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HAEMATOLOGICAL INDICIES, CARCASS YIELD AND ORGAN WEIGHTS OF GROWING RABBITS FED DIETS CONTAINING VITAMINS C AND E IN A HOT HUMID TROPICAL ENVIRONMENT

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Abstract: An eight-week study was conducted to determine the response of growing rabbits to varying dietary levels of vitamins C and E under a hot humid tropical environment. Thirty-six hybrid (Chinchilla × New Zealand white) growing rabbits of both sexes with initial average weight of 0.80 kg were randomly divided into nine groups of four rabbits each and assigned to 9 diets a in a 3×3factorial arrangement involving three vitamin C levels (0, 200 and 400mgkg^{-1}diet) and three vitamin E levels (0, 200 and 400mgkg^{-1}diet) in a completely randomized design. Each treatment was replicated 4 times with one rabbit constituting a replicate. Data were collected on haematology and carcass characteristics. Results showed that haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), mean cell haemoglobin (MCH) and differentials (neutrophils, lymphocytes, monocytes, eosinophils and basophils) were significantly ($p<0.05$) affected by dietary treatments, while mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were not significantly ($p>0.05$) affected by the dietary treatments while the white blood cell count (WBC) of rabbits on treatments 5 and 7 increased significantly.Treatments had significant effects on the carcass traits (live weight, carcass weight and dressing percentage) but had no effect on organ characteristics of the rabbits. It was concluded that a combination of 200mgkg^{-1}diet of vitamin C and 200mgkg^{-1}diet of vitamin E can be successfully added to the diet of growing rabbits during the dry season, without having any negative effect on their carcass yield, relative organ weights and haematological parameters.

Keywords: Antioxidants, Growing rabbits, haematology, carcass yield, organ weights.

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Introduction: The rabbit (*Oryctolagus curiculus)* described as a micro-livestock species (Vietmeyer, 1985), appears to be the cheapest and sustainable means of producing high quality protein for the expanding populations of the less developing countries like Nigeria. Rabbit production may provide the impoverished urban population and the resource poor rural dwellers

the opportunities to earn additional income on a sustainable basis. The above possibilities derive from the exceptional attributes of rabbits which include: affordable or low cost management requirement, small body size, short generation interval, high fecundity, rapid growth rate, and genetic diversity, ability to utilize forage and agricultural by-products, and adaptation to a wide range of ecological environment (Onifade *et al*., 1999).

In tropical and sub- tropical countries, climatic heat is the major constraint on animal productivity. Production and reproduction are impaired as a result of the drastic changes in biological functions caused by heat stress (Marai *et al*., 2002). Heat stress results from a negative balance between the net amount of energy flowing from the animal to its surrounding environment and the amount of heat energy produced by the animal (Farooq *et al.*, 2010). During stress episode, reactive oxygen species (ROS) generation exceeds the body's antioxidant production capacity, and oxidative stress develops (Roth, 2000). In the rabbit, stress associated with exposure to high ambient temperatures decreases growth performance, possibly because of excessive production of reactive oxygen species (ROS) that oxidize and destroy cellular biological molecules (Liu *et al.*, 2011).

Rabbits normally responds to stimuli (stressors) with pattern of behavioural, endocrine, neural, immune, hematological and metabolic changes designed to restore homeostasis in order to be adaptive or to promote survival (Knowles and Wariss, 2000; Wariss, 2010; and Muir, 2004). Heat stress triggers several physiological responses, particularly increased concentration of primary mediators (Stress related hormones glucocorticoids, catecholamines and beta-endorphines) (Von Borell, 2001; Terlouw *et al;* 2008), which in turn leads to changes in the biochemical and hematological components of the animal. (Nemec Svete *et al*., 2012).

Dietary supplementation with vitamins C and E has been proved to be a simple and convenient strategy to introduce a natural antioxidant that may effectively inhibit the oxidation reactions (Botsoglou *et al*., 2004). The synergies of vitamin E and vitamin C on the hemetological indices of rabbits are yet to be substantiated. Against this backdrop, the present study was therefore conducted to determine the response of growing rabbits to varying dietary levels of vitamins C and E under a hot humid tropical environment.

