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Palliative Effects of Vitamins C and E on Blood Parameters of Growing Rabbits Fed Crude Oil-Contaminated Diets

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Abstract. The palliative effects of vitamins C and E in rabbits that ingested crude oil were investigated on the animals' blood parameters. 24 males New Zealand White rabbits weighing between 770-835 grams were acquired for the study from one source in one batch and randomly allocated to three dietary treatments of 8 rabbits per treatment. The animals had similar diet and management conditions for 2 weeks to properly pre-condition them for the study after which they were presented with their experimental diets as: T_0 (was the negative control group; no crude oil and experimental vitamins C and E), T_1 received crude oil at 1.5g or 0.15% /kg of diet while the T_2 group also received dietary crude as in T_1 but in addition received 200mg (100mg of vitamin C + 100mg of vitamin E)/kg of diet, respectively for 2 weeks. The hemoglobin (Hb) concentrations of the T_0 animals were significantly higher ($P < 0.05$) than those of T_1 and T_2 animals. However, those of T_2 animals were significantly higher ($P < 0.05$) than those of T_1 animals. This trend was mirrored in the packed cell volume (PCV), red blood cell (RBC) counts and white blood cell (WBC) counts, respectively. It was concluded that ingestion of crude oil induced anemia and leukemia in rabbits. However, the intake of vitamins C and E had some palliative attributes in reducing the degrees of anemia and leukemia induced by crude oil ingestion in the rabbit.

Key words: Crude oil, Blood parameters, Vitamins C and E, Palliative effects and Rabbits

Introduction

Crude oil is a liquid fuel located underground. It consists of about 50-97% hydrocarbons, 6-10% of organic components, such as nitrogen, oxygen and Sulphur and less than 1% of metals, such as vanadium (Ita & Udofia, 2011). However, crude oil composition differs depending on the site from where they are obtained. During explorations, the crude oil is constantly being spilled leading to the contamination of the ecosystem and by extension the organisms living in the environment (United Nations Environment Program [UNEP], 2011).

Accordingly, the ingestion of crude oil-contaminated diet had been demonstrated to depressed growth, organ developments and other important physiological functions in rabbits (Berepubo, Johnson & Sese, 1994). Blood parameters, namely red and white blood cells, including the white blood cells differentials as well as packed cell volume and hemoglobin have recently been shown to be one of the major indices of assessing the physiological, pathological, nutritional statuses of the animal (Ekenyem & Madubuike, 2006) and as such can be used to judge the health status of the animal. Animals in the Niger Delta region of Nigeria are often exposed to crude oil-contaminations due to the intensive crude oil explorations activities in the region (UNEP, 2011).

We have also recently demonstrated that the combinations of antioxidant vitamins C and E improved hematological parameters in the growing pig (Johnson, Okejim & Amakiri, 2020). It has also been advanced that there is a strong synergy between these two vitamins physiologically, principally due to the ability of vitamin C to regenerate vitamin E that is involved in generating glutathione and other antioxidant molecules, thereby aiding in maintaining strong defense system of the animal (Salonen et al., 2000). This therefore suggests that vitamins C and E may be able to show some palliative effects in animals that ingested crude oil. Different independent studies have shown that ingestion of crude oil caused

leukemia, reduced red and white blood cells counts as well as hemoglobin (Ovuru & Ekweozor, 2004). Therefore, the objectives of this study are to investigate the palliative effects of dietary vitamins C and E on blood parameters of rabbits fed crude oil-contaminated diets.

Materials and Methods

Animals and Management

Prior to the introduction of the animals into their hutches, the hutches, feeding and the water troughs were thoroughly cleaned to ensure a pathogen-free environment. After drying, twenty-four (24) male New Zealand White rabbits weighing 770-835 grams were acquired from one source in a single batch and weighed after which they were randomly assigned to their hutches with 8 rabbits per treatment, since there were only three treatments. The animals were pre-conditioned to their new environment for two weeks. During the pre-conditioning period the animals were similarly fed and administered prophylactic coccidiosis as well as a broad spectrum antibiotics (terramycin) to properly stabilize the animals according to the method of Berepubo, Johnson and Sese (1994) prior to presenting the experimental diets.

Crude Oil Contamination

The crude oil used in this study is the Bony Light acquired from Agip Oil Company Nigeria Limited. Prior to using the crude oil in contaminating the experimental diets, it was exposed for 24 hours in shallow pans according to the method of Ovuru and Ekweozor (2004) to enable its light fractions to evaporate leaving the stable product that resembles the natural pollution form.

Experimental Diets

The rabbits were fed with grower mash supplemented with *Centrosema pubescens* to enable the animals practice caecotrophy (Grant, 1985). T_0 served as the negative control group whereas T_1 and T_2 groups were the positive control groups as: T_1 animals received 1.5g of crude oil (0.15%)/kg of diet and T_2 animals received similar amount of dietary crude oil as T_1 but in addition received 200mg of vitamins C and E (100mg of vitamin C + 100mg of vitamin E)/kg of diet for 2 weeks. Therefore, the trial lasted for four weeks consisting of 2 weeks of pre-conditioning period and 2 weeks of receiving experimental diets, respectively.

