

# Effect Of Oral Vitamin E Supplementation On Blood And Some Electrolyte Parameters Of Rabbit With Short-Term Hypothermia

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**Abstract:** Effects of oral vitamin E supplementation on blood parameters and some electrolytes in acute hypothermia of rabbits were investigated. 24 New Zealand White rabbits, weighing 960 – 1000g were randomly divided into one of three experimental groups: 1 (control, without cooling); 2 (hypothermic) and 3 (hypothermic with vitamin E supplementation). The rabbits of group 3 received daily supplementation of 460mg/kg body weight (BW) for 4 days before inducing hypothermia. 24 hours after the last vitamin E supplementation, the rabbits of groups 2 and 3 were cooled by immersion into cold water (10 – 12°C) while the control rabbits were immersed into water of body temperature (37°C) up to their necks for 5 minutes without any anesthetic or tranquilizer. Rectal body temperatures of groups and blood samples were taken for biochemical analyses after cooling and were immediately snaps frozen. The body temperatures of hypothermic animals were lower ( $P < 0.05$ ) ( $T_2$ ,  $34 \pm 1$ ) and ( $T_3$ ,  $34.2 \pm 0.7$ ) compared with the control ( $T_1$ ,  $37.9 \pm 0.5$ ). However, white blood cell (WBC), lymphocytes (LYM), sodium ( $Na^+$ ) and chloride ( $Cl^-$ ) of hypothermic animals + vitamin E were higher ( $P < 0.05$ ) than those of control animals. The neutrophils (NEU) of the control and group 2 animals were higher ( $P < 0.05$ ) than those of group 3 animals. All other parameters of group 3 and the control animals were not significantly ( $P > 0.05$ ) different. It was concluded that oral supplementation of vitamin E can improve the quality of life by stimulating the immune system, maintain enhanced levels of RBC, Hb, WBC as well as those of  $Na^+$  and  $Cl^-$  of domesticated rabbits during short-term hypothermia. Therefore, vitamin E can improve rabbit quality of life during hypothermia.

**Key words:** Hypothermia, Vitamin E, Blood Parameters, Electrolytes and Rabbit.

## 1 INTRODUCTION

Rabbit production is currently one of the major micro-livestock productions receiving attention, especially in the developing countries, including Nigeria [10]. The reasons for this are not far-fetched. Rabbit production requires modest investment. Rabbit keeping is a source of low cost but high quality protein that uses only local forages and food wastes that are of no direct value to man [10]. Additionally, rabbit keeping is not restricted by any traditional or religious taboos as in the case with other animals, such as the pig and cattle that are forbidden by some religious groups, respectively. During emergencies where other protein sources are absent the rabbit with proper feeding regimens can produce high quality protein very rapidly, especially with the New Zealand White breed [10]. Rabbit is also not a smelly or noisy animal and as such can be reared near residential homes compared with other livestock and poultry [9; 12]. However, one of the factors that can militate against its production is the ambient temperature in the hutch microclimate. Although the rabbit originated from the temperate region it is a homeothermic animal. This means that rabbit must keep its body temperature within certain limits if it is to remain productive and alive. Rabbit normal body temperature ranges between 37 – 39.5°C [6-7]. Its ambient temperature ranges between 16 – 19°C [6]. In rabbit therefore, hypothermia is defined as a core temperature of less than 35°C. Moderate hypothermia occurs when temperature falls between 35°C and 32°C and severe hypothermia occurs when rectal temperature descends below 32°C [4]. During hypothermia the ambient temperature often descends to between 10 – 12°C [4]. Therefore, during hypothermia rabbit production efficiency and health may be compromised. During such

periods blood profiles and electrolyte balances may be distorted which can lead to poor health by inducing acid-base imbalances of the animal [12]. Vitamin E has been demonstrated to be the most potent antioxidant vitamin, compared to vitamins A, C and D [8; 12]. The possibility therefore exists that supplementing rabbit with vitamin E during hypothermia may rescue the animal from the drawbacks of hypothermia. Therefore, the objectives of this study are:

1. To determine the effect of vitamin E on the blood profiles of rabbits exposed to short- term hypothermia;
2. To determine the effect of vitamin E on some electrolytes in rabbits exposed to short- term hypothermia.

## 2 MATERIALS AND METHODS

### Animals, Housing and Management

Prior to the introduction of animals into their hutches, the hutches, feeding and water troughs were thoroughly washed with detergent/ hypochlorite and water to ensure that the animal environment is much cleaned. After cleaning it was allowed for one week to dry before introducing the experimental animals into their new environment. Twenty-four (24) male New Zealand White rabbits were acquired from EC ONE Nigeria Limited, Elibrada, Emuoha Local Government of Rivers State. The animals on arrival at the rabbitry wing of the Rivers State University Teaching and Research Farm were weighed to obtain their initial body weights and randomly assigned to their hutches in three treatment groups of eight rabbits each as:

1. Treatment 1 (control), no vitamin E and no hypothermia;
2. Treatment 2, no vitamin E + hypothermia; and
3. Treatment 3, vitamin E supplementation + hypothermia.

