Physiological Responses Of Rabbits To Oral Vitamin E Supplementation During Oxidative Stress Induced By Short-Term Hypothermia

Johnson, N. C., Diri, M., Owen, O. J.

Rivers State University, Animal Science Department, Faculty of Agriculture
Port Harcourt, Nigeria , +2349094624825
ntinya@alumni.uoguelph.ca

Rivers State University, Animal Science Department. Faculty of Agriculture
Port Harcourt, Nigeria +2348063995693
dirimoses@yahoo.com

Rivers State University, Animal Science Department.
Port Harcourt, Nigeria +2348033309939
congomfx@yahoo.com

Abstract: Physiological responses of rabbits to oral vitamin E supplementation during oxidative stress induced by short-term hypothermia were studied. Twenty four (24) male New Zealand White rabbits of 8 – 10 weeks of age and weighed 800-1000g were used in the experiment. The rabbits were randomly assigned to their treatment groups: T_0 (control), T_1 (hypothermia) and T_2 (hypothermia + vitamin E). The experiment duration was six weeks. The animals were fed similar diets throughout the experimental duration except that T_2 in the last four days of the experiment received 460mg/kg body weight of oral vitamin E (dl alpha tocopheryl acetate) supplementation daily. 24 hours after the last administration of vitamin E T_0 rabbits were dipped into a water of body temperature (37^{0}C) whereas T_1 and T_2 rabbits were dipped into a water of 10-12^{0}C with their heads up for five minutes after which their blood samples were immediately collected and snap frozen. Body temperatures of the animals after dipping were T_0 (37.9 ± 0.5), T_1 (34 ± 1) and T_2 (34.2 ± 0.7). Catalase (CAT) showed no significant differences ($p > 0.05$) in all the treatment groups. Superoxide dismutase (SOD) was significantly lower ($p < 0.05$) in T_2 compared with T_0 and T_1 which had similar levels ($p > 0.05$). . Glutathione (GSH) was significantly different ($p < 0.05$) among treatments but T_1 animals showed the lowest value. Glutathione Peroxidase (GSH-Px) was significantly different ($p < 0.05$) for all treatments but T_1 animals showed the lowest concentration. Oxidized form of glutathione (GSSH) concentration was significantly different ($p < 0.05$) for all treatments but T_2 animals demonstrated lowest concentration. Vitamin E concentration was significantly different ($p < 0.05$) among treatments with T_2 animals showed the highest concentration. Malondialdehyde (MDA) concentration was significantly different ($p < 0.05$) for T_0, T_1 and T_2 but T_0 demonstrated the lowest value. It was concluded that administration of oral vitamin E at 460kg/mg of body weight can up-regulate the activities of anti-oxidants while simultaneously reduce pro-oxidants in rabbits during hypothermic condition.

Key words: hypothermia, oxidative stress, rabbit and vitamin E

Introduction

At present rabbit production has become very popular. The reason for this is not far-fetched. Rabbit meat is widely accepted by most religious and traditional groups around the globe (1). Additionally, its meat has little or no fat; in this way, it helps in reducing cardiovascular diseases when compared to the consumption of other meats like beef and pork (2). According to (1), (3) rabbits is one of the animals known for its fast growth rate as rabbit can attain about 1.3 - 2kg body weight in 6 – 10 weeks of age. Again, compared to poultry and other livestock production, rabbit can easily be reared in small space even near residential areas as rabbit is not smelly like other livestock, including poultry. In addition they have a short gestation period (3), (4). Although, rabbit originated from the Western Hemisphere, environmentally areas where they are reared is very important. Hypothermia is the decrease in body temperature to less than 35^{0}C (5). Reduction in the body temperature can result to the production of reactive oxygen species (ROS) leading to oxidative stress (6). At times like this the animal health and welfare are compromised resulting in reduction in the animal productivity with its attendant reduction in the profit margin of the rabbit farmer (7). Vitamin E is the post potent antioxidant vitamin (8) and as such is also very potent in protecting the animal during oxidative stress. This assertion has been confirmed from recent studies (9) To date, there is no study that has investigated the effect of oral vitamin E supplementation in rabbits exposed to very cold temperature. Therefore, the objectives of this study are; to determine the effect of oral vitamin E supplementation on some antioxidant enzymes (CAT, SOD, GSH, and GSH-px) in rabbit exposed to short-term hypothermia and also to determine the effect of oral vitamin E supplementation on serum GSSH and MDA in rabbit exposed to short-term hypothermia.