Materials and Method

Experimental Materials: The two antioxidants, vitamins C and E (Hoffman la Roche®) used for the experiment were procured from a pharmaceutical store at the University Market Road, Nsukka. Other ingredients used were obtained from different locations within Nsukka Local Government Area, Enugu State, Nigeria and used to formulate the experimental diets.

Location and Duration of Study: The study was conducted at the Rabbitry Unit of the Department of Animal Science, University of Nigeria, Nsukka. The experiment lasted nine (9) weeks between March 25th to May 19th 2015. The climate during the period of study was characterized by dry to wet season, low to high relative humidity (Range: 4% and 85% at 10am) and higher average temperature of 36.7^{0}C to 41^{0}C that is higher than in previous months, few rainy and therefore cloudy days.

Nsukka lies in the derived savannah region and is located at longitude 6°41`N and 7°24`E (Ofomata, 1975) with an altitude of 447m above sea level (Breinhalt *et al.*,1981).The daily mean ambient temperature range is between 21-32.8^0 C (Ingrid Jensen, 2015).

The two Antioxidants (vitamin C and E) used for the experiment were procured from a pharmacy at the University Market Road Nsukka.

Experimental Animals and Management: A total of 36 growing rabbits of about 6 weeks of age and mixed sexes sourced from the Rabbit Farms in Nsukka were used for the study. The rabbits were housed in individual hutches on arrival and fed compounded ration and during

the first two weeks of life, the rabbits were allowed to acclimatize with the new environment. Feed and water were served *ad libitum* throughout the experimental period.

The rabbits were treated for coccidiosis by mixing the coccidiosat in their drinking water and administerd to the rabbits for three days. Ivermectin was administered at 0.2ml per rabbit subcutaneously to prevent external and internal parasites.

At about 6 weeks of the experimental period, snuffles were noticed in some rabbits and oxytetracylin (antibiotic) was administered subcutaneously at 0.2ml to all the rabbits to treat the infected rabbits and to prevent the spread to healthy rabbits.

Experimental Procedure: In the experiment 6 weeks old hybrid (Chinchilla × New Zealand white) growing rabbits of both sexes with initial average weight of 0.8 kg were randomly divided into nine groups of four rabbits each. The groups were randomly assigned to nine treatment diets containing 0, 200 and 400mg of vitamin C and E and their combinations. The percentage composition of the experimental diets is presented in Table 1. Each treatment group was repeated four times with a rabbit constituting a replication place in a four-tier rabbit cage that had a total of 12 hutches per tier. The cages were located inside a building equipped with nets and windows for proper ventilation. Each hutch, which accommodated 1 rabbit, was partitioned with metal sheets and wire mesh and fitted with metallic trays (for collection of faecal droppings) and stainless feeders and drinkers.

The rabbits were provided feed and water *ad libitum* for 56 days of the experimental period. The rabbits were weighed at the beginning of the experimental feeding and subsequently on a weekly basis to determine the daily weight gain. Forages like Bermuda grass, elephant grass, alfalfa and glover weed were used to supplement the experimental diet.

Feed intake was determined daily by the weight back technique. Feed conversion ratio was calculated from the weight gain and feed intake values.

Chemical and Data Analysis: Experimental diet and faecal samples were assayed for proximate composition by the method of AOAC (1990). Gross energy of experimental diets and faecal samples were determined in a Parr Oxygen adiabatic bomb calorimeter.

Data collected were subjected to a one-way analysis of variance using SAS (1990). Significantly different means were separated using Duncan's New multiple Range test (Duncan, 1955).

