



Vitamin E Can Completely Reverse the Toxicological Effects of Crude Oil on the Glutathione System of the Growing Pig without Selenium

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ABSTRACT

The study investigated the reversal effects of vitamin E or vitamin E + selenium in pigs following crude oil ingestion. 24 growing pigs were used in the study. 6 Pigs were randomly assigned to 4 dietary treatments: T1 (the control diet contained 0gram of crude, no addition of vitamin E or selenium/kg of diet; T2 (contained 15gram of crude oil without addition of vitamin E or selenium/kg of diet); T3 (contained 15gram crude oil + 200mg of vitamin E/kg of diet) and T4 (contained 15gram of crude oil + 200mg of vitamin E + 5mg of selenium/kg of diet). The pigs received their respective experimental diets for 4 weeks. At the end of trial, blood samples were collected from all the 6 pigs in each treatment group for antioxidants' [glutathione, (GSH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)] and oxidant malondialdehyde (MDA) analyses. The GSH serum levels of T1, T3 and T4 animals were similar ($P > 0.05$) and significantly ($P < 0.05$) higher compared with those of T2 animals. The serum levels of GSH-Px, SOD and CAT mirrored GSH pattern. MDA serum levels of T3 and T4 animals were similar ($P > 0.05$) and significantly ($P < 0.05$) lower than those of T1 animals with the T2 animals showing significantly ($P < 0.05$) the highest levels compared with other treatment groups. It was concluded that crude oil ingestion causes oxidative stress in the growing pig and vitamin E without selenium restored antioxidants and oxidant statuses to control levels.

INTRODUCTION

Due to dangerous occurrences in the animal habitat, such as oxidative stress that often leads to sudden deaths in farm animals; the animal has developed an inherent mechanism of reducing or preventing oxidative stress (NRC, 2012). In other words, the body develops complex defensive systems consisting of enzymatic and non-enzymatic components that defend the animal against the negative effects of oxidative stress. The enzymatic components include SOD, CAT and GSH-Px with MDA as the major by-product (Traber *et al.*, 2011; NRC, 2012). These enzymes operate in various subcellular compartments and respond in concert when cells are exposed to oxidative stress (Traber *et al.*, 2011). Glutathione is the major non-enzymatic component of the glutathione defense system as is involved in the preservation of many antioxidant molecules and thus termed the mother of all antioxidants (Traber *et al.*, 2011).

The glutathione (GSH) system play some vital functions identified as being responsible in keeping the animal healthy during oxidative stress. Some of the functions are scavenging for free radicals and other reactive species which results in the removal of oxygen and lipid peroxides, acting as proton donor in the presence of reactive oxygen species (ROS), aids in the proliferation of cells, such as lymphocytes and intestinal epithelial cells and also essential for mounting successful immune response when the host is immunologically challenged just to mention but few. These functions can thus be summed up as antioxidant defense, nutrient metabolism and regulation of pathways essential for whole body homeostasis (Wu *et al.*, 2004).

It has shown previously that crude oil contamination at 15g of crude oil/kg of diet was beyond the threshold growing pigs could tolerate as judged by its compromised effect on the GSH defense system of the growing pig (Johnson *et al.*, 2020). Vitamin E has been identified as a very potent antioxidant vitamin in up-regulating the GSH defense system of the animal (Hu *et al.*, 2015; Leskovec *et al.*, 2018). Furthermore, it has also been postulated that vitamin E and selenium work synergistically in eliciting enhanced activities of the GSH defense system (Aslam *et al.*, 2010; Elblehi *et al.*, 2015). Therefore, the objectives of this study are to investigate the potential reversal effects of vitamin E alone or in combination with selenium on the GSH defense system following oxidative stress induced by crude oil ingestion in the growing pig.

MATERIALS AND METHODS

Experimental site: This study was carried out at the Teaching and Research Farm of the Rivers State University, Nkpulu-Oroworukwo, Port Harcourt. The

farm is situated at latitude 4° 48'N and longitude 6° 48'E at the Rivers State University campus.

Animals and Management: Twenty-four (24) growing pigs of average body weight (BW) of 16.5 ± 1.1 kg were acquired from a commercial pig producer from Port Harcourt, Rivers State, Nigeria and used in the study. The animals on arrival at the teaching and research farm of the Rivers State University 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 and administered a broad spectrum antibiotics (terramycin) to stabilize them. Prior to the introduction of the animals into their pens, pens, feeding and the water troughs were thoroughly cleaned to ensure a 'pathogen-free' environment and allowed to dry thoroughly. At the end of the pre-conditioning period, pigs were presented to four dietary treatments with 6 pigs per treatment (6 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 animals were fed with Pfizer grower mashTM except that they contained different levels of dietary crude oil, vitamin E and selenium as: T₁ (the control diet or treatment; contained 0gram of crude, no additional vitamin E or selenium/kg of diet, T₂ (contained 15gram of crude oil without addition of vitamin E or selenium/kg of diet), T₃ (contained 15gram of crude oil + 200mg of vitamin E/kg of diet) and T₄ (contained 15gram of crude oil + 200mg of vitamin E + 5mg of selenium/kg of diet), respectively. Vitamin E (E50) and selenium were obtained from Bio-organics Nutrient Systems Limited, Lagos, Nigeria. Animals received their respective experimental diets for 4 weeks.

