



Vitamin E, Liver and Kidney Functions of Growing Pigs During Hypothermia

*Diri M; Fakae, LB; Ogbamgba, VM; Johnson, NC

Department of Animal Science, Rivers State University, Nigeria. PMB 5080

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*Corresponding Author

Diri Moses

E-mail: dirimoses@yahoo.com

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ABSTRACT

The effect of oral vitamin E supplementation on the health status of liver and kidney of growing pigs during oxidative stress induced by hypothermia was studied. 27 Landrace pigs, with an average weight of 14 ± 1.02 were used. Pigs were randomly assigned to three treatments (T1, T2, and T3) with 9 pigs per treatment. Each treatment had 3 replicates with 3 pigs per replicate and the study lasted for six (6) weeks. All treatments received similar diets (Pfizer Growers Marsh TM) except that T3 was administered vitamin E orally from the 4th week at the rate of 470mg/kg body weight daily. 24 hours after the last administration, T3 and T2 were immersed into a water body temperature of 120C up to their neck while same was done for T1 in temperature of 360C water. Immediately after immersion, body temperatures were taken and blood samples were collected and preserved. Results showed that Alanine Amino Transferase (ALT) had higher significant differences ($P < 0.05$) between T3 and T2. The T3 and T1 groups had similarities (43.66 and 44.32) in Aspartic Amino Transferase (AST) while T2 had significant differences ($P < 0.05$) in figures. Alkaline phosphatase had significant differences ($P < 0.05$) in all treatments. Blood urea nitrogen (BUN), T2 had significant differences ($p < 0.05$) and T3 and T1 had similar values ($p < 0.05$). Creatinine had higher value in T2 ($P < 0.01$) with T1 and T3 with similar values ($p < 0.01$).

INTRODUCTION

It is an established fact that oxidative stress could lead to death of cells in liver, kidney and other visceral organs in animals if intensive measure is not taken. To this point therefore, it is pertinent to work out modalities on how to effectively care for pigs during a case of oxidative stress that may be induced by hypothermia. Johnson *et al.*, (2020) demonstrated that

presence of hypothermia induced oxidative stress to weaner rabbits. Also, the media is inundated with literatures confirming that vitamin E (dl- Alpha tocopheryl Acetate) has antioxidant properties and as such can be used to combat the effect of lipid peroxidation in animals when supplemented in diets (Fulya *et al.*, 2012). This is achieved by its donation of hydrogen atom from the hydroxyl group and the hydrogen atom reacts with hydroxyl radicals thereby

detoxifying them to water and rendering reactive oxygen species inactive to cells and tissues of animals (Aslan and Meral, 2007; Johnson *et al.* 2023).

During cold weather, like the hammatan and rainy seasons which mimics winter, the tissues and visceral organs of pigs may be compromised as a result of the production of reactive oxygen species in the cells caused by cold conditions and this may lead to apoptosis (programmed cell death) or even death of pigs if proper measure is not taken to cushion the effect of cold which may be achieved by detoxification of free radicals produced by cold conditions. Aslan and Marel, (2007) showed that vitamin E was able to up-regulate the activities of liver enzymes in pigs.

High ALT and AST level in blood serum of animals is an indication of abnormality in liver functionality. Thus, this has proved that ALT and AST are the major liver enzymes, confirmed to be a good biomarker of oxidative stress induced by extremely cold season. Also, blood urea nitrogen and creatinine concentrations are higher in the serum when the kidney has compromised its functions (Fulya *et al.*, 2012; Lalita *et al.*, 2016), thus, making them good kidney biomarkers of oxidative stress.

The objective of this study is to determine the effect of oral vitamin E supplementation on the health statuses of liver and kidney of growing pigs during oxidative stress induced by hypothermia.