Table 1: Gross Composition of Experimental Diets

Vitamin C levels($mgkg^{-1}$)	0			200			400		
Vitamin E levels($mgkg^{-1}$)	0	200	400	0	200	400	0	200	400
Ingredients (%)/Diets	1	2	3	4	5	6	7	8	9
Maize	37.35	37.35	37.35	37.35	37.35	37.35	37.35	37.35	37.35
Wheat offal	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Soya bean	11.20	11.20	11.20	11.20	11.20	11.20	11.20	11.20	11.20
PKC	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Fish meal	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Groundnut cake	20	20	20	20	20	20	20	20	20
Bone meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit-mineral mix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated composition									
Crude protein %	15.38	14.81	16.99	15.09	15.80	16.73	15.46	16.45	16.51
Energy (Mj/kg ME)	12.01	12.05	12.07	12.01	12.01	12.06	12.02	12.06	12.06
Crude fibre (%)	5.16	4.51	5.14	5.35	4.86	5.15	5.02	5.41	5.38
Determined composition:									

Dry matter	67.2	66.5	64.8	70.5	72.5	64.6	65.8	68.2	71.3
Crude protein	15.38	14.81	16.9	15.1	16.5	15.5	15.8	16.7	16.5
Crude fibre	5.16	4.51	5.14	5.35	5.41	5.02	4.86	5.15	5.38
Ether extract	8.55	4.22	4.16	3.77	5.66	4.16	3.61	3.06	3.42
Ash	3.58	3.06	2.50	3.31	3.88	2.87	2.94	3.55	3.00
Nitrogen free- extract	46.06	54.9	49.5	54.5	52.1	51.9	51.9	5.71	54.3

*Vit A – 10,000.00 iu, D_3-2,000 iu, B1-0.75g, B_2-5g, Nicotinic acid – 25g, Calcium pantothenate 12.5g, B_12 – 0.015g, K_3-2.5g, E-25g, Biotin – 0.050g, Folic acid – 1g, Manganese 64g, Choline chloride 250g, Cobalt – O.8g, Copper 8g, Manganese 64g, Iron – 32G, Zn-40g, Iodine-0.8g, Flavomycin-100g, Spiramycin 5g, Dl-methionie-50g, Selenium 0.6g, Lysine 120g, BAT-5g.

Results

Haematological Parameters: Table 2 show that significant ($p<0.05$) differences existed among treatments in haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), mean cell haemoglobin (MCH) and differentials (neutrophils, lymphocytes, monocytes, eosinophils and basophils). However, the mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were not significantly ($p>0.05$) influenced by dietary treatments.

White blood cell count (WBC) values of rabbits on treatments 5 and 7 were similar ($p>0.05$), and these were significantly ($p<0.05$) higher than the values obtained from rabbits on other treatments. Rabbits on treatments 6 and 8 also had similar WBC values and these were significantly ($p>0.05$) higher than the WBC values of rabbits on treatments 1, 2, 3, 4 and 9. The least WBC value was obtained from rabbits on treatment 2.

The red blood cell (RBC) values of rabbits on treatments 3, 4 and 9 were significantly ($p<0.05$) higher than the RBC values of those on treatments 1, 2 and 8, but were similar to the RBC values of rabbits on treatments 5 and 6. Rabbits on treatment 1(control) had the least RBC value. The Hb values of rabbits on treatments 5, 7 and 9 were similar to the Hb values of rabbits on treatments 6, and these were significantly ($p<0.05$) higher than the Hb values of rabbits on other treatments. The control group had the least haemoglobin value.

The packed cell volume (PCV) value of rabbits on treatments 5 and 9 were similar to the PCV values of rabbits on treatments 2, 3, 4, 6, 7 and 8, and there were significantly ($p<0.05$) higher than the PCV values of rabbits on treatment 1 (control). Rabbits on treatment 1 had similar PCV values with those on treatments 2, 3, 4, 6, 7 and 8.