Materials and Method

The study was carried out at the Rabbitry Unit of the Teaching and Research Farm of the Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria. The farm is situated at latitude 4^0 48'N and longitude 6^0 48' E of Rivers State University campus.Prior to the introduction of the experimental animals to the experimental site, the hutches, feeding and water troughs, including the floor were washed thoroughly with detergent, water and hypochlorite. It was then allowed to dry for one week to ensure that the site is pathogenic-free before the rabbits were introduced into the hutches. Twenty four (24) male New Zealand White rabbits of 8-10 weeks of age and weighing between 800-1000g were used in the experiment.The 24 rabbits on arrival at the rabbitry wing of the Rivers State Teaching and Research farm were weighed to obtain their initial body weights and

randomly assigned to their hutches in three (3) treatment groups of eight (8) animals each as:

1. T_0 (control), no vitamin E, no hypothermia
2. T_1, no vitamin E + hypothermia
3. T_2, vitamin E supplementation + hypothermia.

During the experimental period, the ambient and body temperatures of the rabbits were closely monitored using Millet Digital Thermometer (Beijing, China). The rabbits were kept in their separate hutches for five (5) weeks under similar management condition during which they were fully acclimatized to the research environment. Furthermore, during this period all animals were fed growers diet (pellet) ad libitum twice per day with Centrosema pubecen forage supplementation. The experiment lasted for a duration of six weeks.In addition to their daily regiment feeding, rabbits in the T_2 group received oral supplementation of vitamin E (dl-Alpha Tocopheryl Acetate) via drinking water at the level of 460mg/kg of body weight for 4 days in the sixth week of the experiment. Twenty four (24) hours after the last vitamin E supplementation, hypothermia was introduced as:T_0 (Control) rabbits were immersed into water of body temperature (37^0C) up to the neck for 5 minutes without any anaesthetic or tranquilizer. T_1 and T_2 rabbits were cooled by immersion into cold water (10-12^0C) also for 5 minutes. The temperatures of the water were confirmed using Millet water Thermometer (Beijing, China). Blood samples were collected from individual rabbits immediately after dipping and snap frozen in all the treatment groups using a 2ml syringe via their ear vein and placed in well labeled vial tubes of equal number of non-ethylene diamine tetracetic acid treated tubes for vitamin E analysis and ethylene diamine tetracetic acid treated vial tubes for anti-oxidants and oxidants, respectively. Additionally, as the animals were dipped rectal temperatures were taken and recorded for all animals in each group.

Blood Sample Analysis

Blood samples were analyzed for antioxidant enzymes, as well as pro-oxidant namely, GSSH and MDA Catalase was measured according to (10). Briefly, 0.1ml sample was pipette into curette containing 1.9ml of 50mm phosphate buffer of pH 7. Reaction initiated by adding 0.1ml freshly prepared 30% w/v hydrogen peroxide. Absorbance was read at 240nm wavelength. SOD was measured using the methods of (11), (12). 1ml of sample was diluted with 9mls of distilled water to make 10ml dilution an aliquor of diluted sample was added to 25ml of 0.05m carbonate pH 10.2 to calibrate the spectrophotometer. Reaction initiated by adding 0.3ml of adrenaline and absorbance was read at 430nm wavelength. GSH, GSH-px and GSSH activities were determined according to (13). For GSH, three tubes containing blank, standard and sample were used. The blank consists of 4 reagents; standard was prepared using 1ml of 20µml/L of GSH standard, 1.25ml of reagent 2 solution, 0.25ml of reagent 3 and 0.05ml of reagent 4. Sample was prepared using 1ml of supernatant, 1.25ml of reagent 2 application solution, 0.25ml of reagent 3 and 0.05ml of reagent 4. The blank, standard and sample tubes were fully mixed and kept for 15 minutes at room temperature. Spectrophotometer was set to zero with distilled water and optical density (OD) was measured for each test tubes at 420nm. GSH-Px: GSH-Px = GSH + 0.26mg/L. reduced GSH was obtained by adding water to GSH. GSSH = GSH + O_2. MDA was measured by the reaction of MDA with thiobarbituric acid reactive substances (TBARS) to form MDA- TBARS that absorb light at 532nm. 0.4ml reaction mixture with sample was quenched with 0.5ml at 30%. TCA was added to 1.6ml of trihydroxymethyl methylamine potassium chloride at pH of 7.4 of 8% tiobarbituric acid, incubated for 45 minutes and then centrifuge at 1400rmp for 5 minutes. Absorbance was taken at 532nm. Data obtained were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS (27). Treatment means were compared using Tukey's test. The experimental design used was the completely randomized design and therefore the model was$Y_{ij} = u + X_i + E_{ij}$.