During the experimental period ambient and rectal temperatures of the rabbits were closely monitored and were within the normal ranges using millet digital thermometers (Songhai, China).

#### Experimental Diets, Vitamin E Administration, Hypothermia and Blood Collection

The experiment lasted for six weeks. During the experimental period, the animals were fed grower diet in pellet forms ad libitum twice daily at 0900 h and at 1600 h, respectively with *Centrosema pubescence* forage supplementation. In the six week of the study, animals in treatment 3 received oral supplementation of vitamin E at 460mg/kg of body weight for 4 days. Twenty-four hours after the last vitamin E supplementation, hypothermia was introduced followed by blood sample collections. Blood samples were collected by humanely euthanizing the animals and blood collected using a 2 ml syringe into EDTA treated tubes for blood parameters analyses and untreated tubes for electrolytes analyses, respectively. Blood samples were collected from all the experimental animals. However, before blood sample collections, animals in group 1 were immersed into water of body temperature (37<sup>0</sup>) up to the neck for five minutes without any anaesthetic or tranquilizer. Animals in groups 2 and 3 were cooled by immersion into cold water of 10 – 12<sup>0</sup>C temperature also up to their necks for 5 minutes. After each immersion for each animal blood sample were collected and snaps frozen. Rectal temperatures were also taken immediately after dipping for each animal before taking blood samples. Packed cell volume (PCV), Red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC) and their differentials (neutrophils, lymphocytes, eosinophils, monocytes and basophils) were analysed for. For blood electrolytes, sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>) and chloride (Cl<sup>-</sup>) were analysed for. The blood samples were analysed by haematology auto-analyser machine (BC-2300) while electrolytes were analysed for by the flame photometric and spectrophotometric methods according to [1]. Briefly, final values were obtained by dividing results obtained by optical density of test sample by optical density of standard x concentration of standards.

#### Design and Statistical Analysis

The designed used in this study was the completely randomized design (CRD). Therefore, the model is:  $Y_{ij} = \mu + X_i + E_{ij}$ ; where  $Y_{ij}$  = individual observation,  $\mu$  = population mean,  $X_i$  = treatment effect and  $E_{ij}$  = the error term. Data were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure of SAS. Treatment means were compared using Tukey's test and a  $\alpha$ -level of 0.05 was used for all statistical comparison to represent significance.

### 3 RESULTS

All animals in the three treatment groups were healthy throughout the experimental period as they were closely monitored and seen to readily consume their diets normally. The results of the effects of hypothermia on the rabbit blood profiles regarding the animals' PCV, Hb and RBC are shown in Table 1.

**Table 1: RBC count, Hb concentration and PCV of rabbits with or without hypothermia.**

Item	Treatments			SE M	P-value
	Treatment 1 (n = 8)	Treatment 2 (n = 8)	Treatment 3 (n = 8)		
RBC(x10 <sup>9</sup> /l)	7.13 <sup>ab</sup>	6.50 <sup>a</sup>	7.25 <sup>b</sup>	0.28	0.03
Hb (g/dl)	13.63 <sup>ab</sup>	13.25 <sup>a</sup>	14.13 <sup>b</sup>	0.27	0.01
PCV (%)	41.13	41.25	41.38	0.44	0.85

<sup>a,b</sup>Means with different superscripts within the same row are significantly ( $P < 0.05$ ) different; SEM = standard error of the mean. Legend: RBC = Red blood cell. Hb = Haemoglobin. PCV = Packed cell volume.

For the RBC, treatments 1 and 3 had similar contents but treatment 3 was significantly ( $P < 0.05$ ) higher than that of treatment 2. This was also mirrored in the Hb levels. The Hb of treatments 1 and 3 were similar but that of treatment 3 was significantly ( $P < 0.05$ ) higher than that of treatment 2. However, there were no significant ( $P > 0.05$ ) differences in the PCV percentages for all treatment groups. Table 2 shows the results of short-term hypothermia on WBC and its differentials (neutrophils, lymphocytes, eosinophils, monocytes and basophils).

**Table 2: Total and differential WBC counts in rabbits with or without hypothermia.**

Item	Treatments			SE M	P-value
	Treatment 1 (n = 8)	Treatment 2 (n = 8)	Treatment 3 (n = 8)		
WBC(x10 <sup>9</sup> /l)	7.63 <sup>ab</sup>	7.00 <sup>a</sup>	7.75 <sup>b</sup>	0.25	0.02
NEU (%)	50.13 <sup>a</sup>	50.63 <sup>a</sup>	45.63 <sup>b</sup>	1.05	0.001
LYM (%)	47.88 <sup>a</sup>	46.63 <sup>a</sup>	51.75 <sup>b</sup>	1.25	0.014
MON (%)	1.50	1.75	2.00	0.35	0.76
EOSI (%)	0.50	0.63	0.50	0.31	1.00
BASO (%)	0.00	0.38	0.13	0.11	0.77

<sup>a,b</sup>Means with different superscripts letter are significantly different ( $P < 0.05$ ); SEM = standard error of the mean. LEGEND: WBC = white blood cell. NEU = neutrophils. LYM = lymphocytes. MON = monocytes. EOSI = eosinophil. BAS = basophils.

