

Assuaging Effects of Ingestions of Vitamins C and E on the Blood Parameters of Growing Pigs Fed Crude Oil-Contaminated Diets

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**Abstract.** The assuaging effects of the dietary ingestions of vitamins C and E on the hematological parameters of growing pigs that consumed crude oil-contaminated diets were investigated. 18 Landrace growing pigs weighing 9 – 10 kg were used in the study. There were 3 dietary treatments as: diet 1 {(T<sub>0</sub>) the negative control diet}, diet 2 {(T<sub>1</sub>) containing crude oil at 15g/kg of diet} and diet 3 {(T<sub>2</sub>) (as in T<sub>1</sub> + 200mg of vitamins C and E/kg of diet) as 100mg of vitamin C and 100mg of vitamin E, respectively with 6 replications per treatment. The experiment lasted for 4 weeks. The hemoglobin (Hb) concentrations, packed cell volume (PCV) and red blood cell (RBC) counts of the negative control diet animals (T<sub>0</sub>) were significantly higher ( $P < 0.05$ ) than those of animals on diet 2 (T<sub>1</sub>). However, animals on diet 3 (T<sub>2</sub>) had similar ( $P > 0.05$ ) RBC counts with those of T<sub>0</sub> diet. T<sub>2</sub> animals also had significantly higher ( $P < 0.05$ ) Hb and PCV values compared with animals on the T<sub>1</sub> diet. In the white blood cell (WBC) counts, animals of the T<sub>0</sub> diet had significantly higher ( $P < 0.05$ ) value compared with animals of the T<sub>1</sub> and T<sub>2</sub> diets. However, animals of the T<sub>2</sub> diet demonstrated significantly higher ( $P < 0.05$ ) WBC counts compared to those of T<sub>1</sub> diet. It was concluded that vitamins C and E have assuaging potentials on the adverse effects of crude oil on the hematological parameters of the growing pig.

**Key words:** Crude oil, Assuaging Impacts of Vitamins C and E, Blood Parameters and the Pig

### Introduction

Young growing animals including the growing pig often suffer from environmental stressors. At present one of the major factors intensifying these stresses on the pig is the exploitation and exploration of crude oil. During these processes the crude oil spills into the environment, including its fumes and via the ecosystem get to the rearing animals through their feeding regimes (Amakiri, 2006; United Nations Environment Programme [UNEP], 2011). Crude oil ingestion has been shown to have deleterious effects on the pig such as reducing growth rate and its adverse effects on the animal blood parameters thereby reducing the animal's quality of life (Johnson, Okejim & Amakiri, 2020). To this extent therefore, nutrition still remains one of the fundamental keys in dealing with the situation although animal wholesomeness depends on multifarious factors.

From nutrition standpoint therefore, nutrition is the mainstay or foundation in the prevention-modulation reflecting a specific index on diet as an essential strategy in the preservation of animal well-being, especially in maintaining its hematological characteristics. Thus nutritional science has to move towards the development of strategies for dietary ingredients particularly as it relates to the use of micro-nutrients, such as vitamins in the maintenance of sound animal health status (NRC, 2012). There are well-documented data in the literature showing strong synergy between vitamins C and E in maintaining optimal functions of the immune system and therefore may be a means in retaining good health status (Salonen et al., 2000).

Antioxidant vitamins, including vitamins C and E play special roles in the generation of antioxidant molecules such as glutathione and its cohorts which in turn play major functions in health-related benefits in the animal (Finkelstein et al., 2011). However, there is paucity of information on the potentials of vitamins C and E in assuaging the health conditions of the pig

in the face of crude oil ingestion, such as its hematological characteristics. Therefore, the objectives of this study are to examine the assuaging effects of vitamins C and E on the hematological parameters of the growing pig following ingestions of crude oil-contaminated diets.

