

Full Length Research

Effects of Dietary Inclusion of Vitamins C and E on Nutrient Digestibility, Haematological and Carcass Characteristics of Rabbits in a Hot Humid Tropical Environment

*Okachi, V. C.W. and Ani, A. O.,

Department of Animal Science, University of Nigeria Nsukka, Nigeria

*Corresponding author Email: vwestleyc@gmail.com; augustine.ani@unn.edu.ng

Accepted 12th July, 2016.

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 0.60-1.0kg were randomly divided into nine groups of four rabbits each and assigned to 9 diets in a 3×3 factorial 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. Feed and water were supplied to the animals *ad libitum*. Data were collected on nutrient digestibility, haematology, carcass and organ weights of rabbits. Results showed that although dietary treatments had no significant effects on dry matter (DM) and crude protein (CP) intakes by rabbits, they had significant effects ($p < 0.05$) on the intakes of crude fibre (CF), ether extract (EE) and nitrogen-free extract (NFE) by rabbits. The diet containing 200mg/kg of vitamin E (treatment 4) produced the highest dry matter (DM) digestibility coefficient which was similar ($p > 0.05$) to that of the control and the diet containing 400mg/kg vitamins C and E. 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 ($p < 0.05$). Treatments had significant ($p < 0.05$) effects on the carcass traits (live weight, carcass weight and dressing percentage) and organ characteristics (relative lungs and large intestine weights) of the rabbits. Rabbits on treatment 4 had significantly ($p < 0.05$) higher live weight, dressed carcass weight and dressing % than the control group. It was concluded that a combination of 200mg of vitamin C per kg diet and 200mg of vitamin E per kg diet can be successfully added to the diet of growing rabbits in a hot humid tropical environment, without having any negative effect on carcass yield, relative organ weights and haematological parameters of rabbits.

Keywords: Rabbits, vitamins C and E, nutrient digestibility, haematology, carcass, organ weights

INTRODUCTION

The ever increasing human population with the attendant insufficient animal protein intake is the major problem confronting developing world economies like

Nigeria. 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. Alleviation of poverty, attainment of food security and provision of adequate nutrition are some of the millennium development goals that Nigeria has to meet. This makes intensive rabbit production in Nigeria one of the surest ways of solving the problems associated with low protein intake particularly, as rabbits have fast growth, high fecundity and short generation interval. Other exceptional attributes of rabbits include as follows: affordable or low cost management requirement, small body size, ability to utilize forage and agricultural by-products, adaptation to a wide range of ecological environment and genetic diversity (Onifade *et al.*, 1999). However one of the factors that militate against increased rabbit production in Nigeria is heat stress, especially during the hot dry season.

Nigeria is close to the equator and characterised by high ambient temperatures between 27°C and 44°C, which might be detrimental to the performance of exotic animals introduced into the country. In tropical and subtropical 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). Live body weight and gain were decreased by exposure to heat stress. Higher temperature and higher humidity are most conducive for growth and proliferation of disease producing microbes. Within body systems, a higher body temperature implies all metabolic reactions taking place at a higher rate (Q10 effect) and reducing body's capacity to fight the production are factors that influence heat load, but the underlying physiological, behavioural or genetic mechanisms are largely unknown (Hall, 2004). 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). Dietary supplementation with vitamin 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).

Antioxidants are substances that inhibit oxidation, especially one used to counteract the deterioration of cells in the body. Under normal conditions, the body has sufficient antioxidant reserves to cope with the production of free radicals (oxidants), which are