Where; Y_{ij}= individual observation of treatment
u= population mean
X_i= treatment effect and
E_{ij}= the error term.

An α-level of 0.05 was used for all statistical comparisms to represent significance.

Results and Discussion

Rabbits in all treatment groups fed normally and was seen to be in good health throughout the experimental period as they were observed and monitored on daily basis. Mean body temperature of control group was significantly higher (37.9 ± 0.5) than those of hypothermic group (34 ± 1.0) and hypothermia group with vitamin E supplementation (34.2 ± 0.7), respectively. This was indication that hypothermia was experienced with animals in treatments T_1 and T_2, respectively. The effect of hypothermia on anti-oxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, and glutathione), vitamin E and malondialdehyde, including vitamin E levels are shown in Table 4.1

Table 4.1 Anti-oxidant Levels, GSSH, MDA and vitamin E Concentration in Serum of Rabbits with or without Hypothermia

Items	Treatments				
	T_0 (n=8)	T_1 (n= 8)	T_2 (n=8)	SEM	P-value
CAT (u/ml)	21.33	21.30	21.46	0.07	0.97
SOD (u/ml)	1.90^a	1.90^a	1.84^b	0.01	0.001
GSH (umol/g)	3.84^a	2.66^b	3.55^c	0.03	0.001
GSH-px (umol/g)	1.73^a	1.13^b	1.58^c	0.02	0.001
GSSH (umol/g)	1.03^a	1.38^b	0.95^c	0.02	0.04
MDA (nmol/ml	2.74^a	3.83^b	2.93^c	0.03	0.001
Vit E (1mg/ml)	1.90^a	1.61^b	2.36^c	0.02	0.001

Means with different superscripts within the same row are significantly different at (P < 0.05)
SEM= standard error of the mean
Legends: CAT= Catalase, SOD= superoxide dismutase, GSH = glutathione, GSH-px = glutathione peroxidase, GSSH = oxidized form of glutathione, MDA = malondialdehyde and Vit E = vitamin E.

Hypothermia and vitamin E had no effect on CAT as there were no significant differences (p > 0.05) in all the treatment groups. Hypothermia had no effect on Superoxide dismutase