Rabbits on treatment 8 had similar MCH value with those in treatments 1 and 2 and this was significantly ($p<0.05$) higher than the MCH values of rabbits on treatments 3, 4, 5, 6, 7 and 9. Rabbits on treatments 1, 2, 7 and 9 had similar MCH values. Rabbits on treatments 3 and 4 had the least MCH values. Rabbits on treatment 5 had the highest lymphocytes value, while those on treatment 7 had significantly ($p<0.05$) higher lymphocytes value than rabbits on treatments 1, 2, 3, 4, 6, 8 and 9. The neutrophils of rabbits on treatment 1 (control), 3 and 4 were similar to the values obtained from rabbits on treatments 2, 8 and 9, and these were significantly ($p<0.05$) higher than the values obtained from rabbits on treatments 5, 6 and 7. Rabbits on treatments 2, 8 and 9 and those on treatment 6 had similar neutrophils and these were significantly ($p<0.05$) higher than the values obtained from rabbits on treatments 5 and 7. Rabbits on treatment 5 had the least neutrophils value. The monocytes of rabbits on treatments 2, 3, 4

and 5 were similar, and these were significantly ($p<0.05$) higher than the monocytes of rabbits on treatments 1, 6, 7, 8 and 9 which were also similar. The value of basophils of rabbits on treatment 4 was similar to the values obtained from rabbits on treatments 2, 3 and 5. Rabbits on treatments 1, 6, 7, 8 and 9 had similar values of basophils with those on treatments 2, 3 and 5 and these were significantly lower than the values obtained from rabbits on treatment 4. Rabbits on treatment 5 had significantly ($p<0.05$) higher eosinophil value than those on other treatments.

Table: 2Haematological values of growing rabbits fed diets containing varying levels of Vitamins C and E

Vitamin E levels ($mgkg^{-1}$)		**0**			**200**			**400**		
Vitamin C levels ($mgkg^{-1}$)	0	200	400	0	200	400	0	200	400	
Parameters / treatments	1	2	3	4	5	6	7	8	9	**SEM**
White blood cell ($\times10^3/mm^3$)	10750^{e}	10550^{f}	11450^{cd}	11550^{c}	12400^{a}	11750^{b}	12450^{a}	11800^{b}	11350^{d}	**148**
Red blood cell ($\times10^6/mm^3$)	10.525^{e}	10.88^{d}	11.62^{a}	11.64^{a}	11.57^{ab}	11.61^{ab}	11.52^{b}	10.97^{c}	11.62^{a}	**0.096**
Hemoglobin (g/100ml)	12.40^{e}	12.75^{d}	12.95^{cd}	13.05^{bc}	13.35^{a}	13.30^{ab}	13.50^{a}	13.05^{bc}	13.50^{a}	**0.085**
Packed cell volume (%)	36.0^{b}	42.5^{ab}	40.5^{ab}	45^{ab}	13.35^{a}	38.5^{ab}	43^{ab}	43.5^{ab}	13.50^{a}	**0.956**
Mean cell hemoglobin (%)	11.75^{ab}	11.69^{abc}	11.13^{e}	11.18^{e}	11.53^{cd}	11.45^{d}	11.68^{bc}	11.85^{a}	11.59^{bcd}	**0.057**
Mean cell hemoglobin (%) concentration	34.45	30.05	31.98	29	29.10	34.53	31.41	30.33	31.39	**0.656**
Mean cell volume (μm^3)	34.19	39.15	35	38.6	39.9	33.1	37.3	39.6	37.1	**0.79**
Lymphocytes (%)	70^{cde}	69.5^{cde}	68.5^{de}	68^{e}	79^{a}	71.5^{c}	76^{b}	69.5^{cde}	70.5^{cd}	**0.677**
Neutrophils (%)	30^{a}	28^{ab}	30^{a}	29.5^{a}	21^{d}	27^{b}	25^{c}	28.5^{ab}	28^{ab}	**0.856**
Monocytes (%)	0^{b}	2^{a}	1^{a}	1^{a}	12400^{a}	0^{b}	0^{b}	0^{b}	0^{b}	**0.147**
Basophil (%)	0^{b}	0.5^{ab}	0.5^{ab}	1^{a}	11.57^{ab}	0^{b}	0^{b}	0^{b}	0^{b}	**0.00**
Eosionophil (%)	0	0	0	0	13.35^{a}	0	0	0	0	**0.00**