produced continuously during metabolism and may increase as a result of pathological and other circumstances. When oxidants generation exceeds the body's antioxidant production capacity, oxidative stress develops (Roth, 2000). The formation of these oxidants is counteracted by natural anti-oxidants. The α -tocopherol (vitamin E) is a highly effective natural antioxidant that protects cellular membranes against oxidative damage. According to Morrissey *et al.* (1994) Vitamin C can reduce the generation of oxidants and regenerates α -tocopherol from its oxidation form (Reed, 1992). Based on that anti-oxidant action, both vitamins have been used on rabbits under stress conditions to improve the performance *in vivo*. Meshreky and Shaheed (2003) and Corino *et al.* (2007) and others working on vitamin E, and Abdel-Hamid (1994) and Sedki *et al.* (2002) working on vitamin C, reported a growth promoting action for the two vitamins in rabbits. However, other studies failed to prove such response (Castellini *et al.*, 1998, 2001; Oriani *et al.*, 2001; Del Bosco *et al.*, 2004; Botsoglou *et al.*, 2004; Selim *et al.*, 2004). Vitamin C supplementations have been reported to aid in the control of increase in body temperature and plasma corticosteroid concentration, improves shell and egg quality through its formation of the shells organic matrix in birds, protect immune system and reduce mortality of growing rabbits (Rao and Sharma, 2001). Puron *et al.* (1994) reported that addition of ascorbic acid (200-600mg) in diets, improves growth, feed efficiency, and livability in heat stress. Bain (1996) revealed that vitamin C aids conversion of protein and fat into energy for production and survivability through increase corticosteroid secretion. According to Sahin and Kucuk (2001), vitamin C supplementation increases performance and carcass yield in broilers reared under heat stress conditions (32°C). However, the synergic effects of dietary inclusion of vitamins E and vitamin C on nutrient intake and digestibility, haematology and carcass characteristics of growing rabbits in a hot humid environment are yet to be substantiated. The study was therefore conducted to evaluate the effects of dietary inclusion of vitamins C and E on nutrient digestibility, haematological and carcass characteristics of rabbits in a hot humid tropical environment.

MATERIALS AND METHOD

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 for nine (8) weeks. The climate during the period of study was characterized by dry to wet season, low to high relative humidity (Range:

Table 1: Percentage Composition of Diets Containing Varying Levels of Vitamins C and Experimental Diets

Vitamin levels(mgkg ⁻¹)	E	0	200	400	0	200	400	0	200	400
Vitamin levels(mgkg ⁻¹)	C	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		67.16	66.39	64.87	70.55	72.54	64.67	65.82	68.16	71.31
Crude protein		15.38	14.81	16.99	15.09	16.45	15.46	15.80	16.73	16.513
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	54.95	49.27	54.46	52.10	51.98	51.92	5.712	54.33

*Vit A – 10,000.00 iu, D₃-2,000 iu, B₁-0.75g, B₂-5g, Nicotinic acid – 25g, Calcium pantothenate 12.5g, B₁₂ – 0.015g, K₃-2.5g, E-25g, Biotin – 0.050g, Folic acid – 1g, Manganese 64g, Choline chloride 250g, Cobalt – 0.8g, 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.

4% and 85% at 10am) and higher average temperature of 36.7°C to 41°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 641°N and 724°E (Ofomata, 1975) with an altitude of 447m above sea level (Breinhalt *et al.*, 1981). The daily mean ambient temperature range is between 26-32.8°C (Uzodinma and Ofoelule, 2009).

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.

Experimental Animals and Management

Thirty-six hybrid (Chinchilla × New Zealand white) growing rabbits of both sexes with initial average weight of 0.60-1.0kg were randomly divided into nine groups of four rabbits each and assigned to 9 diets a in a 3×3 factorial 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. The percentage composition of the experimental diets is presented in Table 1. Each treatment group was replicated four times with a rabbit constituting a replicate placed in a four-tier rabbit cages that had a total of 16

hutches per tier. Each hutch measures 0.6m x 0.5m x 0.4m.

The cages were located inside a building equipped with vents and windows for proper ventilation. Each hutch, which accommodated 1 rabbit, was partitioned with metallic sheets and wire mesh and fitted with metallic trays (for collection of faecal droppings) and with stainless feeders and drinkers. The rabbits were provided feed and water *ad libitum* twice daily at 0.800h and 16.00h for 56 days of the experimental period.

Digestibility Study

During week 8 of the experiment, a seven-day faecal collection from three rabbits per treatment was carried out to determine the nutrient digestibility coefficients by the rabbits. During this period, weighed quantity of feed (90% of the daily feed intake) was offered to each rabbit daily. Daily feed consumption was recorded as the difference between the quantity offered and the quantity left after 24 hours. Faecal samples were collected daily from separate cages in detachable trays placed beneath the wire mesh floor of the cages, oven-dried for 24hrs at 60°C to achieve complete dryness and weighed over a seven-day period. At the end of the collection period, all faecal samples from each rabbit were bulked and preserved for analysis. Data collected were used to determine the digestibility coefficients.