as T_0 and T_1 rabbits had similar levels of SOD ($p > 0.05$). However, hypothermia reduced SOD level in the presence of vitamin E (T_2) as SOD level was significantly lower ($p < 0.05$) in T_2 rabbits compared with T_0 and T_1 groups. Hypothermia had effect on GSH as GSH level is significantly lower ($p < 0.05$) in T_1 and T_2 compared with T_0. However, GSH level was significantly higher ($p <0.05$) in T_2 compared with T_1, demonstrating that vitamin E improved GSH level during oxidative stress induced by hypothermia. Hypothermia had effect on GSH-Px as GSH-Px level was significantly lower ($p < 0.05$) in T_1 rabbits compared with T_0 and T_2 rabbits. However, GSH-Px level was significantly higher ($p < 0.05$) in T_2 compared with T_1 indicating that vitamin E improved the concentration of GSH-Px during hypothermic condition. Hypothermia induced oxidative stress in T_1 rabbits as GSSH level was significantly higher ($p < 0.05$) in T_1 rabbits compared with T_0 and T_2. GSSH level was also significantly lower ($p < 0.05$) in T_2 rabbits compared with T_0 indicating that vitamin E can protect rabbits against hypothermia via their glutathione defense system. MDA was significantly higher ($p < 0.05$) in T_1 and T_2 rabbits compared with T_0 group. However, MDA in T_2 rabbits was significantly lower ($p< 0.05$) compared with T_1 rabbits demonstrating that hypothermia induced oxidative stress but vitamin E was capable of reducing the level of oxidative stress as evidenced by the significant lower ($p < 0.05$) value of MDA in T_2 group of animals compared with T_1 group. The level of vitamin E was significantly lower ($p < 0.05$) in T_0 and T_1 compared with T_2. T_1 group actually demonstrated the lowest level of vitamin E while T_2 group showed the highest level of vitamin E Catalase is an antioxidant enzyme located in the cytosol of living cells. They protect the cells, tissues and organs from oxidative damage by catalyzing the degradation of hydrogen peroxides to water and oxygen (15). In this study, hypothermia and vitamin E had no effects on CAT as its levels were similar ($p> 0.05$) in all the three groups. This finding is not surprising in this study since CAT is mostly found in the cytosol of the cell. Therefore, the five minutes hypothermia in this study probably was not long enough for cold penetration into the cytosol. This finding agrees with (15) which demonstrated that vitamin E had no effect on CAT during oxidative stress induced by physical exercise. However, this finding disagrees with the finding of (16) which investigated the effect of vitamin E on CAT in rats during oxidative stress induced by high cholesterol levels and found that vitamin E up-regulated or increased CAT levels. This can partially be explained with the fact that vitamin E is a fat soluble vitamin and thus cholesterol is one of the major carriers of vitamin E (17) which might had aided its potency in increasing CAT and protecting the cell against damage. Superoxide dismutase is a major antioxidant enzyme found in the cells of animals. Their main function is to catalyse the dismutation of superoxides, breaking them down to simple molecular oxygen and hydrogen peroxides which are further degraded by catalase enzyme to water (18). In this study, hypothermia had no effect on SOD as SOD levels were similar ($p > 0.05$) in T_1 and T_0 rabbits. This could probably be that hypothermia was not long enough for cold penetration into the mitochondria of the cell where SOD is found. Furthermore, SOD was significantly lower ($p < 0.05$) in T_2 rabbits compared with T_1 rabbits. This finding agrees with that of (15) which demonstrated that there was a decrease in SOD level when vitamin E was supplemented in the diets of rats on oxidative stress induced by physical exercise. However, the finding did not agree with that of (16) which investigated the effect of vitamin E on SOD in rats on oxidative stress induced by high dietary cholesterol and found that vitamin E supplementation up-regulated SOD level. This can partially be explained again with the fact that vitamin E is a fat soluble vitamin and thus, cholesterol being one of the major carriers of vitamin E (17) might had aided its potency as evidenced in that study to enhance SOD levels and prevented the cell against damage (16) Glutathione (GSH) is primarily known as the body master antioxidant found in all cells of living organisms. Glutathione protect the cells, tissues, and organs from oxidative damage (19). When GSH is used by the animal for defense it is oxidized to GSSH. In this study, hypothermia had effect on GSH as GSH level was significantly lower ($p < 0.05$) in T_1 and T_2 rabbits compared with the T_0 group. Nevertheless, GSH level was significantly higher ($p < 0.05$) in T_2 rabbits compared with T_1 group indicating that vitamin E up-regulated GSH level in T_2 animals as GSH level in T_2 was significantly higher ($p < 0.05$) compared with T_1 group. This finding agrees with that of (19), (14), (21). In these studies it was shown that vitamin E improved GSH levels in cells of rats and poultry during oxidative stress induced by dimethoate and exogenous tyroxine treatment according to these workers. GSH-Px is an enzyme found in the cells of organisms which aids in protecting the cells from oxidative damage (21). GSH-Px is involved in ensuring normal cellular levels of GSH by converting GSSH back to GSH; thereby ensuring the continuous availability of GSH in protecting the animal. It also removes hydrogen peroxides (22). In this study, hypothermia had effect on GSH-Px as GSH-Px was significantly lower ($p< 0.05$) in T_1 and T_2 rabbits compared with T_0 group. However, GSH-Px level was significantly increased ($p< 0.05$) in T_2 rabbits compared to T_1 group indicating that vitamin E increased GSP-Px level in the rabbits during hypothermic condition. Therefore, the significant lower ($p < 0.05$) GSSH concentration in T_2 rabbits compared with T_1 group in this study was not surprising. This finding agrees with those of Michael et al., (2010) and (14) who found that vitamin E aided in improving GSH-Px level in the cells of rats during oxidative stress in their independent studies, respectively. Oxidized form of glutathione (GSSH) reduces disulfide bonds made within the cytoplasm proteins to cysteine by donating an electron. In this process, GSH is converted to GSSH. However, GSSH can also be restored back to its reduced form (GSH) by glutathione peroxidase using nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor (20). In this study, hypothermia had effect on GSSH as GSSH was significantly higher ($p < 0.05$) in T_1 rabbits compared with T_0 group. This could be that hypothermia induced oxidative stress that would have triggered the conversion of GSH to GSSH particularly in the animals of T_1 group. Also, vitamin E had effect on GSSH as GSSH was significantly lower ($p < 0.05$) in T_2 compared with T_0 and T_1 indicating that vitamin E would have better protected rabbits in T_2 during hypothermic conditions via their glutathione defense system. This can explain in part the increased significant level ($p < 0.05$) of GSH and the significant lower level ($p < 0.05$) of GSSH in T_2 group. This finding agrees with those (23) who showed that there was a significant decrease in GSSH level when Albino rats on oxidative stress induced by Carbamazepine were treated with vitamin E, and (5) which

