Crude Oil ingestion and Kidney Health Amelioration Effects of Vitamin E in the Growing Pig

[1]Johnson, N. C. [1]Eli, O. H [1]Fakae, L. B. [1*]Diri, M.

Department of Animal Science, Rivers State University, Port Harcourt, Nigeria

Corresponding Author: dirimoses@yahoo.com

Abstract

This primary objective of this study was to investigate the amelioration effect of vitamin E on kidney health of growing pigs fed high crude oil-contaminated diet. 24 landrace pigs with average body weight of 15.9 ± 1.2 kg were used in the trial. The pigs on arrival at the animal wing of the Rivers State University Teaching and Research Farm were randomly assigned to three dietary treatment groups of 8 pigs per treatment. They were allowed to be fully adapted to their new environment for 7 days during which they were similarly managed. Water was provided *ad libitum* and pigs were de-wormed by ivermectin injection subcutaneously. A broad spectrum antibiotic (amoxicillin) was intramuscularly given to ensure good health status. At the end of the adaptation period, pigs were fed their experimental diets: treatment 1 (T_1, contained 0 gram of crude oil and no supplemental vitamin E/kg of diet), treatment 2 (T_2, contained 15 gram of crude oil/kg of diet), treatment 3 (T_3, contained 15 gram of crude oil + 460 mg of vitamin E/kg of diet), respectively for 4 weeks. At the end of 4-weeks of treatment administration, blood samples were collected from the 8 pigs in each of the treatment groups and immediately snap-frozen in ice for later analyses. Blood urea nitrogen (BUN) and creatinine levels were analyzed for. There were significant ($P < 0.05$) differences in the BUN serum concentrations. BUN levels of animals in T_1 and T_3 animals were similar and significantly lower than those of T_2 animal group. Similarly, the serum concentrations of creatinine in T_1 and T_3 animals' groups were statistically similar but significantly ($P < 0.5$) lower than those of animals in the T_2 group. It was concluded that vitamin E has an ameliorative effect on kidney health following high crude oil ingestion in the growing pig.

Key words: Crude oil, Kidney health, Blood Urea Nitrogen Creatinine, Vitamins E and the Pig

INTRODUCTION

The kidney just like any other organ in the animal's body performs special functions in ensuring the overall health of the animal. It is also a known fact that Blood urea nitrogen is a component of urea and the means by which the body eliminates its excesses is by filtration by the kidney and it is a by-product of protein metabolism leading to amino acids giving rise to ammonia that is later converted to urea that is transported in the blood to the kidney that finally causes its filtration and thus eliminated from the body via urine. Different independent studies (Lin *et al.,* 2011; Fulya *et al.,* 2012 and Lalita *et al.,* 2016) had demonstrated that high level of BUN is highly correlated with damage of the kidney leading to the malfunctioning of the kidney leading to the inability of the kidney to filter urea. To this point therefore, one of the best means of testing for optimal functioning of the kidney is by BUN test.

Another known practice of testing for normal functioning or kidney integrity is by measurement of blood creatinine level. Again, the studies of Fulya *et al.* (2012) demonstrated that increased blood creatinine levels were as a result of damage to the kidney usually associated with kidney failure as a result of the inability of the kidney to filter creatinine in the blood for excretion in urine. Creatinine is usually produced in the body during protein metabolism.

To our knowledge via the literature search, no study has investigated the effect of crude oil-contaminated diet and the amelioration of vitamin E on kidney health in the growing pig. Therefore, the objectives of this study are to investigate the responses of growing pigs' ingestion of crude oil-contaminated diet and the amelioration effect of vitamin Eon kidney health based on the use of kidney health biomarkers, namely BUN and creatinine.

MATERIALS AND METHODS

Location of the Study

The study was carried out at Rivers State University teaching and research farm.

Animals and Management: Twenty-four (24) landrace pigs weighing on average 15.9± 1.2 kg were acquired from a local market at Aluu, Ikwerre Local Government of Rivers State and used in the study. The animals on arrival at the Rivers State University Teaching and Research Farm were

randomly allotted to their individual pens and pre-conditioned for one week according to the method of Berepubo *et al*. (1994) and similarly managed during the pre-conditioning period. During the pre-conditioning period the animals were similarly fed Growers mash diet and administered a broad spectrum antibiotics (terramycin) to properly stabilize the animals. Prior to the introduction of the animals into their pens, the pens, feeding and water troughs were thoroughly cleaned to ensure a 'pathogen-free' environment and allowed to dry thoroughly. After the pre-conditioning, pigs were offered three dietary treatments with 8 pigs per treatment (8 replications) for 4 weeks.

Crude oil contamination: The crude oil used in this study is the Bonny 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 Berepubo *et al*. (1994) to enable its light fractions to evaporate leaving the stable product that mimics natural crude oil pollution form.