Haematological Evaluation

At the end of the 8th week of the experiment, two rabbits were randomly selected from each treatment group. Blood samples were collected with sterile syringes aseptically from the ear vein of the animal and transferred into sterile universal bottles containing a pinch of dried ethyl-diamine-teracetic acid (EDTA) and refrigerated before analysis. Haematological parameters that were determined included haemoglobin concentration (HbC), packed cell volume (PCV), white blood cell (WBC) count, and red blood cell (RBC) count. The PCV was determined by the microhaematocrit method described by Schalm *et al.* (1975), and Mitruka and Rawnsley (1977) using a microhaematocrit centrifuge and reader (Hawksley and Sons Ltd, England). The Hb was determined using a haemoglobinometer (Marienfeld, Germany), while the WBC counts were carried out by the haemocytometer method using an improved Neubauer counting chamber (Hawksley, England) and avian RBC and WBC diluting fluids as described by Campbell and Coles (1986) and Lamb (1991). Serum metabolites (total protein (TP), glucose albumin, globulin, creatine, cholesterol and

calcium) were also determined according to the methods described by Campbell and Coles (1986).

Carcass and Organ Evaluation

At the end of the feeding trials, two (2) rabbits from each treatment were selected randomly; they were starved for 12hrs, weighed and slaughtered. Eviscerated carcasses were roasted to remove fur and weighed to determine carcass yield. Body organs (liver, kidney, lungs and heart) were removed from each rabbit and weighed fresh. The intestines (large and small) were also weighed for each rabbit and all the organ weights were expressed as percentages of the live weights.

Proximate and Statistical Analyses

The experimental diets and faeces were assayed for proximate composition by the method of AOAC (1990). Data collected were subjected to analysis of variance (ANOVA) for completely randomized design (CRD) using a Stat Graphic Computer Package (SPSS, 2007) Model. Significantly different means were separated using Duncan's New Multiple Range Test (Duncan, 1955) option in SPSS.

RESULTS AND DISCUSSION

Proximate Composition of Experimental Diets

Table 2 shows the proximate composition of the experimental diets.

Effects of Varying Inclusion Levels of Vitamins C and E on Nutrient Intake of Growing Rabbits

Table 3 shows the data on nutrient intake of growing rabbits fed diets containing varying levels of vitamins C and E. Significant differences ($p < 0.05$) existed among treatments in crude fibre (CF), ether extract (EE) and nitrogen-free extract (NFE). There were no significant ($p > 0.05$) differences among treatment groups in dry matter (DM) and crude protein (CP). The crude fibre intake values of rabbits on treatments 4, 8 and 9 were similar ($p > 0.05$) to the values observed in treatments 1, 3, 5, 6 and 7, but significantly ($p < 0.05$) higher than the value observed in treatment 2. Rabbits on treatment 2 had similar crude fibre intake value with those on treatments 1, 3, 5, 6 and 7. The ether extract (EE) intake value of rabbits on treatment 1 was higher than the values obtained from rabbits on other treatment groups. Rabbits on treatment 6 had the least ether extract (EE)

Table 2: Proximate composition of the experimental diets

Vitamin E levels(mgkg ⁻¹) Vitamin C levels(mgkg ⁻¹) Components (%)	0			200			400		
	0	200	400	0	200	400	0	200	400
	1	2	3	4	5	6	7	8	9
Dry matter	67.16	66.39	64.87	70.55	72.54	65.82	64.67	68.16	71.31
Crude protein	15.38	14.81	16.99	15.09	16.45	15.80	15.46	16.73	16.513
Crude fibre	5.16	4.51	5.14	5.35	5.41	4.86	5.02	5.15	5.38
Ether extract	8.55	4.22	4.16	3.77	5.66	3.61	4.16	3.06	3.42
Ash	3.58	3.06	2.50	3.31	3.88	2.94	2.87	3.55	3.00
Nitrogen free- extract	46.06	54.95	49.27	54.46	52.10	51.92	51.98	5.712	54.33

Table 3: Nutrient intake of rabbits fed diets containing varying levels of Vitamins C and E