also showed that GSSH levels significantly decreased when pigs on oxidative stress induced by hypothermia were treated with oral vitamin E. Malondialdehyde is the major by-product of lipid peroxidation normally released into the blood serum when there is damage in the cells and tissues of organs. Thus, its quantification is a measure of oxidative stress in animals (24). In this study, hypothermia significantly increased serum MDA as MDA level was significantly higher ($p < 0.05$) in T_1 group compared with T_0 and T_2 groups. However, MDA concentration was significantly reduced ($p < 0.05$) in T_2 rabbits compared with T_1 group. This suggests that vitamin E reduced the effect of oxidative stress induced by hypothermia in rabbits of T_2 group. This finding agrees with those of (18), (14), (23) and (24) who found that vitamin E aided in reducing the level of MDA in the serum of animals during oxidative stress induced by exhaustive exercise, dimethoate, carbamazephine and melathion in rats according to their independent studies. Vitamin E is a very potent anti-oxidant vitamin that had been identified in reducing oxidative stress in living organisms (6). Its antioxidant activities are related to regulating or modulating glutathione and glutathione peroxidase (22). In this study, the observed significantly higher ($p < 0.05$) level of vitamin E in T_2 group compared with T_1 was expected as T_2 animals received oral vitamin E. Hypothermia had effect on vitamin E concentration as vitamin E level was significantly lower ($p< 0.05$) in T_1 rabbits compared with T_0 group. This could probably be that the dietary antioxidant vitamins, especially vitamin E in the T_1 rabbits were mostly used up by their glutathione defense system suc**3BDF7HBXCXN6**h that animals in T_1 group became more prone to lipid peroxidation (26) due to oxidative stress induced by hypothermia. This finding agrees with those of (28), (9) and (21) who demonstrated that vitamin E works synergistically with antioxidant enzymes in reducing or preventing oxidative stress in animals, including humans (25).

Conclusion

Administration of vitamin E at 460mg/kg of body weight can improve the quality of life of rabbits by alleviating lipid peroxidation-induced disturbances during cold periods. Vitamin E can do this by up-regulating body antioxidant enzymes, such as GSH and GSP-Px; while simultaneously reduce oxidant and pro-oxidant levels, such as MDA and GSSH respectively.

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