## Materials and Methods

### Animals and Management

Eighteen (18) growing Landrace growing pigs weighing between 9 to 10 kg BW were used in the study. The animals on arrival at the venue of the experiment were weighed to obtain their initial BW and randomly assigned to their individual pens. The first 2 weeks were used to properly pre-condition the animals to their environment and fed similar grower mash diets. Six pigs were assigned to each dietary treatment and fed at 5% of their BW (as-fed-basis) which is very close to *ad libitum* feeding in the commercial setting. Animals were fed twice daily at 09:00h and at 16:00 h, respectively according to the method of Johnson, Okejim and Amakiri (2020). Animals received their experimental diets for 2 weeks and also had unlimited access to drinking water. Animals' pens were kept clean throughout the experimental duration.

### Experimental Diets

Three corn-soybean meal-based diets similarly formulated to be isocaloric and also isonitrogenous were used in the current study. However, while the negative control diet (T<sub>0</sub>) contained no crude and experimental vitamins C and E diet 2 (T<sub>1</sub>) contained dietary crude oil at 15g/kg of diet whereas diet 3 (T<sub>2</sub>) contained crude oil as in T<sub>1</sub> but in addition contained vitamins C and E at 200mg/kg of diet as (100mg of vitamin C + 100mg of vitamin E), respectively. There were 6 pigs per dietary treatment. The experiment lasted for 4 weeks consisting of 2 weeks of pre-conditioning to their environment and 2 weeks of ingesting their respective experimental diets. Crude oil was treated as described by Ovuru and Ekweozor (2004) before incorporation into the diets.

### Blood Sample Collections

At the end of study, blood samples were collected from individual pigs from each of the dietary treatment groups into ethylene diamine tetracetic acid (EDTA) treated tubes between 9 and 10 a.m. on the same day and immediately snap-frozen. Blood samples were analyzed using a hematology auto-analyzer (BC-2300). Hb concentration, PCV, RBC count and total and differential WBC counts of each dietary group were finally determined by standard laboratory procedures.

### Statistical Analysis

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

## Results

The results of the effects of ingesting vitamins C and E following the consumption of crude oil-contaminated diets on Hb, PCV and RBC of growing pigs are shown in Table 1.

**Table 1. Hb concentration, PCV and RBC counts of growing pigs that ingested vitamins C and E and crude oil-contaminated diets.**

Item	Diets			SEM	P-value
	T <sub>0</sub> n = 6	T <sub>1</sub> n = 6	T <sub>2</sub> n = 6		
Hb (g/dl)	14.12 <sup>a</sup>	7.55 <sup>b</sup>	11.58 <sup>c</sup>	0.22	0.02
PCV (%)	40.09 <sup>a</sup>	20.47 <sup>b</sup>	35.54 <sup>c</sup>	1.93	0.001
RBC (x 10 <sup>9</sup> /l)	5.99 <sup>a</sup>	3.11 <sup>b</sup>	5.45 <sup>a</sup>	0.39	0.03

Note: 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 effects on the Hb concentrations of the pigs as its levels were significantly reduced ( $P < 0.05$ ) in the pigs that received diet two compared with the negative control diet. However, the intake of vitamins C and E significantly improved Hb levels in diet 3 (T<sub>2</sub>) animals than those of diet 2 (T<sub>1</sub>), although they were significantly lower ( $P < 0.05$ ) than those of the negative control group (T<sub>0</sub>). This observation therefore, lay some credence to some degree the assuaging impact of the ingested vitamins suggesting that the vitamins had some palliative effects on the animals in respect to crude oil negative effects on Hb component of the pigs' blood. The trend was also similar in the PCV. In the RBC the assuaging impact of the vitamins were most evident as the RBC counts of the negative control diet (T<sub>0</sub>) and those of diet 3 (T<sub>2</sub>) were similar and significantly higher ( $P < 0.05$ ) than those of diet 2 (T<sub>1</sub>) confirming that although ingestion of crude oil suppressed the RBC counts, the effects of the ingestions of vitamins C and E were able to assuage it (Table 1). The results of the effects of crude oil and the ingestions of combined vitamins C and E on WBC counts and their differentials in the growing pig are shown in Table 2.