Vitamin E levels(mgkg ⁻¹) Vitamin C Levels(mgkg ⁻¹) Components	0			200			400			SEM
	0	200	400	0	200	400	0	200	400	
	1	2	3	4	5	6	7	8	9	
Dry matter	67.16	66.39	64.87	70.55	65.82	68.16	64.67	72.54	71.33	0.97
Crude protein	15.38	14.81	16.99	15.09	15.80	16.13	15.46	16.45	16.53	0.23
Crude fibre	5.16 ^{ab}	4.51 ^b	5.14 ^{ab}	5.35 ^a	4.86 ^{ab}	5.15 ^{ab}	5.02 ^{ab}	5.41 ^a	5.38 ^a	0.79
Ether extract	8.55 ^a	4.22 ^c	4.16 ^c	3.77 ^{cd}	3.62 ^{cd}	3.06 ^e	4.16 ^c	5.66 ^b	3.42 ^{cd}	0.29
Nitrogen free-extract	46.1 ⁱ	54.9 ^a	49.4 ^h	54.5 ^b	51.9 ^g	52.7 ^d	51.9 ^f	52.1 ^e	54.3 ^c	0.47

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

value. Rabbits on treatment 2 had significantly ($p < 0.05$) higher nitrogen-free extract (NFE) intake value than rabbits on other treatments. Rabbits fed the control diet had the least NFE intake value.

As shown in Table 3, dietary treatments had no significant effect on dry matter (DM) and crude protein (CP) intakes by rabbits (Table 3). However, treatments had significant effects ($p < 0.05$) on the intakes of crude fibre (CF), ether extract (EE) and nitrogen free extract (NFE) by rabbits. The results show that the inclusion of vitamin C in the diets enhanced nutrient intake. West (1999, 2003) had shown that high temperature, above the critical threshold is related to reduce feed intake and has deleterious effect on the physiological status of rabbits.

Nutrient Digestibility of Growing Rabbits Fed Diets Containing Varying Levels of Vitamins C and E

Table 4 shows the data on nutrient digestibility % of rabbits fed diets containing varying levels of vitamins C and E. Significant differences ($p < 0.05$) existed among treatments in dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and nitrogen free extract (NFE) digestibility coefficients. The dry matter digestibility % of rabbits on treatment 4 was similar to that of rabbits on treatment 9, and this was significantly

($p < 0.05$) higher than the values observed in other treatment groups. Rabbits on treatment 9 had similar ($p < 0.05$) DM % value with those on treatments 2, 5, 7 and 8, and this was significantly ($p < 0.05$) higher than the values observed in treatments 1, 3 and 6. Rabbits on treatments 1, 2, 3, 6 and 7 had similar Dm % values. The crude protein digestibility % of rabbits on treatment 6 was similar ($p > 0.05$) to the values obtained from rabbits on treatments 1, 5, 8 and 9, but significantly ($p < 0.05$) higher than the values obtained from rabbits on treatments 2, 3, 4 and 7. Rabbits on treatments 1, 2, 3, 4, 5, 7, 8 and 9 had similar CP digestibility coefficients. Rabbits in the control group had the highest crude fibre digestibility %, but this was similar ($p > 0.05$) to the values obtained from rabbits on treatments 5 to 9. Rabbits on treatment 4 had the least CF digestibility % value, although this value was similar to that of rabbits on treatment 3. The ether extract digestibility coefficients of rabbits on treatments 1, 2, 3 and 6 were similar to those of rabbits on treatments 4, 5, 7 and 8. Rabbits on treatment 9 had the least ether extract digestibility % value. The Nitrogen-free extract digestibility coefficient of rabbits in the control group was significantly ($p < 0.05$) higher than the values obtained from rabbits on other treatment groups. Rabbits on treatment 9 had the least NFE digestibility coefficient value, but this was similar to that of rabbits on treatment 7.