Blood Sample Collections

At the termination of trial, blood samples were collected from individual rabbits from each of the three treatment groups into ethylene diamine tetracetic acid (EDTA) treated tubes between 9 and 10 a.m. and immediately snap-frozen for later analyses using hematology auto-analyzer (BC-2300). Hb concentration, PCV, RBC count and total and differential WBC counts of each group were finally determined using standard laboratory procedures.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS. Treatment means were compared using Tukey's test. The experimental design was the CRD. Therefore, the model was $Y_{ij} = \mu + X_i + E_{ij}$; where: Y_{ij} = individual observation of any animal receiving a treatment, μ = population mean, X_i = effect of the i^{th} treatment (i = 1, 2, 3) and E_{ij} = the error term. An *α-level* of *0.05* was used for all statistical comparisons to detect significance.

Results

The results of the effects of crude oil and vitamins C and E ingestions on rabbits are shown in Table 1.

Table 1. Hb concentration, PCV and RBC counts of rabbits fed crude oil-contaminated diet and combined dietary vitamins C and E

Item		Diets		SEM	*P-value*
	T_0 n = 8	**T_1** n = 8	**T_2** n = 8		
Hb (g/dl)	12.54^a	5.14^b	8.78^c	0.34	0.03
PCV (%)	39.23^a	18.61^b	30.86^c	2.11	0.001
RBC (x 10^9/l)	6.65^a	3.09^b	5.58^a	0.45	0.01

Note: ^{a,b,c} means with different superscripts within the same row are significantly ($P < 0.05$) different. SEM = Standard error of the mean

Crude oil ingestion had significant depressive effects on Hb concentrations as its levels were significantly suppressed ($P < 0.05$) in the animals' that received T_1 diet compared with the control. However, the ingestion of vitamins C and E (T_2 diet) though significantly lower than those of the negative control diet was significantly higher ($P < 0.05$) than those of T_1 diet animals that did not receive the vitamins suggesting that the vitamins had some palliative effects on the animals in terms of reducing crude oil ingestion negative impacts on these blood components; nevertheless, the palliative effect was somewhat marginal for Hb. These trends were mirrored in the PCV and RBC, respectively confirming that ingestion of crude oil suppressed the volumes of blood circulation in the animals as well as the circulating levels of the RBC counts. However, the vitamins ingestions resulted in significant relief in the RBC counts in T_2 animals as there were no significant differences ($P > 0.05$) between animals of T_0 and T_2 groups. The results of the effects of crude oil and the vitamins ingestions on WBC counts and their differentials are shown in Table 2.

Table 2. Total and differential WBC counts in rabbits fed crude oil-contaminated diets and combined dietary vitamins C and E

Item		Diets		SEM	*P-value*
	T_0 n = 8	**T_1** n = 8	**T_2** n =8		
WBC (x10^9/l)	6.77^a	2.51^b	5.15^c	0.27	0.001
NEU (%)	50.50	45.00	46.40	3.36	0.07
LYM (%)	48.20	54.70	53.00	4.04	0.08
MON (%)	1.30^a	0.30^b	0.60^c	0.03	0.043
EOSI (%)	-	-	-	-	-
BASO (%)	-	-	-	-	-

Note: ^{a,b,c} means with different superscripts within the same row are significantly different. SEM = standard error of the mean.

The effects of crude oil ingestion like with other blood parameters significantly reduced ($P < 0.05$) the WBC counts of T_1 diet animals' compared with those of the negative control. However, the ingestion of dietary vitamins C and E had a palliative influence on the animals that ingested crude oil demonstrating that these vitamins can reduce the leukemia effects of crude oil ingestion. Although crude oil reduced the WBC counts it had no significant effects

on the ratios of NEU to LYM as there were only tendencies of altering their ratios. However, crude oil had a significant effect ($P < 0.05$) on MON concentrations (Table 2).

Discussion

Crude oil has been known as an environmental pollutant of the environment and games that live within the vicinity or around crude oil polluted areas (Berepubo, Johnson & Sese, 1994). One of the means of assessing the health status of the animal is via its nutrition on growth and hematological parameters (Ekenyem & Madubuike, 2006). Although growth parameters were not measured in this study, those of hematology were assessed. Ingestion of crude oil depressed Hb concentrations, PCV levels and RBC counts indicating that the crude oil ingestion induced anemia in the animals that consumed it. This agrees with the findings of Monsi, Kwuinji and Akpan (1991) and Ngodigha (2009). However, in the animals that ingested the crude oil in addition to vitamins C and E, the degree of anemia induced by the crude oil were remediated demonstrating that these vitamins had some palliative effects on the rabbits in the study. This finding is not surprising as we have previously showed that vitamins C and E often work synergistically together in improving the health of the animal, including their hematology status (Johnson, Okejim & Amakiri, 2020). The synergy existing between vitamins C and E might also be related to the ability of vitamin C to regenerate vitamin E that is implicated in modulating the animal defense system by triggering the glutathione anti-oxidant systems of the animal due to the need of protecting the animal in the presence of a stressor, such as oxidative stress induced by crude oil ingestion (Salonen et al., 2000; Gatellier et al., 2000).