^{abc}means with different superscripts across a given row differ significantly ($p<0.05$). SEM= standard error of mean

Carcass traits and Organ Characteristics

In table 3Significant ($p<0.05$) differences were observed among treatments in live weight, carcass weight and dressing percentage of the rabbits. There were no significant ($p>0.05$) differences between treatments of relative organ weight. Rabbits on treatment 4 had significantly ($p<0.05$) higher mean live weight and mean dressed carcass weight than those on treatments 1, 2, and 6 but were similar to those obtained in treatment3, 5, 7, 8 and 9 which were similar to the values observed in treatments 1, 2and 6. Dressing % values of treatment 4 and 8 were similar ($p>0.05$) to dressing % of rabbits in treatment 2, 7, 8 and 9 but were significantly ($p<0.05$) higher than those obtained in treatment 1, 3, 5 and 6 which were similar ($p>0.05$) to those obtained in treatment 2.

Table: 3Effects of Varying Inclusion Levels of Vitamins C and E on Carcass traits and relative Organ Characteristics of growing rabbits

Vitamin Elevels(mgkg^{-1})		0			200			400		
Vitamin Clevels(mgkg^{-1})	0	200	400	0	200	400	0	200	400	
Parameters/treatments	1	2	3	4	5	6	7	8	9	**SEM**
Live weight (kg)	1.27^{b}	1.32^{b}	1.42^{b}	1.87^{a}	1.55^{ab}	1.27^{b}	1.50^{ab}	1.47^{ab}	1.50^{ab}	**0.05**
dressed weight (kg)	0.72^{b}	0.77^{b}	0.83^{ab}	1.11^{a}	0.89^{ab}	0.73^{b}	0.89^{ab}	0.89^{ab}	0.89^{ab}	**0.39**
Dressing (%)	56.8^{b}	58.3^{ab}	57.9^{b}	62.8^{a}	57.9^{b}	57.7^{b}	59.3^{a}	60.2^{a}	59.3^{a}	**0.59**
Lungs (%)	0.04	0.05	0.04	0.03	0.03	0.04	0.04	0.04	0.04	**0.02**
Liver (%)	0.39	0.28	0.25	0.21	0.30	0.36	0.22	0.29	0.29	**0.02**
Heart (%)	0.01	0.02	0.01	0.01	0.01	0.02	0.02	0.02	0.02	**0.01**
Kidney (%)	0.04	0.05	0.04	0.03	0.03	0.05	0.04	0.04	0.04	**0.02**
Large intestine (%)	0.31	0.25	0.21	0.16	0.24	0.31	0.18	0.26	0.22	**0.02**
Small intestine (%)	0.16	0.19	0.17	0.12	0.13	0.18	0.14	0.13	0.15	**0.1**

^{abc}means with different superscripts across a given row differ significantly (p<0.05). SEM= standard error of mean