Table 4: Digestibility coefficients of growing rabbits fed 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
Dry matter	98.17 ^c	98.79 ^{bc}	98.47 ^{cd}	99.33 ^a	98.90 ^b	98.47 ^{cd}	98.77 ^{bc}	98.90 ^b	99.07 ^{ab}	0.07
Crude protein	55.03 ^{ab}	45.20 ^b	47.03 ^b	46.20 ^b	51.87 ^{ab}	59.7 ^a	43.73 ^b	53.57 ^{ab}	50.17 ^{ab}	1.37
Crude fibre	75.13 ^a	63.10 ^b	61.70 ^{bc}	53.93 ^c	62.87 ^{ab}	66.33 ^{ab}	70.48 ^{ab}	68.90 ^{ab}	66.03 ^{ab}	1.36
Ether extract	95.43 ^a	92.17 ^a	94.20 ^a	87.37 ^{ab}	89.67 ^{ab}	91.47 ^a	81.43 ^{ab}	88.77 ^{ab}	65.29 ^c	1.89
Nitrogen free-extract	35.5 ^a	20.13 ^{ef}	29.c ^b	25.07 ^{cd}	20.13 ^{de}	27.57 ^{bc}	17.94 ^g	23.2 ^d	16.10 ^g	1.16

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

Data on digestibility coefficients (Table 4) show that dietary treatment 4 (diet containing 200mg/kg of vitamin E and 0mg/kg of vitamin C) produced the highest dry matter (DM) digestibility coefficient even though it was similar to that of dietary treatment 9 (diet containing 400mg/kg of vitamin E and 400mg/kg of vitamin C (treatment 9)). Crude protein digestibility was highest in treatment 6 (diet containing 200mg/kg of vitamin E and 400mg /kg of vitamin C). However, the CP digestibility coefficient of rabbits in treatment 6 was similar to the CP digestibility coefficients of rabbits in treatments 1, 5, 8 and 9. The crude fibre digestibility coefficient of rabbits in treatment 1 (control) was the highest, although the value was similar to the values obtained in treatments 5 to 9. Treatments 1 to 9 had similar ether extract digestibility coefficients while treatment 9 had the least ether extract digestibility coefficient. Nitrogen-free extract digestibility was highest in dietary treatment 1 (control). The results obtained in the present study show that dietary inclusions of vitamins C and E did not have a remarkable influence on the nutrient digestibility coefficients with the exception of DM digestibility.

Effects of Varying Inclusion Levels of Vitamins C and E on Haematological Parameters of Growing Rabbits

Table 5 show the effects of varying inclusion levels of vitamins c and e on haematological parameters of growing rabbits. 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 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

Table5: Haematological values of growing rabbits fed diets containing varying levels of Vitamins C and E

Vitamin Eleveles(mgkg ⁻¹)	0			200			400			
Vitamin Cleveles(mgkg ⁻¹)	0	200	400	0	200	400	0	200	400	
Parameters / traments	1	2	3	4	5	6	7	8	9	SEM
White blood cell ($\times 10^3/\text{mm}^3$)	10750 ^e	10550 ^f	11450 ^{cd}	11550 ^c	12400 ^a	11750 ^b	12450 ^a	11800 ^b	11350 ^d	148
Red blood cell ($\times 10^6/\text{mm}^3$)	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}	33.35 ^a	38.5 ^{ab}	43 ^{ab}	43.5 ^{ab}	33.50 ^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

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.

As shown in Table 5, 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. Vitamin E is known to play 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, 4 and 6. Rabbits in treatment 1 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). Puroh *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 5) also show that rabbits on treatments 2 to 9 had 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

Table 6: Effects of Varying Inclusion Levels of Vitamins C and E on Carcass traits and relative Organ Characteristics of growing rabbits

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
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}	0.051
Carcass weight (kg)	0.72 ^c	0.97 ^{bc}	1.01 ^{bc}	1.47 ^a	1.16 ^{ab}	0.90 ^{bc}	1.14 ^{ab}	1.12 ^{ab}	1.13 ^{ab}	0.054
Dressing percentage (%)	56.80 ^b	73.15 ^a	70.65 ^a	78.65 ^a	70.05 ^a	70.65 ^a	75.95 ^a	76.25 ^a	75.60 ^a	1.61
Lungs(g)	0.52 ^{abc}	0.66 ^a	0.56 ^{abc}	0.45 ^c	0.55 ^{abc}	0.57 ^{abc}	0.56 ^{bc}	0.51 ^{abc}	0.59 ^{ab}	0.016
Liver (g)	3.27	3.74	3.63	3.65	4.25	3.37	4.19	3.80	4.35	0.071
Heart (g)	0.19	0.24	0.2	0.19	0.25	0.25	0.25	0.19	0.22	0.074
Kidney (g)	0.55	0.64	0.59	0.51	0.53	0.58	0.59	0.53	0.55	0.017
Large intestine (g)	2.55 ^b	3.27 ^{ab}	3.06 ^{ab}	3.22 ^{ab}	3.35 ^{ab}	2.83 ^b	4 ^a	2.90 ^b	3.37 ^{ab}	0.119
Small intestine (g)	2.05	2.61	2.54	2.02	1.97	2.11	2.30	2.22	2.32	0.072