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

Item	Diets			SEM	P-value
	T <sub>0</sub> n = 6	T <sub>1</sub> n = 6	T <sub>2</sub> n = 6		
WBC (x10 <sup>9</sup> /l)	6.62 <sup>a</sup>	3.42 <sup>b</sup>	6.01 <sup>c</sup>	0.19	0.01
NEU (%)	52.00	40.60	47.30	3.49	0.07
LYM (%)	48.00	59.40	52.70	4.44	0.06
MON (%)	-	-	-	-	-
EOSI (%)	-	-	-	-	-
BASO (%)	-	-	-	-	-

Note: Means with different superscripts within the same row are significantly ( $P < 0.05$ ) different.

The effects of crude oil ingestion by the growing pig significantly suppressed ( $P < 0.05$ ) the WBC counts of diet two (T<sub>1</sub>) animals' compared with those of the negative control diet. However, the ingestions of dietary vitamins C and E had a significant ( $P < 0.05$ ) assuaging impact on the animals that ingested crude oil which is suggestive of the fact that these antioxidant vitamins especially in their combined forms have the potentials to assuage or reverse leukemia induced by crude oil ingestion. Furthermore, although crude oil ingestion reduced WBC counts there were no significant effects ( $P > 0.05$ ) on the ratios of NEU to LYM; nevertheless, there were tendencies of altering their ratios.

## Discussion

Crude oil has been identified as a toxicant to both domesticated animals (Monsi, Kwuinji & Akpan, 1991) and game animals (Berepubo, Johnson & Sese, 1994). We have also previously demonstrated that pigs were easily susceptible to the ingestion of crude oil, particularly when it is above the growing pig threshold. To this point, we have shown that crude oil ingestion beyond 10g/kg of diet suppressed growth and other important physiological functions in the pig (Johnson, Okejim & Amakiri, 2020). Therefore, the observation of the suppressive effects of crude oil on the hematological parameters of the growing pig in this study is not surprising.

On the other hand, we have also shown that the ingestion of combined anti-oxidant vitamins, especially those of vitamins C and E improved pig performance and anti-oxidants status molecules, such as glutathione of the pig (Johnson, Popoola & Owen, 2019). This is an indication that these vitamins can improve or aid in maintaining good health status of the animal. In the Niger Delta region of Nigeria, animals are often exposed to environmental stressors, including crude oil due to its incessant spills during exploration and its eventual entry into the ecosystem (UNEP, 2011). Some of these environmental stressors also accounts for the 'sudden death syndrome' in the pig. This has an overall effect of negatively affecting the hog farmer, including reduction in profit margins (NRC, 2012). It is a known fact that livestock production should be on the increase as a result of continuous and more demands for animal protein. Hematological components are one of the major indices in measuring the animal's well-being. Nutritional science has made tremendous improvements in the development of nutritional strides in terms of developing strategies in managing animals in the presence of stressors. To this extent, the developments and recommendations for optimal dietary ingredients as it relates to micro-nutrients in dealing with some of these environmental challenges should be developed to aid in maintaining good health of the growing animal as not to compromise their productivity. Different independent studies have shown that crude oil ingestion decreased RBC counts and PCV (Ovuru & Ekweozor, 2004), decreased Hb, mean corpuscular hemoglobin and WBC counts (Summonu & Oloyede, 2008), respectively. The United Nations Environmental Protection Agency (UNEP) reported that some of the effects of crude oil exploration in the Niger Delta region include: impairment of animal and human health, vegetation hazards and loss of biodiversity in breeding grounds just to mention but few culminating in reduced quality of life (UNEP, 2011). These negative effects of crude oil were also observed in this current study.

However, in this study, the ingestion of vitamins C and E in the presence of crude oil significantly assuaged the negative impacts of crude oil ingestion on major blood parameters of the growing pig. This finding is in agreement with those of Okejim, Johnson and Amakiri (2018). Therefore, the use of combined vitamins C and E in the range and ratio used in this study should be employed in the management of assuaging the adverse effects of crude oil in the growing pig. At this range and ratio the negative effects of crude oil on the blood parameters of the growing pig were assuaged, particularly the RBC and WBC counts.

## Conclusions

From the findings of this study, it was concluded that the use of the antioxidant vitamins C and E should be employed in assuaging the adverse effects of crude oil in the growing pig, especially as it relates to the hematological parameters of the pig.

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