MATERIAL AND METHOD

The study was conducted at the piggery section of The Rivers State University, Teaching and Research Farm, Port Harcourt. Pigs were purchased in a local market within Port Harcourt City. Twenty seven (27) landrace pigs with an average weight of 14 ± 1.02 kg were purchased and used for this study. Prior to the arrival of the animals, the floor of the pens were thoroughly washed with water and hypochlorite and allowed to dry for one week before introducing the animals into their pens. They were randomly placed to their individual pens and were given a week of adjustment to enable them acclimatizing with the new environment. All animals were similarly managed during the adjustment period. Thus, during the period the animals were similarly fed ad-libitum prior to the commencement of the experiment. Proper hygiene was observed throughout the study period. The Pigs were placed on three dietary treatments having nine (9) pigs per treatment. Each treatment had 3 replicate, with 3 pigs in each of the replicate and the experiment lasted for 6 weeks. All pigs were fed Pfizer Grower MarshTM and vitamin E (dl- Alpha tocopheryl acetate) was administered at the rate of 470mg/kg body weight in diets of T₂ and T₃ from the last two (2) weeks of the study whereas, T₁ (control diet) was not supplemented with Vitamin E. Twenty-four (24) hours after the last administration of Vitamin E, pigs in the control (T₁) group were dipped into a water of body temperature of

36°C with their head up for 5 minutes while hypothermia was introduced to T₂ and T₃ by dipping them into a water body of 12°C up to their neck for 5 minutes without any form of tranquilizer or sedative, after which their body temperatures were taken rectally and blood samples were collected. At the last day of the sixth week of the study, blood samples were taken from all treatment groups using 2ml syringe via their external jugular vein and placed in a well labeled non-ethylene diamine tetracetic acid (non-EDTA) treated vial tubes between 7 and 8 am and immediately placed inside a deep freezer for later analysis.

Liver Enzymes (ALT, AST and ALP) were analyzed. ALT and AST were analyzed by the method of Reitman and Frankel (1957) while ALP was determined by the method of Aaron (1930). Kidney Biomarkers: Test for blood urea nitrogen and creatinine was tested for; to determine the functionality of the kidney. Urea concentration was determined by the method of Machado and Horizonte (1958) while creatinine was determined by the method of Max (1886)

Statistical Analysis

Data obtained from the study was subjected to analysis of variance (ANOVA) using the general linear model of SAS (SAS Inst., Cary, NC, 1988). Treatment means were compared using turkey test. Completely Randomized Design was used as the experimental design with linear model: $Y_{ij} = \mu + X_i + E_{ij}$

Where E_{ij} is error involved in monitoring animal receiving *i*th treatment in the study.

X_i is effect of *i*th level of treatment

μ is the population mean

Y_{ij} is the individual observation of any animal receiving treatment.

RESULTS and DISCUSSION

Pigs in all the treatment groups were fed normal and seen to be in good condition all through the study period as they were monitored regularly. After introduction of cold, mean body temperature for T₁ group was 36.8 ± 0.5 , T₂ group had 33 ± 1.2 and T₃ group was 33.2 ± 0.8 respectively. This showed that slight cold was experienced for T₂ and T₃ treatment groups respectively. Data in Table 1 show that hypothermia and vitamin E actually had effect on the liver biomarkers. For ALT and AST, there were significant differences ($P < 0.05$) in T₃ compared to T₂. However, T₃ and T₁ have similarities. For ALP, there were significant differences ($P < 0.05$) across board in all treatment groups.

ALT is one of the chief enzymes found in the liver of animals. ALT aids in breaking down food into energy. A damaged liver will release more ALT into the blood stream and this is why its test is conducted in most cases to determine the functionality of the liver,

thus making it a biomarker of oxidative stress. T₂ group had a higher significant difference ($p < 0.05$) compared to other treatment groups. This is a clear indication that cold affected ALT and oxidative stress was induced by the presence of cold. This is in conformity with the findings of Johnson, *et al* (2019). Also there were similarities between T₁ and T₃ groups, this could have been that vitamin E supplemented to T₃ group was able to reverse the effect of oxidative stress caused by cold. This agrees with findings of some researchers (Aslan and Meral, 2007; Johnson *et al.*, 2019; Johnson *et al.*, 2023) which posited that oral supplementation of vitamin E reversed the effect of oxidative stress induced by cold and crude oil respectively by reducing ALT levels in blood serum.