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

at 200mg/kg, the 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 defence 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).

Carcass traits and Organ Characteristics of Growing Rabbits Fed Diets Containing Varying Levels of Vitamins C and E

Data on carcass traits and organ characteristics of rabbits fed the experimental diets are summarized in Table 6. Significant ($p < 0.05$) differences were observed among treatments in live weight, carcass weight and dressing percentage of the rabbits. Significant ($p < 0.05$) differences also existed among treatments in some of the relative organ weights such as lungs and large intestine of the animals. Rabbits on treatment 4 had significantly ($p < 0.05$) higher mean live weight and mean dressed carcass weight than those on treatments 1, 2, 3 and 6. However, the mean live weight values of rabbits on treatments 5, 7, 8 and 9 were similar to the values observed in treatments 1, 2, 3, 4, 5, 6, 7, 8 and 9. Similar mean dressed carcass weight values were also observed in treatments 1, 2, 3 and 6. The mean carcass dressing % values of rabbits on treatments 2 and 9 were similar and significantly ($p < 0.05$) higher than that observed in the control treatment. Rabbits on treatment 2 had the highest relative lungs weight but this was

similar ($p > 0.05$) to the values for rabbits on treatments 1, 3, 5, 6, 8 and 9. Rabbits on treatment 4 had the least value. Rabbits on treatment 7 had relative large intestine weight that was similar to those of rabbits on treatments 2, 3, 4, 5 and 1 and this was significantly ($p < 0.05$) higher than the values observed in treatments 1, 6 and 8. Rabbits on treatments 1, 2, 3, 4, 5, 6, 8 and 9 had similar large intestine weights.

As shown in Table 6, 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 show 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

The results obtained in the present study show that a combination of 200mg of vitamin C per kg diet and 200mg of vitamin E per kg diet can be successfully added to the diet of growing rabbits in a hot humid tropical environment, without having any negative effect

on carcass yield, relative organ weights and haematological parameters of rabbits.