Crude oil ingestion also caused leukemia in the animals that ingested it. However, the ingestions of vitamins C and E significantly reduced the crude oil-induced leukemia. The finding that crude oil induced leukemia is in agreement with those of a recent study (Akporhuarho, Okpara & Oridje, 2015). Additionally, the palliative effects of vitamins C and E in reducing the degree of leukemia induced by crude oil again was not surprising as these vitamins are implicated in protecting the health of animals in oxidative stresses induced by environmental stressors, such as crude oil as previously alluded to. Furthermore, the data of House and Fletcher (2003) and Stahly et al. (2007) had demonstrated that the recommended levels of vitamins for animals, especially the fast growing species, including rabbits and especially the New Zealand White rabbits was below the requirements for most fast growing species by the Nutrient Research Council. Therefore, the possibility exists for vitamins to truly show some palliative effects in rabbits involving their mega doses in the presence of oxidative stress induced by crude oil ingestion. Our next set of studies would investigate this hypothesis.

Conclusions

Crude oil ingestion caused anemia and leukemia in rabbits that consumed it. However, dietary vitamins C and E demonstrated some palliative effects on the animals that ingested crude oil. Therefore, these two vitamins can be used to reduce the negative effects of crude oil ingestion in the rabbit, especially as it relates to hematological parameters of the animals.

References

Akporhuarho, P. O., Okpara, O., & Oridje, S. O. (2015). Performance and blood indices of finisher broilers given graded level of crude oil contaminated water. *J. Bio. Agric. Healthcare, 5*(16), 22-25.

Berepubo, N. A., Johnson, N. C., & Sese, B. T. (1994). Growth potentials and organ weights of weaner rabbits exposed to crude oil contaminated feed. *Int. J. Anim., 9*, 73-76.

Ekenyem, B. U. & Madubuike, C. (2006). Hematology and serum biochemistry of grower pigs fed varying levels of Ipomea asarofolia leaf meal. *Pakistan J. Nutr., 6*(6), 603-606.

Gatellier, P., Mercier, Y., Rock, E., & Renerre, M. (2000). Influence of dietary fat and vitamin E supplementation on free radical production on lipid and protein oxidation in turkey muscle extracts. *J. Agric. Food Chem., 48*, 1427-1433.

Grant, D. R. (1985). *A study of some aspects of commercial rabbit production and management*. B. Sc. Hons. Thesis, Department of Agriculture, School of Agriculture. University of Edinburgh, Edinburgh, Scotland.

House, J. D. & Fletcher, C. M. T. (2003). Response of early weaned piglets to graded levels of dietary cobalamin. *Can. J. Anim. Sci., 83*, 247-255.

Ita, S. O. & Udofia, U. A. (2011). Comparative study of some haematological parameters in rats following ingestion of crude oil (Nigerian Bonny Light) petrol, kerosene and diesel. *Asian J. Biol. Sci., 4*(6), 498-505.

Johnson, N. C., Okejim, J. A., & Amakiri, A. O. (2020). Effects of feeding graded levels of crude oil-containing diets on hematological parameters of growing pigs. *Intern. J. Adv. Res. Public., 4*(3), 137-140.

Monsi, A., Kwuinji, Z. N., & Akpan, O. U. (1991). Responses of broiler chickens to dietary and water administered Nigerian (Bonny Light) crude petroleum. *J. Anim. Prod. Res., 11*(1), 99-110.

Ngodigha, F. M. (2009). Haematological characteristics and performance of West African Dwarf goats fed crude oil contaminated forage. *Afr. J. Biotechnol., 8*(4), 82-91.

Ovuru, S. S. & Ekweozor, I. K. E. (2004). Haematological change associated with crude oil ingestion in experimental rabbits. *Afr. J. Biotech., 3*(6), 346-348.

Salonen, J. P., Nyyssonnen, K., Salonen, R., Lakka, H. M., Kaikkonen, J., Kala-Sarataho, P., Voutilainen, E. et al. (2000). Antioxidant supplementation in atherosclerosis prevention (ASAP) study: a randomized trial of the effect of vitamin E and C on a 3-year progression of carotid atherosclerosis. *J. Intern. Med., 248*, 377-386.

Stahly, T. S., Williams, N. H., Lutz, T. R., Ewan, R. C., & Swenson, S. G. (2007). Dietary B vitamin needs of strains of pigs with high and moderate lean growth. *J. Anim. Sci., 85*, 188-195.

United Nations Environment Program (UNEP). (2011). Environmental Assessment of Ogoni land, Nairobi, Kenya: UNEP, 8-17.