The WBC of treatments 1 and 3 were similar but that of treatment 3 was significantly ( $P < 0.05$ ) higher than that of treatment 2. However, the NEU of treatments 1 and 2 were similar and significantly ( $P < 0.05$ ) higher than that of treatment 3. Conversely, the LYM of treatments 1 and 2 were similar and significantly ( $P < 0.05$ ) lower than that of treatment 3. There were no significant ( $P > 0.05$ ) differences for MON, EOSI and BASO for all treatment

groups. The results of the effects of hypothermia on electrolytes, namely Na, K and Cl in rabbits with or without hypothermia are shown in Table 3.

**Table 3.**  $Na^+$ ,  $K^+$  and  $Cl^-$  serum concentrations in rabbits with or without hypothermia.

Item	Treatment			SE M	P-value
	Treatment 1 t 1 n = 8	Treatment 2 2 n = 8	Treatment 3 t 3 n = 8		
$Na^+$ (mmol/l)	132.13 <sup>a</sup>	130.88 <sup>a</sup>	136.88 <sup>b</sup>	0.38	0.01
$K^+$ (mmol/l)	4.25	4.13	4.25	0.15	0.83
$Cl^-$ (mmol/l)	95.25 <sup>a</sup>	96.13 <sup>a</sup>	101.25 <sup>b</sup>	0.81	0.01

<sup>a,b</sup>Means with different superscripts are significantly ( $P < 0.05$ ) different; SEM = standard error of the mean. LEGEND:  $Na^+$  = Sodium ion.  $K^+$  = Potassium ion.  $Cl^-$  = Chloride ion.

The sodium levels of treatments 1 and 2 were not significantly ( $P > 0.05$ ) different. The sodium level of treatment 3 was significantly ( $P < 0.05$ ) higher than those of treatments 1 and 2. However, the potassium levels for all treatment groups were similar ( $P > 0.05$ ). Nevertheless, the chloride level of treatment 3 was significantly ( $P < 0.05$ ) higher than those of treatments 1 and 2 that had similar levels.

#### 4 DISCUSSION

The studies indicated that the five minutes' dip of animals of treatments 2 and 3 in cold water experienced hypothermia as confirmed by their lowered body temperatures. This was expected because cold shock was induced as the temperature of the water was below the animals' comfort zone [12]. The study also demonstrated that rabbits of all treatments had similar PCV values. This is an indication that short period hypothermia had no effect on PCV. However, the study showed that hypothermia reduced the Hb levels of rabbits in the absence of vitamin E as animals of treatment 2 had lowered levels of Hb. The mechanism through which hypothermia reduces Hb concentrations at present is not well understood. Similarly, the study demonstrated that rabbit quality of life may be compromised during cool period when ambient temperature is below the rabbit's lower critical temperature [11] as hypothermia reduced the level of RBC and that vitamin E is to the rescue; as vitamin E increased RBC in treatment 3 compared with treatment 2. This preliminary study of the use of vitamin E to improve the quality of life during hypothermia indicated that rabbits that received vitamin E had a preponderance of WBC as animals in treatment 3 were observed to have significant higher levels of the WBC counts compared with treatment 2. Furthermore, vitamin E significantly increased the ratio of lymphocyte to neutrophil when compared with treatments 1 and 2. These findings therefore, support the earlier data of previous workers that vitamin E stimulates immune functions [3; 5]. This can also be used to explain in part the role of

vitamin E in activating the natural killer cells; the function of T-lymphocytes [3]. Hypothermia and vitamin E had no effects on monocytes, eosinophil and basophils. Hypothermia and vitamin E had no effects on serum potassium. However, in the presence of hypothermia, vitamin E significantly increased the serum levels of sodium and chloride. This finding is suggestive of a mild hypernatremic and hyperchloremic effects probably due to physiologic cold shock in ensuring the animal acid-base balance. To this point therefore, vitamin E in addition to its other functions may favour an improvement in renal function by increasing sodium and chloride re-absorption [2]. This secondary function of vitamin E may also serve in relieving hypertension by its effect on possible increase in renal blood flow which also enhances renal reabsorption of basic electrolytes like sodium and chloride.

#### 5 CONCLUSION

Oral supplementation of vitamin E can alleviate the negative effects of short-term hypothermia in rabbits by improving the animal's quality of life through the stimulation of the immune functions, maintaining enhanced levels of RBC, Hb, WBC as well as those of  $Na^+$  and  $Cl^-$  of the animal. It was concluded that vitamin E can be used to improve quality of life of domestic rabbits during hypothermia, such as during the long cold seasons.

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