REFERENCES

- Abdel-Hamid (2006). *Effect of Adrenal Hormone and Ascorbic Acid on Resistance of Growing Rabbits. PhD. Thesis*, Faculty of Agriculture, Alexandria University, Egypt.
- Abdel-Hamid E. (1994). *Effect of Adrenal Hormone and Ascorbic Acid on Resistance of Growing Rabbits. PhD. Thesis*, Faculty of Agriculture, Alexandria University, Egypt.
- AOAC (1990). Association of Official Analytical Chemists. Official Methods of Analysis (21st edition) Washington D.C. USA.2330.
- Bain, B. S. (1996). *The Role of Vitamin c in Stress Management*.World Poultry, 12(4) 34-41.
- Baker FJ, Silverton RE, Pallister CJ (1998). *Bakers and Silvertons Introduction to Medical Laboratory Technology*.7th ed. Butterworth-Heinemann, Oxford, 339 – 73.
- Beyer, R.E. (1994). The Role of Associate In Antioxidant Protection of Biomolecules Interaction With Vitamin E And Coenzyme Q. *J. Bioenerg Biochem* 26: 349 – 58.
- Botsoglou, N., Florou-Paneri, P., Christaki, E., Giannenas, I., Spais, A. (2004). *Performance of Rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with Oregano essential oil*.Arch. Animal Nutrition. 58(3), 209-218.
- Breinholt, K.A, Gowen, F.A and Nwosu C.C (1981).Influence of Environmental and Animal Factors on Day and Night Grazing Activity of Imported Holstein Freisian Cows in the Humid Lowland Tropics of Nigeria.Trop. Animal Produc.6:4.
- Campbell, T.W. and Coles, E.H. (1986). Avian clinical pathology. In: Coles E.H (ed) Veterinary Clinical Pathology. 4th edn. Saunders, Philadelphia, pp 279-291
- Castellini, C., Del Bosco, A., Bernardini, M. (2001). Improvement of lipid stability of Rabbits and oxidative stability of rabbit meat by Vitamin C and E administration: *Journal of the science of food and agriculture*, 81, 46-53.
- Castellini, C., Del Bosco, A., Bernardini, M., Cyril, H. (1998). *Effects of dietary vitamin e on oxidative stability of raw and cooked rabbit meat*.Meat science, 50, 153-161.
- Chen, H, Tappel AL (1995). *Protection of vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q0, coenzyme Q10, betacarotene, canthaxanthin, and (+)-catechin against oxidative damage to rat blood and tissues in vivo*.Free Radic Biol Med; 18: 949 – 53.
- Corino, C., Lo-Fiegn, D., Macchionio, P., Pastorelli, G.,DiGiancamillo, A., Domeneghini, C., Possi, R. (2007). *Influence of dietary conjugated linoleic acids and Vitamin E on meat quality and adipose tissues in Rabbits*. Meat science.79, 19-28.
- Del Bosco A., Castellini C., Bianchi L., Mugnai C. (2004). *Effects of dietary α -linolenic acid and Vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat*. Meat Science,66, 407-413.
- Duncan D. B.(1955). Multiple Range and Multiple F-test.Biometrics, 11:1-42.
- Farooq U, Samad, HA, Shehzad F, Qayyum A (2010).Physiological Responses of Cattle to Heat Stress.*World Appl. Sci. J.* 8 (Special Issue of Biotechnology and Genetic Engine Habeeb et al., 1992).
- Hall, S.J.G. (2004). *Livestock biodiversity.Genetic Resources for the Farming of the Future*.Blackwell
- Ibrahim WH, Blagavan HW, Chopra RK, Chow CK (2000). Dietary coenzyme Q10 and vitamin E alter the status of these compounds in rat tissues and mitochondria. *J Nutr*; 130: 2343– 9.
- Lamb, G. N. (1991). *Manual of Veterinary Laboratory Technique*. CIBA – GEIGY, Kenya. Pp. 92 – 109.
- Lass A. and Sohal, R.S. (2000). Effect of Coenzyme Q10 and Alphanatocopherol Content of Mitochondria on Production Of Superoxide Anion Radicals. *FASEB J*; 14: 87 – 94.
- Liu H., Dong X., Tong J., Zhang Q. (2011). A Comparative Study of Growth Performance and Antioxidant Status of Rabbits When Fed With or Without Chestnut Tannins Under High Ambient Temperature. *Animal Feed Sci. and Techno.*, 164, 89-95.
- Marai IFM, Ayyat MS, Gabr HA and Abdel– Monem UM. (1996). *Effect of Summer Heat Stress And Its Amelioration on Production Performance of New Zealand White Adult Female And Male Rabbits, Under Egyptian Conditions*. Proceedings of 6th World Rabbits Congress, Toulouse, France, 2: 197-208.
- Marai, I.F.M., Ayyat, M.S and Abdel-Monem, U.M. (2002). *Growth Performance and Reproductive Traits at First Parity of New Zealand White Female Rabbits as Affected by Heat Stress And Its Alleviation, Under Egyptian Conditions*. Tropical Animal Health and Production 33, 1-12.
- Meshreky S., Saheed I. (2003). Efficiency of Vitamin E And Selenium Administration on Growth, Puberty, Anatomical And Histopathological Traits of Female Genitalia in New Zealand White Rabbits. *Egyptian Journal of Nutrition and Feeds*.6(special

