction in haemoglobin, packed cell volume and erythrocyte count demonstrate and suggest an anemic condition in the dump site treated rabbits as against the control (non-dumpsite). These toxic components present in the dumpsite forages change blood chemistry and induce anaemia by causing bone marrow hypoplasia and interfered with platelet production in the animals, hence the reduced values (Sudakov, 1992). This assertion is supported by Snyder (1987) who demonstrated that benzene is activated in the bone marrow. Thus bone marrow failure as indicated in this study is characterized by inadequate production of red cell and other formed elements. Lowered RBC count, decreased Hgb and PCV, MCH and MCV are other concordant hematological changes found in the dumpwhich contaminated site group forage was administrated is indicating of anemia which is in the form of microcytic and hypochromic. This might be as a result of the effects of the toxicity effects of the dumpsite forages. This tendency to microcytosis and hypochromia hematopoiesis is commonly observed in the liver especially in the cases of toxicity. This decrease in hemoglobin was also found by Bersenyi et al, (2003) in rabbits poisoned by lead, by Jarup, 2003 in mice exposed to cadmium chloride, for Ognjanovic et al., (2003) in rats exposed to cadmium chloride. Also, Mercury and the combination of high concentrations of cadmium and mercury could inhibit heme synthesis of red blood cells and cause anemia signs described by Bottomley and Muller-Eberhard (1998).

The primary function of white blood cells appears to be to defend the body against foreign bodies, which is achieved by leucocytosis and antibody production (Rio, 2001). Total white blood cell count decreased (Table 4.2). The observation in this study is similar to the findings of Ngodigha et al., (1999) in which there was a reduction in total white cell count in goats as the level of crude oil concentration increased. Neutrophils and eosinophils values increased however in the dump-site treatment. This increase is an indication of stress imposed by toxic elements in the diets and confirmed Selye's (1963) finding that a stress stimulus elicits a defense response.

CONCLUSION

From the results of this study, it is hereby suggested that heavy metals is an environmental stressor which causes depression of total white cell and red cell count, thus, having serious consequences on hematological parameters in rabbits. This investigation showed heamatoxic effects of these heavy metals on the erythrocytes and leukocytes following long-term exposure in rabbits. Severe changes in blood indices by these toxic elements that were found in the present investigation and other studies indicate the necessity of even more concerns about the bio-environment pollution of heavy metals. Designation and provision of the health programs to limit causal exposure to these toxic elements is highly important for human health and in animal production.

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