AST is one of the major enzymes domiciled in the tissues of liver organ of animals. Reactive oxygen species produced by some toxic factors caused damage to the liver thereby releasing large volume of AST in the blood stream (Ekhatu, *et al.*, 2014). This is an indication that AST just like ALT is a good biomarker of oxidative stress as such induced by hypothermia. From the result of this study, oxidative stress was induced in T₂ group and oral vitamin E supplementation

was able to normalize the effect of hypothermia as evidenced in the T₃ group and T₁ group having similarities in value. This consolidates findings that confirms that vitamin E up-regulated the activities of antioxidants in blood serum of animals subjected to oxidative stress (Mahaswari, *et al.*, 2015; Johnson, *et al.*, 2023)

ALP is an enzyme found in almost all part of the body, especially in the liver. ALP test is usually done when there is a suspected case of liver, bone or kidney abnormality. The higher the volume in the blood serum, the tendency of a major damage in the liver. Judging the result of this study, T₂ group had a higher value of ALP which is an indication that presence of hypothermia actually had a negative effect on the liver. Also in T₃ group, vitamin E was able to reduce the effect of hypothermia as evidenced in the lower value when compared to T₂ group. However, vitamin E didn't actually bring down the value to normalcy as evidenced in the result when T₃ group was compared to T₁ group. This may be that the introduction of hypothermia may have not only affected the liver but have had little or no effect on the bones of the pigs, since hypothermia was introduced by immersion.

Table1. Serum Levels of Liver enzymes with or without hypothermia.

Item	T ₁ (control)	T ₂ (Hypo)	T ₃ (vit E + SEM Hypo)	P-Value	
ALT [iu/l]	102.55 ^a	112.20 ^d	104.12 ^a	0.8	0.026*
AST [iu/l]	44.32 ^b	51.20 ^a	43.66 ^b	0.6	0.001**
ALP [iu/0]	43.68 ^c	47.86 ^a	45.50 ^b	0.5	0.012**

a,b,c, means within the same row with different superscripts are significantly ($P < 0.05$) different; SEM = standard error of mean. Iu/l means international units per liter, and Hypo means hypothermia

In Table 2 BUN had a significant increase ($p < 0.05$) in T₂ pigs compared to T₁ and T₃ that had similarities as BUN was significantly reduced ($p < 0.05$). Creatinine was significantly higher ($p < 0.01$) in T₂ pigs compared with T₁ and T₃ which had similarities in their concentrations ($p < 0.01$).

. BUN occurs in larger volume in the serum as evidenced in T₂ group compared to other treatment groups. This may be that the kidney failed to eliminate some nitrogenous wastes via urine. In other words, the kidney compromised its function and this is a clear indication that cold induced oxidative stress which led to the kidney compromising. This agrees with the research work which shows that rabbits exposed to hypothermic conditions actually had a higher volume of BUN in their serum which kept the rabbits in an oxidative stress conditions (Johnson and Diri, 2020). Also, the similar values obtain in T₃ and T₁ groups showed that the presence of vitamin E was able to reverse the effect of oxidative stress induced by cold. This corresponded with the research which showed that vitamin E reduced the level of creatinine in the serum of rabbits on

oxidative stress induced by hypothermia (Johnson and Diri, 2020). However, this did not agree with the study which showed that there were no significant differences on rabbits administered vitamin E during oxidative stress induced by partial bladder outlet obstruction when compared to their control group (Lin, *et al.*, 2011)

Creatinine compound are by products of creatinephosphatate. It is produced in the body during metabolism of creatine and usually excreted by the urine after it has been filtered by the kidney. Increased level of creatinine in the serum is an indication that the kidney malfunctioned as seen in T₂ group compared to other treatment groups. In other words, T₂ had a higher significant differences ($p < 0.01$) compared to T₃ and T₁ groups. Vitamin E had effect on creatinine volume as it was able to reduce creatinine level as T₃ group had similarities ($p < 0.01$) with T₁ group. This result disagreed with this study of Lin, *et al* (2011) but agrees with the studies of Mahaswari, *et al.*, 2015; Johnson and Diri 2020; and Johnson, *et al.*, 2023.

Table 2. Serum levels of kidney biomarkers with or without hypothermia

Items	T ₁ (control)	T ₂ (Hypo)	T ₃ (vit E + SEM)	P-Value
BUN (mmol/l)	4.42 ^b	6.01 ^a	4.45 ^b	0.046*
Creatinine (mmol/l)	78.42 ^b	89.22 ^a	76.25 ^b	0.001**

Means with different superscripts within the same row are significantly different ($P < 0.05$); ($P < 0.01$) SEM = error of mean, and mmol/l means millimole/liter

CONCLUSION

Oral vitamin E supplementation at 470mg/kg body weight can help reduce the effect of oxidative stress in pigs during hypothermic conditions such as the raining, winter and hammatan seasons.

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