Experimental diets: All pigs were fed with Pfizer grower mash^{TM} except that the control did not contain crude oil and vitamin E (α-tocopheryl acetate); whereas treatment T_2 contained 15gram/kg of diet and treatment T_3 contained 15gram crude oil + 460 mg of vitamin E/kg of diet, respectively. That is: T_1 (the control diet or treatment; contained 0gram of crude/kg of diet, T_2 (contained 15gram of crude oil/kg of diet), T_3 (contained 15gram crude oil + 460 mg of vitamin E/kg of diet). The pigs received their respective experimental diets for 4 weeks.

Blood sample collections: At the termination of feeding trial, blood samples were collected from individual pig from each of the three dietary treatment groups into ethylene diaminetetracetic acid (EDTA) treated tubes between 9 and 10 a.m. to avoid heat stress and immediately snap-frozen for later analyses for BUN and creatinine concentrations. BUN was analyzed for according to the method of Machado and Horizonte (1958). Creatinine was determined according to the method of Max (2011).

Statistical analysis and Experimental Design: 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} diet (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 BUN and creatinine serum concentrations are shown in Table 1.

Table 1.BUN and creatinine levels of pigs fed crude oil-contaminated diet and vitamin E

	TREATMENTS				
Parameter	T_1, n = 8	T_2, n = 8	T_3, n = 8	SEM	*P-value*
BUN (mmol/l)	4.65^b	6.78^a	4.55^b	0.10	0.043
Creatinine (mmol/l)	81.34^b	99.48^a	80.55^b	1.32	0.003

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

BUN level was significantly ($P < 0.05$) higher in treatment 2 (T_2) when compared with the T_1 and T_3 that have similar ($P > 0.05$) concentrations of BUN. Creatinine serum concentrations mimicked BUN pattern. Creatinine concentration level of the T_2 was significantly ($P < 0.05$) higher when compared with the T_1 and T_3 that have similar ($P > 0.05$) creatinine levels.

DISCUSSION

We have reported previously that organ health biomarkers particularly those of the liver and kidney in the growing pigs were compromised when they ingested crude oil-containing diet at 15gram of crude oil/kg of diet (Johnson *et al.,* 2020).That is, this level of crude oil is very high for the pigs as they were not able to handle this level of crude oil-contaminated diet. In other words, this means that this level of crude oil-contamination is beyond the threshold the growing pig could tolerate as confirmed in our early study (Johnson *et al.,* 2020). Vitamin E is the most potent known anti-oxidant vitamin that had been identified in reducing oxidative stress in the body of the animal (Anderson *et al.,* 2014). This might also be related to vitamin E activity in stimulating the animal's defense system such as the glutathione defense system (Kohrle *et al.,* 2000) to protect the animal.

The primary objective of this study was to investigate the ameliorative effect of vitamin E following the ingestion of high crude oil contaminated diet. One of the major hypothesis of this

study is that crude oil ingestion would compromise kidney health in the growing pig based on our previous data and vitamin E may be to the rescue by maintaining the integrity of the kidney and its physiological functions via its biomarkers.

From the result obtained in this study, Blood Urea Nitrogen levels in the T_2 animals' group was significantly increased indicating that crude oil ingestion had a profound impeded effect on urea elimination via the kidney suggesting that filtration by this group of animal was significantly impeded whereas BUN levels in the T_1 and T_3 animals' groups were significantly lowered compared with the BUN levels in the T_2 animals demonstrating that vitamin E ameliorated the negative effect of crude oil ingestion in the T_3 animal group. The observation in this study is in agreement with the findings of Leyla and Ismail (2007) that found that vitamin E supplementation significantly reduced BUN level to the control guinea pigs exposed to oxidative stress by hypothermia.

Creatinine is another by-product that is also eliminated from the body by filtration into urine by the kidney (Leyla and Ismail, 2007). Therefore, serum accumulation of creatinine is usually as a result of the inability of the kidney to eliminate it through urine indicating kidney failure and malfunctioning. Consequently, the accumulation of serum creatinine of the animals in the T_2 animals demonstrated that the ingestion of crude oil compromised the functions of their kidneys compared to animals of T_3 group that demonstrated similar levels of creatinine with the control group (T_1).This findings in this study was a confirmation that vitamin E protected the kidneys of animals in the T_3 group and hence maintained their filtration functions effective. Again, this finding is also in tandem with the report of Tapan *et al*. (2013) that demonstrated that vitamin E supplementation in goats significantly reduced creatinine levels during oxidative stress induced by long term exposure to arsenic. However, our findings in this study was not in agreement with those of Ognik and Wertelecki (2012) that studied the effects of different vitamin E sources on selected oxidative stress indices in blood and tissues of turkey hens and found no differences in the turkey's serum. This disagreement between our study .It could be explained in part based on the levels of vitamin E supplementation (45mg/kg of body weight) used in their studies.. This supplementation level of vitamin E might have been ineffective in maintaining optimal functioning of the kidneys of the animals.

Conclusions: Vitamin E at the concentration of 460mg/kg of diet was effective in maintaining optimal functioning of the kidney in the presence of oxidative stress imposed on the kidney by high levels of crude oil ingestion in the growing pig.

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