- issue),299-312.
- Mitruka, B.M., and Rawnsley, H. M. (1977). Clinical Biochemical and Haematological Reference Values in Normal Experimental Animals, Masson, New York. Pp. 42 – 45.
- Morrissey P., Buckley D., Sheehy P., Monahan F. (1994). *Vitamin E and Meat Quality*. Proc. Nutr. Sci., 53, 289-295.
- Ofomata G.E.K, (1975). Nigeria in Maps Eastern States, Ethiopian Publishing Co. Ltd Benin, Pp 43-45.
- Onifade, A.A.; Abu, O. A.; Obiyan, R.I.; Abanikannda, O.T.F., (1999). *Rabbit Production in Nigeria: Some Aspects of Current Status and Promotional Strategies*. *World Rabbit Science*. 7(2):51-58.
- Oriani G., Canrio C., Pastorelli G., Ritioni A., Salvatori G. (2001). Oxidative status of plasma and muscle in Rabbits supplemented with dietary Vitamin E. *Journal of Nutritional Biochemistry*. 12, 138-143.
- Ozkan C., Kaya A. and Akgul Y. (2012). Normal Values Of Haematological And Some Biochemical Parameters in Serum and Urine of New Zealand White rabbits. *World Rabbit Science* 20 253-259.
- Puron D., Santamaria P., Segura J.C, (1994). Effects of Sodium Bicarbonate, Acetylsalic and Ascorbic acid on Brooder Performance in Tropical Environment. *Journal of Applied Poultry Research*, 3:141-145.
- Rao M. V and Sharma P.S. (2001). *Reproductive Effect of Vitamin E against Mercury chloride in Reproductive Toxicology in Male Mice*. *Reproductive toxicology*, 15(6) 705-712.
- Reed D. (1992). *Interaction of Vitamin E, Ascorbic acid and Glutathione against oxidative damage: Packer L. and Fuchs, Journal Editors, Vitamin E in Health and Disease*. Marcel dekker, New York, 269-281.
- Roth E. (2000). *Oxygen Free Radicals and their Clinical Implications*. *Actachirurgica Hungarica*, 36, 302-305.
- Sahin K., and Kucuk O. (2001). Effects Vitamin E And C on Performance Digestion of Nutrients And Carcass Characteristics In Japanese Quail Reared Under Heat Stress. *Journal of American Physiology and Animal Nutrition*. 85:335-342.
- Schalm, O.W; Jain, N.C; Caroll, E.J.(1975). *Veterinary Haematology*, 3rd edn. Lea and Febiger, Philadelphia. Pp. 471-538
- Sedki A., Ismail A., Abou-El-Ella M., Abou-El-Wafa S., Abdellah A. (2002). Performance and Immune Function of Growing Rabbits As Affected By Vitamin C And E Through The Summer Season. *Egyptian J. Agric. Res.*, 80, 847-864.
- Selim A., Soliman A., Abdel-Khalek A. (2004). Effect of Drinking Water Temperatures And Some Dietary Feed Additives on Performance of Heat Stressed Rabbits. In: *Proc. 8th World Rabbit Congress, 2004 September, Puebla, Mexico, 945-953*.
- Selim N.A., Abdel-Khalek A.M., Nada S.A., El-Medany Sh.A.(2004). *Response of Growing Rabbits to Dietary Antioxidant Vitamins E and C. 2.Effect on Meat Quality*. Animal Production Research Institute, Dokki, ARC, Egypt, Regional Center for Food and Feed, ARC, Egypt
- SPSS.Com.(2007). IBM^(R) SPSS^(R) Advantage for Microsoft^(R) IBM Corporation 2010, IBM Corporation Route, 100, Somers, N4. 10589.
- Tanaka K., Hashimoto T., Tokumaras S., Iguchi H., Kojo S. (1997). Interactions between Vitamin C and E are Observed In Tissues of Inherently Scorbutic Rats. *J Nutr*, 127: 2060 – 64.
- Uzodimma E.O and Ofoefule A. U (2009). Biomass Unit, National Center for Energy Research and Deveelopment, University of Nigeria Nsukka. *International Journal of Physical Sciences vol 4, 91-9*.
- Vietmeyer, N.D., (1985). Potential of Microlivestock in Developing Countries. *J. Applied Rabbit Res.*, 8: 1581-1586.
- West J.W. (2003). Effects of heat stress on production in dairy cattle. *J. Dairy Sci.* 86, 2131-2144.
- West J.W., Hill G.M., Fernandez J.M., Mandebvu P. and Mullinix B.G. (1999). Effects of Dietary Fiber on Intake, Milk Yield, and Digestion By Lactating Dairy Cows During Cool or Hot, Humid Weather. *J. Dairy Sci.* 82, 2455-2465.