Discussion: As shown in Table 2, haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), mean cell haemoglobin (MCH) and differentials (neutrophils, lymphocytes, monocytes, eosinophils and basophils) were significantly ($p < 0.05$) affected by dietary treatments, while mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV were not significantly ($p>0.05$) affected by the dietary treatments. The White blood cell count (WBC) of rabbits on treatments 5 and 7 increased significantly. The increase observed in white blood cell counts of these rabbits under investigation tends to suggest that these animals could have been attacked by disease-causing microbes, and therefore had to increase and mobilize their WBC to combat such diseases. Baker *et al*. (1998) had shown that the WBC counts of rabbits tend to increase as a defensive mechanism, in a disease condition. The Red blood cell values of rabbits on treatments 3, 4, 9 were ($p> 0.05$) were comparable to those of rabbits on treatments 5 and 6, but higher than those produced by rabbits on treatments 1, 2 and 8. These results agree with the findings of Sedki *et al*. (2002) and Meshreky and Shaheed (2003) in studies that investigated the effects of vitamins C and E alone and their combinations on rabbits' haematology. Their findings showed that these vitamins had only appreciable significant effect on lymphocytes % which is a good indicator of increase in the immune efficiency of the investigated rabbits. Badwev and Karmovsky (1980) also reported that vitamin E plays an important role in protecting leukocytes and macrophages during phagocytosis, and protects leukocyte from the toxic products produced from ingested bacteria. The haemoglobin (Hb) values of rabbits on treatments 5, 6, 7 and 9 were comparable ($p> 0.05$), but were higher than the values obtained from rabbits on treatments 2, 3, 4and 6. Rabbits in treatment1 had the least haemoglobin value. The variations observed in the Hb values of the rabbits may be as a result of heat stress and this tends to show that vitamins C and E helped in combating heat stress in rabbits. A similar observation has been reported by Rao and Sharma (2001). Puron *et al*. (1994) had earlier reported that addition of 200-600mg of vitamin in the diets of rabbits, improved livability in heat stress. Results (Table 9) also show that rabbits on treatments 2 to 9had similar ($p> 0.05$) packed cell volume (PCV) values which were higher than that of rabbits on treatment 1. Higher PCV, RBC and HB were also observed in treatments 4,5 and 7. This could be due to the fact that vitamins C and E have anti-oxidant property and this may have favoured the formation of these

blood parameters. However, the values obtained in the present study fall within the normal range reported by Ozkan *et al.* (2012).It was observed that rabbits on treatment 6 had the highest mean cell hemoglobin value affirming that when vitamin C and E are supplemented in the diet of growing rabbits at 200mg/kg, this will help to boost the immune system of rabbits as reported by Rao and Sharma (2001). The protective role of these vitamins is more effective with vitamins E and C administered simultaneously as compared to using vitamin E and C separately. Vitamin E has been reported to be the first line of defense against chemically induced oxidative stress (Ibrahim *et al*., 2000) whereas vitamin C has an important role in the regeneration of reduced form of vitamin E (Tanaka *et al*., 1997). It is well known that antioxidants such as vitamin E and vitamin C can act synergistically to prevent cell destruction (Beyer, 1994; Chen and Tappel, 1995; Lass and Sohal, 2000).

Data on carcass traits and organ characteristics of rabbits fed the experimental diets (Table 3) show that treatments had significant ($p < 0.05$) effects on the carcass traits (live weight, carcass weight and dressing percentage) but not on organ characteristics (relative organ lungs and large intestine weight) of the rabbits. As shown in Table 10, rabbits on treatments 4 had significantly ($p<0.05$) higher live weight, dressed carcass weight and dressing % than the control group. This tends to suggest that rabbits which had access to the diet containing 0 mg/kg vitamin C and 200mg/kg vitamin E had higher carcass yield (carcass weight and dressing percentage) as compared to those in the control group. The significant improvement observed in the dressing percentage of rabbits fed diet containing 200mg/kg diet of vitamin E is in line with the observations made by Abdel-Hamid (2006) with vitamin C and Corino *et al.* (2009) with vitamin E which indicated that dressing % was significantly improved with such supplementations. However this contradicts the reports of Castellini *et al.* (1998), Sedki *et al.* (2002) and Selim *et al.* (2004) which indicated that vitamins C or E had no significant effect on carcass traits of the rabbits used in their studies. The results obtained in this study had affirmed that rabbits which had access to both vitamins had better live weight gain and carcass yield (dressed carcass weight and dressing percentage) than the control group.

Conclusion: Animal production vis-a`-vis rabbit production is seriously affected by high ambient temperature which is widely the case in a hot humid tropical environments such as Nigeria. Results from this study shows that a combination of $200mgkg^{-1}$diet of vitamin C and $200mgkg^{-1}$diet of vitamin E can be successfully added to the diet of growing rabbits during the dry season.

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