Blood collections for antioxidants and MDA analyses: At the end of the study, blood samples were collected from individual pigs from each of the four dietary treatment groups into ethylene diamine tetracetic acid (EDTA) treated tubes between 9 and 10 a.m. and immediately snap-frozen for later analyses for GSH, GSH-Px, SOD, CAT and MDA. GSH, GSH-Px and MDA were analyzed according to the method of Habig *et al.* (1974). SOD was analyzed according to the method of Misra and Fridorich (1972) whereas CAT was analyzed according to the method of Aebi *et al.* (1974).

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 completely randomized design (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 ($i = 1, 2, 3, 4$) 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 ingestion of dietary crude oil, dietary crude oil plus vitamin E and dietary crude oil + vitamin E and selenium, respectively on growing pigs' antioxidant enzymes' status are shown in Table 1.

Table 1: Effects of ingested crude oil, vitamin E and vitamin E + selenium on GSH, GSH-Px, SOD and CAT serum levels of the growing pig

TREATMENTS						
Parameter	T ₁	T ₂	T ₃	T ₄	SEM	P-value
GSH (u/mg)	4.91 ^a	1.95 ^b	4.88 ^a	4.89 ^a	0.01	0.02
GSH-Px (u/mg)	2.52 ^b	1.11 ^c	3.72 ^a	3.67 ^a	0.10	0.001
SOD (u/mg)	0.21 ^b	0.10 ^c	0.34 ^a	0.34 ^a	0.01	0.002
CAT (u/mg)	13.31 ^b	7.85 ^c	15.66 ^a	15.59 ^a	0.30	0.001

^{a,b,c} Means within the same row with different superscripts are significantly ($P < 0.05$) different

The GSH serum levels of T₁, T₃ and T₄ animals were similar ($P > 0.05$) and significantly ($P < 0.05$) higher compared with those of T₂ animals. The serum levels of GSH-Px, SOD and CAT mirrored GSH pattern.

The results of oxidative activities in pigs that ingested crude oil, crude oil plus vitamin E and crude oil plus vitamin E and selenium as indicated by their serum levels as judged by MDA concentration levels are shown in Table 2.

Table 2. Effects of ingested crude oil, crude oil + vitamin E and crude oil and vitamin E + selenium on the serum levels of MDA in the growing pig

TREATMENTS						
Parameter	T ₁	T ₂	T ₃	T ₄	SEM	P-value
MDA (u/mg)	0.32 ^b	0.49 ^a	0.29 ^b	0.30 ^b	0.01	0.000

^{a,b} Means within the same row with different superscripts are significantly ($P < 0.05$) different

The MDA serum levels of T₃ and T₄ animals were similar ($P > 0.05$) and significantly ($P < 0.05$) lower than those of T₁ animals with the T₂ animals showing significantly ($P < 0.05$) the highest levels compared with other treatment groups.

DISCUSSION

As previously stated, it has demonstrated that crude oil contamination at 15g of crude oil/kg of diet was beyond the threshold growing pigs could tolerate as judged by its compromised effect on the GSH defense system of the growing pig (Johnson *et al.* 2020). Usually, in response to oxidative stress, such as the one induced in this study with crude oil ingestion results in changes in the expression levels of antioxidants associated with the GSH defense system of the animal, such as GSH, GSH-Px, SOD and CAT with simultaneously increase in the serum levels of MDA (Johnson *et al.* 2020). This was also observed in this current study with significant reductions in the expression of all the antioxidants studied with a concomitant significant increase in the MDA levels of the T₂ animals (Tables 1 and 2). In these

conditions, the defense system of the animal is significantly weakened and the animal easily becomes susceptible to pathogenic agents. The reduced levels of antioxidants and increased levels of oxidant, such as MDA found in this study following crude oil ingestion is in agreement with the data of Achuba, (2005) and Johnson *et al.* (2020).

In the studies of Bharhan *et al.* (2010) and Okpoghono *et al.* (2018) it was demonstrated that vitamin E attenuated oxidative stress induced by an endotoxin in the rat and crude oil ingestion in the catfish, respectively by restoring serum levels of antioxidants to the control groups' values in their independent studies with simultaneous reduction in MDA values to the levels of the controls'. These

findings were also confirmed in this current study. Again, Manju and Sushovan, (2017) demonstrated that vitamin E in combination with selenium significantly increased serum levels of GSH, GSH-Px, SOD and CAT to normal or to the control values in goats involving oxidative stress induced by arsenic chemical. In this study, it was observed that vitamin E alone restored the antioxidants levels to control values as findings could not identify any significant differences in the values of the antioxidants and even oxidants when vitamin E was provided alone or in combination with selenium; suggesting that vitamin E alone was able to stabilize the animals by restoring GSH and its cohorts to the control animal values as well as the levels of MDA, respectively.

CONCLUSION

It was concluded that crude oil ingestion causes oxidative stress in the growing pig. Furthermore, vitamin E alone can reverse or restore antioxidants and oxidant statuses to normal or control levels following crude oil ingestion without its combination with selenium.

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